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Ecology and physiology of parasitic plants

By

Audrey Haynes

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Integrative Biology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Wayne P. Sousa, Chair
Professor David Ackerly
Professor Todd Dawson
Professor Whendee Silver

Fall 2020

Abstract

Ecology and physiology of parasitic plants

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Doctor of Philosophy in Integrative Biology

University of California, Berkeley

Professor Wayne Sousa, Chair

Parasitic plants are common fixtures in ecosystems. Although traditionally studied primarily for their negative impacts on their hosts, the range of interactions that parasitic plants have and their role in shaping ecosystem structure and function is increasingly recognized. Parasitic plants are defined by a unique set of ecophysiological traits. Accordingly, here I take a primarily ecophysiological approach to understanding parasitic plants and their role in ecosystems. This dissertation is largely organized from narrow to broad in terms of focal species, and explores three major topics within parasitic plant ecophysiology: N-parasitism, nighttime transpiration, and leaf traits.

In the first chapter, I focus on two species of root hemiparasites, *Castilleja applegatei* and *Castilleja wightii*. The N-parasitism hypothesis posits that N limitation drives high transpiration rates in xylem-tapping parasites. Thus, availability of N-fixing hosts may affect parasite's WUE and in turn impact the surrounding plant community. I investigate how the availability of an N-fixing host affects the root hemiparasite, *Castilleja applegatei*, and examine host-mediated effects on community structure and soil moisture. I contrast this work with a removal experiment testing the impact of *Castilleja wightii* on a N-fixing host species. In *C. applegatei* availability of N-fixing hosts corresponded to a significant increase in leaf %N, a distinct $\delta^{15}N$ signature, and an increase in WUE (signified by $\delta^{13}C$). The presence of parasites was associated with a significant decrease in WUE in N-fixing neighbors, but had no effect on the non-N-fixing species. The presence of parasites significantly affected soil moisture but did not impact diversity or percent cover. In contrast to the observational work on *C. applegatei*, I did not find strong evidence for host-parasite interactions between *C. wightii* and available N-fixers in the experimental removal.

In the second chapter, I look at nighttime stomatal conductance in eight species or subspecies of *Castilleja*. Parasitic plants are theoretically released from two of the major drivers of nighttime stomatal closure. First, instead of relying solely on photosynthesis, xylem parasites also derive dilute carbon from their host xylem, a source unaffected by darkness. Second, their access to host xylem also reduces the need to conserve water. Here I measured nighttime stomatal conductance in eight species of *Castilleja*, a widespread genus of hemiparasites that access host xylem via the roots, and common neighboring plants at eight sites in California. All the plants measured displayed some nighttime stomatal conductance, but on average, nighttime stomatal conductance in *Castilleja* was 235% higher than in non-parasites. These data demonstrate that many *Castilleja*

commonly transpire at night, adding these root hemiparasites to the growing group of plants understood to open their stomata at night.

In the third chapter, I use a wider lens to examine leaf traits in parasitic plants across the globe. Utilizing the TRY database, I characterize the state of knowledge on leaf traits in parasitic plants and explore how parasitic plants, with their unique ecophysiology, fit into or deviate from the global leaf economic spectrum (LES). I also compile a dataset of all the known parasitic genera, which is freely available. Heterotrophy in parasitic plants undermines some of the essential functions of leaves, namely C acquisition via photosynthesis, and in theory could lead to departures from the LES. However, despite their unique physiology, parasitic plants largely adhere to the LES although they do have some tendency towards the 'fast' end of the spectrum, that is, towards leaves with shorter lifespans but higher short-term photosynthetic yield. Further research on the physiology of parasitic plants will improve our understanding of patterns in resource acquisition and utilization.

Table of Contents

Acknowledgements

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Countless other colleagues, mentors, mentees, and friends from the wider UC Berkeley community contributed immensely to this work. My utmost gratitude to my dissertation committee, all of whom also served on my qualifying exam committee. Todd Dawson, David Ackerly and Whendee Silver have profoundly shaped this work from the beginning through their pointed and poignant questions, close reading of manuscripts, and invaluable words of encouragement. In particular, my thanks extend to Todd Dawson who served as the chair of my qualifying exam committee and who generously lent me laboratory equipment and space, without which much of this work wouldn't exist. In addition, my thanks to Mary Power, who served on my qualifying exam committee; Stefania Mambelli and Wenbo Yang of the UC Berkeley Stable Isotope Center for Geochemistry, who provided invaluable guidance and help with sample preparation and analysis; all the UC Natural Reserve staff who helped host my projects, in particular Jeff Brown and Faerthen Felix of Sagehen Creek Field Station and Jacqueline Sones of Bodega Marine Lab; the other graduate students in the Sousa Lab with whom I overlapped, in particular, Lindsey Hendricks-Franco, who joined the lab at the same time as me. Last, I am indebted to the many undergraduate researchers who spent countless hours assisting me with lab and field work: Erin Cain, Alyson Ennis, Lena Gavenas, Alexander Goetz, Hannah Grossman, Emma Reich, and Amber Yeh.

This dissertation relied immensely on my ability to access and study ecosystems in California. While ecology professes to be the study of "natural" communities (which we dubiously take to mean without people), the land we study is undeniably shaped by and connected to the peoples who have occupied and interacted with the land, from thousands of years ago up to the present day. I want to acknowledge that fieldwork for this dissertation took place on the ancestral, unceded lands of the Bodega Miwok (Olamentko), Central Sierra Miwok, Eastern Mono/Monache, Western Mono/Monache, Newe (Western Shoshone), Northern Paiute, and Washoe peoples. In addition, UC Berkeley sits on the territory of xučyun (Huichin), the ancestral and unceded land of the Chochenyo speaking Ohlone people.

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Preface

The importance of parasites in natural communities

Parasitism is arguably the most common species interaction on earth (Price 1980), and their negative impacts on host growth and survival are well documented. Parasites' role in ecosystem functioning, however, was once presumed to be minor, in part because they typically represent a small portion of the biomass in an ecosystem (Hudson, Dobson & Lafferty 2006). Biomass, however, does not always correspond to community impacts (Power *et al*. 1996), and indeed parasite-mediated effects are now well-recognized for their importance in shaping communities and ecosystems (Dobson & Hudson 1986; Dobson & Crawley 1994; Marcogliese 2005; Hudson *et al.* 2006; Wood *et al.* 2007; Lefèvre *et al.* 2009; Dunne *et al.* 2013).

Parasitic plants in particular were once largely studied for their role as agricultural pests (Press & Graves 1995) but are increasingly recognized for their complex interactions and ecosystem-level impacts in natural communities. Parasitic plants notably can occupy the same trophic level as the host plants they infect. Interactions with neighboring plants can thus range from parasitism to competition (or facilitation and commensalism) by encompassing both direct negative effects on their hosts and indirect effects on neighboring plants and other trophic levels (Press & Phoenix 2005; Watson 2009). Interactions may not necessarily even benefit the parasite (Atsatt & Strong 1970). This broad range of interactions means that parasitic plants' role in ecosystems is difficult to untangle. In many instances though parasitic plants' interactions lead to considerable impacts on community structure and function (Smith 2000; Aukema 2003; Press & Phoenix 2005; Watson 2009). Parasitic plants are even sometimes considered keystone species (Watson 2001; Watson & Herring 2012; Hartley *et al*. 2015) or ecosystem engineers (Decleer, Bonte & Van Diggelen 2013).

Many of the impacts of parasitic plants are comparable to those of herbivores (Pennings & Callaway 2002). Parasitic plants can influence community composition and biomass allocation by suppressing their host species (Gibson & Watkinson 1991; Pennings & Callaway 1996; Marvier 1998b). In some communities this can indirectly increase diversity via competitive release (Joshi, Matthies & Schmid 2000). In another parallel to herbivores, parasitic plants can also influence nutrient cycling, although the effects are typically not as pronounced. Press (1998) envisioned parasitic plants as 'Robin Hood' characters, which they steal nutrients from dominant species and give to the 'poor' via deposition of nutrient-rich litter. Subsequent experimental evidence suggested that input of nutrient-rich parasite litter can alter the biomass and nutrient status of co-occurring plants, and rates of decomposition (Quested *et al*. 2004; Spasojevic & Suding 2011; Fisher *et al*. 2013; Demey *et al*. 2014).

Parasitic plants may also impact water use in some plant communities. Many parasitic plants have low water-use-efficiency (WUE) as a result of high transpiration rates, which pull water and solutes from host's xylem stream to the parasitic body (Marshall, Dawson & Ehleringer 1994; Ehleringer & Marshall 1995). The high water usage has been shown to impact hosts' water-balance (Press, Tuohy & Stewart 1987; Stewart & Press 1990a) and negatively affect associated plants through decreased drought tolerance and reduced soil moisture (Sala, Carey & Callaway 2001).

Background on parasitic plants

Parasitic plants are defined functionally by the presence of haustoria, a specialized organ which penetrates the host tissue (Nickrent 2020). Parasitism has arisen independently multiple times across the angiosperms (Barkman *et al.* 2007; Naumann *et al.* 2013) and as a result, parasitic plants are diverse in form. They can, however, be sorted along two functional axes: C-dependence on a host and attachment position on a host (Fig 1). Attachment position is divided into stem parasites (e.g. mistletoes such as *Viscum*) and root parasites (e.g. *Castilleja*). Stem parasites lack root systems entirely, while root parasites roots range from fairly typical to reduced and poorly developed (Matthies 2017). C-dependence is largely defined by whether the parasite can photosynthesize. Hemiparasites are photosynthetic and tap into host xylem, from which they derive water and dilute solutes (e.g. *Castilleja*). Holoparasites are incapable of photosynthesis, instead relying on the contents of host xylem and phloem (e.g. *Orobanche*) (Nickrent & Musselman 2004). In practice the divisions between these categories can be blurry and intermediate versions exist (Těšitel 2016). Other work additionally utilizes the dominance of an endophytic stage in the life cycle as a functional axis. In this definition, endophytic parasites are a functional group alongside root hemiparasites, root holoparasites and stem parasites (Těšitel 2016). Because photosynthesis tends to be highly reduced or absent in this group, endophytic parasites have a large overlap with what are termed stem holoparasites above.

Some work has additionally sought to categorize parasitic plants as obligate or facultative (Nickrent 2002). The utility of this definition, however, is not clear. While some root parasites can be grown in greenhouse settings and complete a life cycle without a host (Heckard 1962; Mann & Musselman 1981), parasites have never been documented without a host in the field (Heide-Jørgensen 2013). Importantly, only root hemiparasites are even capable of being facultative. Stem parasites and holoparasites require a host, as they lack roots and chlorophyll respectively.

Host specificity in parasitic plants ranges widely. Some taxa are generalists, capable of attaching to wide range of hosts and often multiple hosts at once, such as *Castilleja* (Marvier 1998a) and *Cuscuta* (Dawson *et al*. 1994). In contrast some species are narrowly host-specific, such as beech drops, *Epifagus virginia*, which only parasitize the beech tree, *Fagus grandifolia* (Tsai & Manos 2010). Importantly, parasitic plant's physiology and interactions with other species are often host-mediated (Stermitz & Harris 1987; Schulze *et al.* 1991; Adler 2000; Schädler *et al.* 2005).

Dissertation

Here, I primarily take an ecophysiological approach to understanding parasitic plants and their role in ecosystems. This is because parasitic plants' unique physiology underlies many of their interactions (Phoenix & Press 2005). This dissertation is largely organized from narrow to broad in terms of focal species, and explores three major topics within parasitic plant ecophysiology: N-parasitism, nighttime transpiration, and leaf traits.

In the first chapter, I focus on two species of root hemiparasites, *Castilleja applegatei* and *Castilleja wightii*. The N-parasitism hypothesis posits that N limitation drives high transpiration rates in xylem-tapping parasites (Ehleringer *et al*. 1985). Thus, availability of N-fixing hosts may affect parasite's WUE and in turn impact the surrounding plant community. I investigate how the availability of an N-fixing host affects the root hemiparasite, *Castilleja applegatei*, and examine

host-mediated effects on community structure and soil moisture. I contrast this work with a removal experiment testing the impact of *Castilleja wightii* on a N-fixing host species. In *C. applegatei* availability of N-fixing hosts corresponded to a significant increase in leaf %N, a distinct $\delta^{15}N$ signature, and an increase in WUE (signified by $\delta^{13}C$). The presence of parasites was associated with a significant decrease in WUE in N-fixing neighbors, but had no effect on the non-N-fixing species. The presence of parasites significantly affected soil moisture but did not impact diversity or percent cover. In contrast to the observational work on *C. applegatei*, I did not find strong evidence for host-parasite interactions between *C. wightii* and available N-fixers in the experimental removal.

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In the third chapter, I use a wider lens to examine leaf traits in parasitic plants across the globe. Utilizing the TRY database, I characterize the state of knowledge on leaf traits in parasitic plants and explore how parasitic plants, with their unique ecophysiology, fit into or deviate from the global leaf economic spectrum (LES). I also compile a dataset of all the known parasitic genera, which is freely available. Heterotrophy in parasitic plants undermines some of the essential functions of leaves, namely C acquisition via photosynthesis, and in theory could lead to departures from the LES. However, despite their unique physiology, parasitic plants largely adhere to the LES although they do have some tendency towards the 'fast' end of the spectrum, that is, towards leaves with shorter lifespans but higher short-term photosynthetic yield. Further research on the physiology of parasitic plants will improve our understanding of patterns in resource acquisition and utilization.

Figures

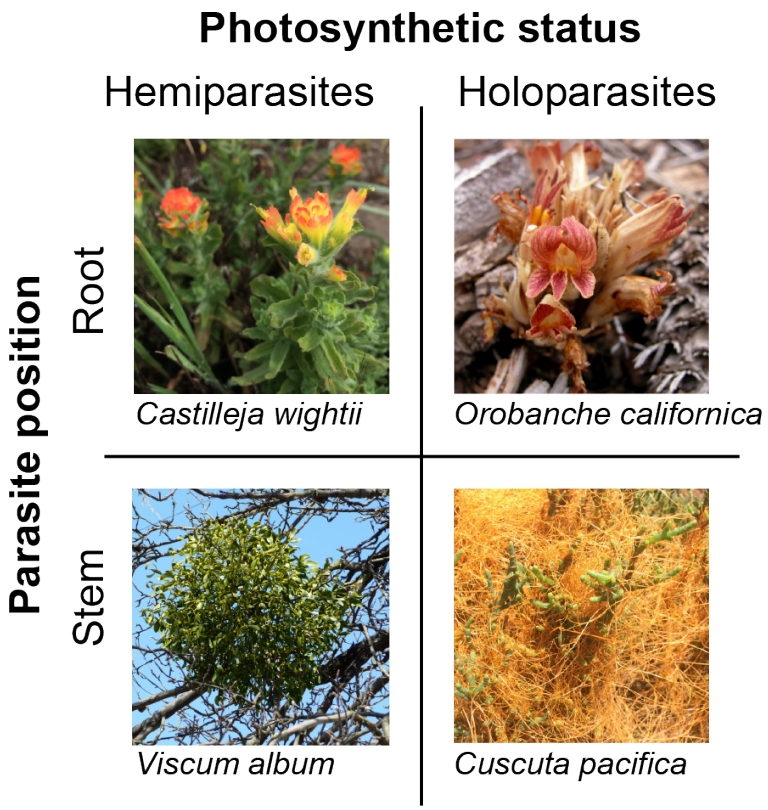

Figure 1 Representative taxa showing major functional divisions in parasitic plants. Photo credits: *Castilleja wightii* and *Cuscuta pacifica* Audrey Haynes, *Viscum album* Wikimedia commons, *Orobanche californica* USFS.

Chapter 1: Contrasting effects of N-fixing neighbors on two species of *Castilleja*, a genus of root hemiparasites

Abstract

Parasitic plants are known for their high transpiration rates and low water use efficiency (WUE), which the N-parasitism hypothesis posits is driven by N limitation. Thus, availability of N-fixing hosts may affect parasite's WUE and in turn impact the surrounding plant community. Here, I investigate how the availability of N-fixing hosts affects two species of *Castilleja*, a genus of the root hemiparasites, and examine host-mediated effects on community structure and soil moisture. I surveyed plant diversity and percent cover, and measured soil moisture in 120 1x1m plots within Sagehen Experimental Forest, CA. Fifty percent included *Castilleja applegatei*. In a subset of plots, I measured leaf N, C/N, $\delta^{13}C$, $\delta^{15}N$ in *C. applegatei*, *Ceanothus prostratus* (a N-fixer), and two non-N-fixing plants (*Artemisia tridentata* and *Wyethia mollis*). In addition, I conducted a removal experiment testing the impact of *Castilleja wightii* on a N-fixing host species in the coastal sand dunes of Bodega Bay, CA. In *C. applegatei* availability of N-fixing hosts corresponded to a significant increase in leaf %N, a distinct $\delta^{15}N$ signature, and an increase in WUE (signified by $\delta^{13}C$). The presence of parasites was associated with a significant decrease in WUE in N-fixing neighbors, but had no effect on the two non-N-fixing species. The presence of parasites significantly affected soil moisture but did not impact diversity or percent cover. For *C. applegatei* higher N availability increases WUE and in turn affects soil moisture, but not plant community structure. These results broadly support the N-parasitism hypothesis and indicate that host type can affect parasite's physiology and downstream effects. However, in contrast to the observational work on *C. applegatei*, I did not find strong evidence for host-parasite interactions between *C. wightii* and available N-fixers in the experimental removal at Bodega Bay.

Introduction

Parasitic plants are a diverse, species-rich group, widespread in natural habitats. Despite representing a relatively small portion of biome-wide aboveground biomass, parasitic plants can play an outsize role in structuring communities (Pennings & Callaway 1996; Marvier 1998b; Smith 2000; Bardgett *et al*. 2006), and in some instances may even be considered keystone species (Press & Phoenix 2005; Watson & Herring 2012). Because parasitic plants are defined by a unique set of ecophysiological traits, understanding their physiology and resource requirement is the entry point for illuminating their ecological roles and unique interactions in plant associations across the globe (Phoenix & Press 2005).

Parasitism has evolved independently multiple times across the angiosperms (Barkman *et al*. 2007; Naumann *et al*. 2013). As parasitic plants are not monophyletic, they are defined functionally by the parasitic uptake of resources from other plants via specialized tissue called haustoria. Within this larger umbrella, parasitic plants can be broken into four functional groups: root hemiparasites, root holoparasites, stem parasites and endophytic parasites (Těšitel 2016). Even within these definitions, however, parasites exhibit a wide-range of forms and physiology (Těšitel 2016). Accounting for the unique and diverse aspects of the functional biology of parasitic plants is instrumental to illuminating their ecological roles and interactions.

Parasitic plants can play important roles in nutrient cycling and plant community structure (Quested 2008; Fisher *et al*. 2013). The decomposition of parasitic plant leaf litter, typically rich in nitrogen (N), may increase overall N mineralization, available soil N, ecosystem productivity, and drive shifts in plant community assemblages (Press 1998; Spasojevic & Suding 2011; Fisher *et al*. 2013). Parasitism may also suppress dominant plant species in a given community, indirectly increasing community diversity via competitive release (Marvier 1998b; Joshi *et al*. 2000; Pennings & Callaway 2002).

Parasitic plants are also known to generally have high transpiration rates and low water-use-efficiency (WUE) (Schulze, Turner & Glatzel 1984; Press *et al*. 1987; Press, Graves & Stewart 1988; Scalon & Wright 2017), defined as the ratio of carbon assimilation (A) to transpirational water loss (E) (denoted as A/E) (Farquhar, O'Leary & Berry 1982). Because water availability has a large bearing on community structure and function (Lauenroth, Dodd & Sims 1978; Stephenson 1990), parasitic plants' rampant water use has the potential to affect the larger community. Parasitic plants penetrate host xylem and then pull water and solutes from host's xylem stream. Maintaining a favorable water potential gradient, achieved through high stomatal conductance, is necessary to redirect the host xylem stream to the parasitic body (Press *et al*. 1987; Stewart & Press 1990b). The resulting low WUE can negatively affect hosts and potentially reduce soil moisture (Sala et al. 2001). Similarly, parasitic plants can decrease drought tolerance in hosts and associated plants (Press *et al*. 1987; Stewart & Press 1990b; Sala *et al*. 2001).

The physiology and subsequent impact of parasitic plants is likely often host-mediated. For example, host type has been shown to affect parasites' palatability to herbivores (Schädler *et al*. 2005). In particular, the N parasitism hypothesis describes an important role for N-fixing hosts, positing that parasites are N limited, and N acquisition then drives high transpiration rates (Schulze *et al*. 1984). Although xylem-tapping parasites do not have access to the host phloem, they acquire dilute C, N and other solutes from the host xylem stream (Bollard 1960) but the low concentrations require profligate transpiration.

Research into the N-parasitism hypothesis has yielded mixed results. Supporting evidence includes observations that N-fixing hosts cause increased growth rates in parasites and a decrease in WUE difference between host and parasite (Schulze & Ehleringer 1984; Schulze *et al*. 1984; Ehleringer *et al*. 1985; Seel & Press 1993, 1994). The fertilization of host plants has also been shown to increase mistletoe WUE efficiency, as a result of increased photosynthesis and stable stomatal conductance (Marshall *et al*. 1994). It is not clear, generally, how much observed increases in WUE are due to a downregulation in transpiration versus an increase in photosynthesis (Seel & Press 1994).

Recently, however, a more comprehensive survey using carbon isotope indicators was conducted of WUE in host-mistletoe pairs (Scalon & Wright 2015). Carbon isotopes are commonly used as a proxy for WUE, which is the ratio of carbon assimilation to transpirational water loss. Carbon isotope composition of plant tissues ($\delta^{13}C$) can be an integrator of the time-averaged c_i/c_a (ratio of internal to ambient [CO^2]) of an individual plant. C_i/c_a is in turn a reflection of the rates of carbon assimilation (demand for CO_2) and stomatal conductance (loss of water). As such, $\delta^{13}C$ has been used to infer the WUE of a particular plant species (Farquhar *et al*. 1982). Scalon and Wright (2015) found no effect of differences in host and parasite foliar N on $\delta^{13}C$, nor any effect of N-fixing hosts on host-mistletoe differences in $\delta^{13}C$. In a follow-up study they also measured nutrient resorption prior to leaf senescence (Scalon, Wright & Franco 2017). But, if N were a limiting nutrient, one would expect N resorption, which they did not find. They did, however, find P resorption suggesting a possible alternative: that P limitation drives high transpiration. In both the above studies, there was a strong effect of site, suggesting that these patterns may be context dependent.

Related, but not mutually exclusive, is the hypothesis that observed low WUE is a byproduct of heterotrophy. The ability of parasites to obtain C from their hosts' transpiration stream biases traditional methods for estimating A/E (i.e. WUE) because A only accounts for carbon gain via photosynthesis (Marshall *et al*. 1994). When heterotrophic C gain is accounted for, estimates of WUE are more similar to host species (Marshall & Ehleringer 1990). However, estimates of the contribution of host-derived C to total C acquisition in parasites are highly variable and not well constrained. In addition, $\delta^{13}C$, used as an estimate of WUE, will reflect not only the parasite's long-term c_i/c_a but also the host's. This ultimately dilutes the signal from the parasite (Bannister & Strong 2001).

The N parasitism hypothesis has largely been investigated in stem parasites. Functionally distinct, root hemiparasites are relatively common and widespread but host-root hemiparasite interactions have been primarily studied in greenhouses (Marvier 1996, Matthies 1997, 2017, Joshi *et al*. 2000, Schädler *et al*. 2005, Sandner and Matthies 2018, but see Marvier, 1998b; Adler, 2002). This paucity of research is understandable: in situ investigation is difficult when the host-parasite connection is hidden from view. Plant physiology methods, in particular stable isotopes, are a powerful tool to elucidate interactions like this, which would be intractable with traditional ecological methods (Dawson *et al*. 2002).

Castilleja, a genus of root hemiparasitic plants, is an ideal group to investigate in this context. Part of the Orobanchaceae family, the second largest family of parasites (Westwood *et al*. 2010), *Castilleja* spp. (paintbrushes) are widespread, common across North America, and occur in a wide range of habitats. Like other parasites, they typically have high transpiration rates, and at least some species benefit from N-fixing hosts (Seel & Press 1993; Matthies 1997). In addition, work in other systems suggests that *Castilleja* substantially affect ecosystem structure and function through depositing N-rich litter, decreases in host biomass, and host-

mediated effects on herbivores (Marvier 1996; Spasojevic & Suding 2011). As root hemiparasites, *Castilleja* can photosynthesize without a host, but obtain nutrients, carbon and water from a wide variety of hosts via haustorial root attachments (Heckard 1962; Stewart & Press 1990b). Although *Castilleja* are generalists, capable of parasitizing a wide variety of hosts, attachment to different types and even different numbers of hosts at once may significantly alter *Castilleja* individuals' physiology and their interactions with neighbors and other trophic levels, such as herbivores and pollinators (Matthies 1997; Marvier 1998a; Adler 2000).

In the present study, I investigate the interaction between two species of *Castilleja*, *C. applegatei* and *C. wightii*, and N-fixing neighbors at two different sites. At the first site, I conducted surveys and measure leaf traits via stable isotopes. At the second, I implement an experimental removal of *Castilleja* individuals and track leaf traits in response. Under the N-parasitism hypothesis, I expected at both sites that when *Castilleja* individuals associated with N-fixing hosts they would exhibit higher N content and higher WUE (as evidenced by their $\delta^{13}C$ values). Because of this higher WUE on the part of the parasite, I also expected that WUE of N-fixer hosts would be less impacted by parasitism than non-N-fixers. In addition, I investigate the effect of parasites on community diversity and productivity at the first site. Because the site has relatively low plant cover, I anticipated that the deposit of N-rich litter would lead to higher percent cover and higher diversity in plots with a parasite and/or a N-fixer. Alternatively parasitism could lead to overall reductions in community-wide plant cover or biomass and N influx via parasitic plant litter deposit could lead to reduced diversity if a competitive dominant takes over (Levine, Brewer & Bertness 1998).

Materials and Methods

Study sites

I conducted fieldwork at two University of California Natural Reserve sites: Sagehen Experimental Forest (39°25.981', -120°14.758') and Bodega Marine Reserve (38°19.351', -123°03.872').

Sagehen Experimental Forest (referred to as Sagehen for the rest of the paper) is located in the Central Sierra Nevada mountain range north of Truckee, CA. Vegetation types include mixed-conifer forest, meadow, shrub and conifer plantations. The shrub type occurs on poor and/or shallow soils unable to support conifer forests and on more productive soils after disturbance (fire, logging). *Ceanothus velutinus*, *Arctostaphylos patula*, *Ceanothus prostratus*, *Ribes cereum*, *Ericameria bloomeri* and *Wyethia mollis* dominate the shrub vegetation (USFS 2008).

Bodega Marine Reserve is located on the Pacific coast at Bodega Head, north of San Francisco, CA. The reserve has 362 acres of terrestrial habitats including sand dunes, where this work took place. The sand dunes are dominated by two introduced species: yellow bush lupine (*Lupinus arboreus*) and a dune grass (*Ammophila arenaria*), which was originally planted for dune stabilization (Wiedemann & Pickart 1996; Danin *et al*. 1998). Some areas still support native scrub communities, including *Lupinus chamissonis*, *Ericameria ericoides,* and a mixture of other grasses and forbs (Barbour 1973; BMR 2018).

Both Sagehen and Bodega have broadly Mediterranean-type climates, characterized by warm, dry summers and cool, wet winters. Bodega's location on the coast results in a relatively temperate year round climate, while Sagehen Creek's location in the mountains means it receives significant snowfall, which accounts for 80% of the annual precipitation in a typical year (USFS

2008). California was in the midst of a historic drought for part of this project (CADWR 2015). The period between Fall 2011 and Fall 2015 was the driest since record keeping began while 2014 and 2015 were the hottest years on record in California. In 2016, average precipitation in Northern California, in combination with above average temperatures, reduced but did not eliminate drought across the state (Griffin & Anchukaitis 2014; Hanak, Mount & Chappelle 2016).

STUDY SPECIES

I focused my investigations on a different N-fixer-parasite pair in each reserve. Each pair is composed of a root hemiparasite in the genus *Castilleja* and an N-fixing neighbor.

At Sagehen Experimental Forest work focused on *Castilleja applegatei* ssp. *pinetorum* (Fernald) T.I. Chuang & Heckard, an N-fixer (*Ceanothus prostratus* Benth.), and two non-N-fixers *(Artemisia tridentata* ssp. *vaseyana* (Rydb.) Beetle and *Wyethia mollis* A. Gray*)*. *C. prostratus* (Rhamnaceae) is a mat-forming shrub found in dry sites in pine forests in the Sierra Nevada and Cascade ranges (Conard *et al*. 1985). *C. prostratus* is actinorhizal, meaning individuals fix N through a symbiotic relationship with a soil actinomycete (Busse 1996). *A. tridentata* (Asteraceae) is an important, often dominant, woody shrub found from the arid lands of the Great Basin to the cooler climes of Western mountains. There is considerable intraspecific variation within *A. tridentata*. The subspecies studied here, mountain sagebrush (*A. tridentata* ssp. *vaseyana*), is found in dry sites in the upper foothills and mountain regions (Winward 1980; Barker & McKell 1983). *W. mollis* (Asteraceae), or mule's ears, is an herbaceous perennial that forms rosettes of large leaves which die back to ground level each winter. It commonly co-occurs with *A. tridentata* in shallow, dry soils and conifer understories in the Sierra Nevada (Parker & Yoder-Williams 1989; Karban 2007).

Castilleja applegatei (Orobanchaceae) is a highly variable species of perennial root hemiparasites, characterized by wavy leaf margins. The subspecies here, *C. applegatei* ssp. *pinetorum*, is typically found in open conifer forest and sagebrush scrub environments throughout the Sierras, the Southern Cascades, the High North coast ranges and into the Modoc plateau (Baldwin & Goldman 2012). At Sagehen, *C. applegatei* ssp. *pinetorum* can be found within the shrub type and on more productive soils after disturbance (fire, logging).

At Bodega Marine Reserve I also focused on an N-fixing plant host, *Lupinus arboreus* Sims, and a parasite, *Castilleja wightii* Elmer. *L. arboreus* is considered native in California from the San Francisco Bay south, but has been widely planted and introduced outside of its natural range and is often considered invasive and damaging to dunes in Northern California and Oregon (Pickart, Miller & Duebendorfer 1998; California Invasive Plant Council 2019). It is a fast-growing perennial shrub, which typically reaches 1-1.5 m in height and width. As a member of the Fabaceae (Leguminosae) family, *L. arboreus* is a productive N-fixer and typically grows in N-poor environments, such as coastal sand dunes, bluffs, and scrub habitats. Soils under lupine stands at Bodega dunes contain significantly more N than otherwise similar soils. This N-rich soil in conjunction with *L. arboreus*' relatively short lifespan (due to herbivory susceptibility) is thought to facilitate invasion by *A. arenaria* (Davison & Barbour 1977; Maron & Jefferies 1999). Wight's Indian Paintbrush, *C. wightii*, is endemic to California, growing primarily in coastal scrub habitats on the Central and North California coast. It is perennial but dies back almost entirely outside of the growing season, flowering from March-August.

Parasite effects on diversity at the Sagehen site

To address the impacts of parasitic plants on community diversity, I established 12 50 m transects throughout the Sagehen Creek Experimental Forest in the summer of 2015. Along each transect 10 1x1 m quadrats were established (120 plots total). I established quadrats every 5 m along each transect, alternating sides until I reached 5 plots with *C. applegatei* and 5 plots without. In order to reach 10 total plots, transects were sometimes extended beyond 50 m (but none exceeded 75 m). I surveyed each quadrat for species presence, count, and percent canopy cover using visual estimation (Meese & Tomich 1992). Volumetric water content (VWC%) was recorded at midday in each plot using a FieldScout TDR 150 (Spectrum Technologies, Aurora, Illinois) soil moisture meter with 12 cm probes at three regular locations in the quadrat, or where the soil was sufficiently soft and rock-free to allow measurement.

Parasite interactions with an N-fixer at the Sagehen site

To address questions about N-parasitism, WUE and host detection, I collected leaf samples for elemental and stable isotope analysis (specifically %N, %C, C/N, $\delta^{13}C$ and $\delta^{15}N$) of the parasite, common N-fixers and non-N-fixers. *C. prostratus* emerged from these surveys as the most common N-fixer available to *C. applegatei*. In a subset of transects, where *C. prostratus* was well represented (see Table 1), I collected leaf samples from *C. applegatei*, *C. prostratus* and the two most common non-N-fixing available hosts: *A. tridentata* and *W. mollis*. For the host species, I collected leaf samples from individuals in quadrats with and without a parasite, *C. applegatei* (n=2x10 each x 3 species=60 total). For the parasite I collected samples from individuals in quadrats with and without an N-fixer (*C. prostratus*) (n=20 each, 40 total). Mature, sun-exposed leaves were collected from each individual.

Experimental removal of parasites from N-fixers at the Bodega site

To further explore the interactions between N-fixers and parasites I implemented a parasite-removal experiment at Bodega in 2016. I established four groups (n=10 each):

A) Parasite with an N-fixer available (defined as *C. wightii* within 1m distance from base of an *L. arboreus* individual;

B) N-fixer with possible parasites removed (defined as *C. wightii* within 1 m distance of base of an *L. arboreus* individual; then all *C. wightii* individuals within 5 m distance from the *L. arboreus* removed);

C) Parasite with no possible N-fixer (defined as no *L. arboreus* within 5 m of a *C. wightii* individual);

D) N-fixer with no possible parasite (defined as no *C. wightii* within 5 m of a *L. arboreus* individual) (Fig 1).

For groups A and B, in cases where there were multiple parasites within 1 m of the N-fixer, I selected the closest individual to the N-fixer to sample from. I did not find cases of multiple N-fixers within 1 m of a parasite. I attempted to transplant the *C. wightii* removed from group B for a fifth group of parasites previously neighboring an N-fixer but all the transplanted individuals died. When removing the *C. wightii* individuals I also attempted to find haustorial attachments to any other plants but was unsuccessful. The bulk of the root mass was small enough (~0.25 m x 0.25 m) to suggest that the distance assumed for no haustorial connection was adequate. To control for soil disturbance effects of removal, I dug a hole approximately the size of a removed *C. wightii* at each site, where no *C. wightii* was removed. I collected mature, sun-

exposed leaves from each individual before removal (March 2016) and 5 months later (August 2016).

LEAF SAMPLE PREPARATION

I transported leaf samples to UC Berkeley and dried them in a 45-55 ºC oven for at least 48 hours. Once dry, I manually removed the midveins with a razor blade and dissecting scope. I ground all leaves from an individual plant together into a fine powder, from which I packed 5-7 mg per sample into a tin capsule for elemental and isotope analysis. Samples were submitted to the Center for Stable Isotope Geochemistry at UC Berkeley for %C, %N, C/N, $\delta^{13}C$ and $\delta^{15}N$ analysis using a CHNOS Elemental Analyzer interfaced to an IsoPrime100 mass spectrometer. The Center for Stable Isotope Geochemistry corrected raw instrument data for drift over time and linearity, and normalized data to the international stable isotope reference scale. The normalization was based on the analysis of three laboratory reference materials with very different carbon and nitrogen delta values. These laboratory reference materials are calibrated annually against IAEA (International Atomic Energy Agency, Vienna, Austria) certified reference materials. For quality control, they used NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) SMR 1577c (bovine liver) previously calibrated against IAEA certified reference materials. Long-term external precision is ± 0.1‰ and ± 0.2‰, respectively for C and N isotope analyses. For the four runs of samples for this work, the analytical standard deviations were ±0.02‰, ±0.07‰, ±0.06‰ and ±0.15 ‰ for ^{13}C and ±0.17‰, ±0.06‰, ±0.12‰ and ±0.28‰ for ^{15}N. All isotope values are expressed in delta notation where $\delta = (R_A / R_S) - 1)$, and R_A and R_S are the ratios of the rare to abundant isotope (e.g. $^{15}N/^{14}N$, or $^{13}C/^{12}C$) in the sample of interest (R_A) and in an international standard (R_S). The primary international isotope standards are Vienna PeeDee Belemnite (VPDB) and atmospheric N_2 for $\delta^{13}C$ and $\delta^{15}N$, respectively.

STATISTICS

I used R to complete all data cleaning and analysis. Unless otherwise noted I used the native R *stats* package for all statistics (R Core Team 2020). For all models below I used visual inspection of the residuals, Q-Q plots and Cook's distance to confirm that the model met all assumptions, and no data points had undue leverage. Where appropriate I also ran Shapiro-Wilk normality tests, F-tests and/or Levene's test to check for normality and heterogeneity of variance.

Diversity, productivity and soil moisture at Sagehen

Using the *vegan* package in R (Oksanen *et al*. 2019), I calculated rarified richness (using the minimum number of individuals [3] within a plot as the subsample size), and two diversity indices: Shannon index and Simpson's Inverse index for each plot at Sagehen. Each index was calculated using both percent cover and counts, resulting in five diversity metrics. In addition, I looked at percent cover and soil moisture (an average of the three measurements in each plot). To examine the effect of *C. applegatei* on diversity, I ran a type III ANOVA on a linear mixed effects model (nlme package) (Pinheiro *et al*. 2017). For each model, the metric was the response variable, binary presence/absence of *C. applegatei* was the parameter, and the transect (i.e. location) was a random effect. I ran a type III ANOVA for each of the five metrics. For these models *C. applegatei* was excluded from the metrics calculation, including percent cover (Spasojevic & Suding 2011). To look for the effect of *C. prostratus* on diversity I repeated this process, using binary presence/absence of *C. prostratus* as the parameter and metrics from which

C. prostratus was excluded. Finally, I ran a type III ANOVA with both presence of *C. prostratus* and *C. applegatei*, and their interaction as parameters. Both species were excluded from metric calculations for these ANOVAs. For soil moisture I only ran one type III ANOVA with both presence of *C. prostratus* and *C. applegatei*, and their interaction as parameters. I then conducted pairwise comparisons on estimated marginal means using a Tukey adjustment for multiple comparisons of means and a 95% family-wise confidence level (*emmeans* package) (Lenth 2019a).

Leaf traits from observational work at Sagehen

For the set of leaf samples from observational work, I looked at the effect of the presence of a parasite (*C. applegatei*) on the N-fixer (*C. prostratus*) and vice versa through four measurements: $\delta^{15}N$, $\delta^{13}C$, %N and C/N. When I found heterogeneity and/or non-normality I initially transformed the data; however, no transformations improved the violations. Two sets of measurements showed significant heterogeneity of variance: %N in *C. applegatei* (F-test: $P < 0.001$, ratio of variances = 0.056) and $\delta^{15}N$ in *C. prostratus* (F-test: $P = 0.045$, ratio of variances = 0.24). For this reason, I subsequently ran Welch two sample t-tests, which allows for unequal variance. Only $\delta^{15}N$ in *C. prostratus* showed significant violation of normality in one of the groups (Shapiro-Wilk test: group = w/o *C. applegatei*, $W = 0.84$, $P = 0.044$). For this measurement I ran the non-parametric Asympototic Wilcoxon-Mann-Whitney test. For the two non-parasite, non-N-fixer species (*W. mollis, A. tridentata*) I ran type III ANOVAs on a linear model fit on $\delta^{15}N$, $\delta^{13}C$, %N and C/N with where both presence of *C. prostratus* and *C. applegatei*, and their interaction were parameters.

I was also interested in the relationship between leaf traits and how that was affected by species and the presence of a parasite and/or N-fixer. To address these questions I ran ANCOVAs for each species looking at $\delta^{13}C$ as a function of leaf N (%) and $\delta^{15}N$ as a function of $\delta^{13}C$. For the parasite (*C. applegatei*) the presence of an N-fixer (*C. prostratus*) was an additional factor and conversely for the N-fixer the presence of a parasite was an additional factor. For the two non-parasitic, non-N-fixing species (*A. tridentata* and *W. mollis*) both presence of a parasite and N-fixer were included as factors. I used a backward model selection, initially including all factors and their interaction terms and dropping factors one at a time, using AIC and BIC to compare models (Zuur, Ieno & Smith 2007). If no factors were significant results are shown for the linear model that just includes the leaf traits (i.e. $\delta^{13}C$ as a function of leaf N (%) and $\delta^{15}N$ as a function of $\delta^{13}C$). One model showed heterogeneity (F-test: $P = 0.0075$, ratio of variances = 8.17): [$\delta^{15}N \sim \delta^{13}C$ *w/ *C. applegatei*] within *C. prostratus*. Various data transformations did not improve the result. In this case I applied a generalized least squares fit by restricted maximum likelihood with a variance structure that allows for different standard deviations in each stratum (*varIdent* variance structure from the *nlme* package) (Zuur *et al*. 2009).

Leaf traits from experimental removal at Bodega

For the set of leaf samples from the removal experiment, I looked at the effect of the treatment group over time, analyzing the two species (*C. wightii* and *L. arboreus*) separately. For the parasite (*C. wightii*) data, I pooled treatment group A and B together for the first time point because they had the same starting conditions and the individuals from group B were removed and therefore were not available in the second time point. This led to two groups in the *C. wightii* analysis: (1) *C. wightii* growing with *L. arboreus* (treatment groups A and B) and (2) *C. wightii* growing without *L. arboreus* (treatment group D). For each leaf trait (Leaf N%, C/N, $\delta^{15}N$, $\delta^{13}C$) I ran a type III repeated measures ANOVA using on a linear mixed effects model where

the leaf trait was the response variable, group and time point were parameters, and the individual was a random effect (lmerTest package) (Kuznetsova, Brockhoff & Christensen 2017). To deal with heterogeneity, I used a logit transformation on leaf N% and a log transformation on C/N. Where significant differences were found, I ran post-hoc tests on a set of pairwise comparisons using a Tukey p-value adjustment and a 95% family-wise confidence level. The pairwise comparisons were between each treatment group within a time period and between time periods within a treatment group (nine comparisons per trait per species).

I was also interested in the relationship between leaf traits and how that was affected by the removal. To analyze this I ran ANCOVAs looking at $\delta^{13}C$ as a function of leaf N (%) and $\delta^{15}N$ as a function of $\delta^{13}C$, with Treatment Group, Time, and their interactions as additional factors. As above, individual was a random effect to account for repeated measures. Initially I looked at each species in isolation because the treatment groups are not fully crossed. However, treatment group was not significant for either species in $\delta^{15}N$ as a function of $\delta^{13}C$. For this relationship I looked at the species together with Species as an additional parameter. I again used a backward model selection, using AIC and BIC to compare models.

Results

DIVERSITY, PRODUCTIVITY AND SOIL MOISTURE

There was no effect of the presence of parasites or N-fixers on plot-level diversity as measured by inverse Simpson's index, Shannon index or rarified richness (Tables 2, 3). There was significant negative effect of *C. prostratus* on percent cover (51% less cover of other species, when cover is calculated without *C. prostratus*, or 157% more when cover includes *C. prostratus*) (Tables 2, 3). The presence of *C. applegatei* had a marginally significant negative effect on plot-level soil moisture while the interaction between a parasite and N-fixer had a significant positive effect (Tables 3, 4). Post-hoc pairwise comparisons, however, showed no differences among groups (Table A1-1).

LEAF TRAITS FROM OBSERVATIONAL STUDIES

At Sagehen the N-fixer and parasites each significantly affected leaf traits in the other. In the parasite, *C. applegatei*, the availability of an N-fixer corresponded to an increase in leaf %N from 1.73% to 3.89% (125.31% increase, $p < 0.001$), a significant decrease in leaf C/N from 25.27% to 11.83% (53.1% mean decrease, $p < 0.001$), a significant decrease in $\delta^{15}N$ values from 1.03‰ to -0.47‰ $\delta^{15}N$ ($p < 0.001$) and significant increase in $\delta^{13}C$ values from -31.01‰ to -29.49 ‰ signifying an increase in WUE ($p < 0.001$) (Table 5, Fig 2). Conversely in the N-fixer, *C. prostratus*, the presence of a parasite corresponded to no significant change in leaf %N and leaf C/N but a significant decrease in $\delta^{15}N$ values from -1.46‰ to -2.18‰ ($p = 0.0019$) and decrease in $\delta^{13}C$ values from -28.13 to -29.18‰ ($p = 0.012$), signifying a decrease in WUE (see Table 5, Fig 3).

The presence of a parasite was not associated with significant changes in leaf traits within the two non-N-fixers, *W. mollis* and *A. tridentata*. The presence of an N-fixer, however, did correspond to a significant decrease in leaf %N from 2.06% to 1.74% (15.79% decrease, $p=0.025$) and a significant increase in leaf C/N from 23.14 to 27.38 (18.33% increase, $p = 0.016$) in *A. tridentata* (Table 5). In *W. mollis*, the presence of an N-fixer corresponded to a significant decrease in $\delta^{15}N$ values from 1.11‰ to -0.28‰ ($p = 0.020$) (Figs 2-3, Table 5).

When controlling for the presence of a parasite and/or N-fixer, $\delta^{13}C$ was significantly correlated with leaf %N in *C. applegatei* ($p = 0.06$, $R^2 = 0.61$), *C. prostratus* ($p = 0.044$, $R^2 = 0.40$) and *A. tridentata* ($p = 0.007$) (Figs 4-5, Table 6). *W. mollis* was the only species where $\delta^{15}N$ was correlated with $\delta^{13}C$ ($p = 0.016$, $R^2 = 0.47$) (Fig 5, Table 6).

LEAF TRAITS FROM EXPERIMENTAL REMOVAL

At Bodega, species and time points were often markedly different but treatment groups generally were not different (Table 7, Fig 6). On average from March to August in the parasite, *C. wightii*, there was a 61% decrease in leaf %N from 2.18% to 0.86% ($p < 0.001$ for all treatment groups) and a 148% increase in leaf C/N from 21.65 to 53.62 ($p < 0.001$ for all treatment groups). In the same time period, on average across all treatment groups there was a parallel 37% decrease in leaf %N from 4.71% to 2.95% ($p < 0.001$ for all three treatment groups) and 59% increase in C/N from 10.14 to 16.13 ($p < 0.001$ for all treatment groups) in the N-fixer, *L. arboreus*. In *L. arboreus*, treatment group B *(L. arboreus* with *C. wightii* removed) compared to treatment group A (*L. arboreus* with *C. wightii*) had 23% lower leaf %N (absolute difference in leaf %N: 0.80%, $p = 0.001$) and 30% higher C/N (absolute difference in C/N: 4.31, $p = 0.001$) in August. There were no significant differences in $\delta^{15}N$ values between time points or treatment groups in either species, with the exception of a significant decrease in $\delta^{15}N$ from March to August in treatment group C (*L. arboreus* without *C. wightii*) (absolute difference: 0.74 ‰, $p=0.049$). In the parasite, *C. wightii*, there were no differences in $\delta^{13}C$ values. In *L. arboreus*, however, there was a significant increase in $\delta^{13}C$ values from March to August from -28.86 ‰ to -26.03‰ on average across all treatment groups (10% increase, $p<0.001$ for all treatment groups) (Tables 7, A2, A3, Fig 6). Controlling for treatment group and time, leaf %N was not correlated with $\delta^{13}C$ in either species, nor was $\delta^{15}C$ with $\delta^{13}C$. However, some interactions included as covariates were significant (Table 8, Fig 7).

Discussion

LEAF TRAITS FROM OBSERVATIONAL STUDIES

As predicted, the availability of an N-fixing host significantly increased leaf N in the parasite, *C. applegatei*. Parasites in plots without an N-fixer had leaf N levels in line with neighboring non-N-fixers, however, the presence of an N-fixer brought the parasite leaf N well above the other species, including even the N-fixer itself. High leaf N is thought to be relatively common in parasitic plants and be an important contributor to indirect effects on plant communities (Pate 1995; Spasojevic & Suding 2011; Fisher *et al*. 2013). These data additionally suggest that leaf N and consequent indirect effects may be host-mediated.

That the parasite's increased N is derived directly from the N-fixer is potentially evidenced by the significant shift in parasites $\delta^{15}N$ values towards the $\delta^{15}N$ signature of the N-fixer. However, a shift of similar magnitude and direction is also observed in *W. mollis* in the presence of the N-fixer, so the effect could be driven by litter deposit (i.e. the N-fixer is dropping leaves and altering the local $\delta^{15}N$ signature in the soil pool). On the other hand, it seems unlikely that the increase in leaf N in *C. applegatei* is derived entirely from leaf litter for two reasons. One: neither of the other two species, *A. tridentata* nor *W. mollis* showed the same increase in leaf N (indeed *A. tridentata* showed a decrease in leaf N). And two: the N-fixer's leaf N was relatively similar to the other species present, thus unless there were large differences in resorption prior to senescence the resulting leaf litter from the N-fixer likely would not be

particularly N rich either. This second point calls into question why an N-fixing host would result in higher leaf N in a parasite if the host's leaf N is not particularly high. Given that N-fixation rates are at least partially controlled by the plant's N demand, one possibility is that N loss to a parasite triggers higher N-fixation rates in compensation (Hartwig 1998). The amount and composition of N in xylem sap is variable, thus it may also be that the N concentration in xylem sap relative to leaf N may be higher in *C. prostratus* (Bollard 1960).

The significant increase in the parasites' $\delta^{13}C$ values when growing with N-fixers supports the N-parasitism hypothesis (Fig 2). Parasites with more access to N (indicated by the availability of N-fixers and the associated increase in leaf N) have a higher WUE. This is further supported by the positive correlation between leaf N and $\delta^{13}C$ among the parasites (Fig 4). Although this does not resolve whether the increase in the parasite's WUE is due to a decrease in transpirational water loss because N needs are met or increased photosynthetic rates brought on by increased N, it adds support to the former explanation. In addition, the negative impact on soil moisture of a parasite alone contrasted with the positive impact of the presence of a parasite and a N-fixer further suggests that the parasite's WUE is impacted by host identity. Although the effect on soil moisture was small in magnitude it demonstrates the possibility of host-mediated effects on the broader plant community.

The positive correlation between leaf N and $\delta^{13}C$ is also seen in the N-fixer itself and one of the non-N-fixers, *A. tridentata* (Fig 5). In theory, some possible drivers behind this pattern in parasitic plants also apply to non-parasites. For one, more N allows for more photosynthesis because it is a key component to the main carboxylating enzyme in leaves, RuBisCO (Chapin *et al*. 1987; Evans 1989). In this case, increases in WUE would be driven by upregulation of photosynthesis rather than downregulation of transpiration. Alternatively, transpiration generally increases the mass flow of nutrients to plant roots (Barber 1962; McDonald, Erickson & Kruger 2002; Matimati, Verboom & Cramer 2014b), thus correlations between leaf N and $\delta^{13}C$ could also be driven by upregulation of transpiration when plant demand for N is higher. However, for non-parasites the potential benefit of high transpiration rates is reduced because they do not obtain N from dilute xylem streams while the relative cost of water is higher because of investment in root architecture.

Previous work has suggested that the deposit of N-rich litter from parasites alters local plant diversity and growth (Quested, Press & Callaghan 2003; Spasojevic & Suding 2011; Fisher *et al*. 2013). Despite evidence that the parasite has relatively high leaf N when N-fixing hosts are available, neither the presence of a parasite nor combination of a parasite and an N-fixer had an impact on plot level diversity or cover. The presence of a N-fixer did have a negative impact on percent cover, likely reflecting *C. prostratus* mat-forming habit, wherein it crowds out other species. It could be that in a system such as the one observed here, the parasites are not a large enough portion of aboveground biomass to have community-level effects. While I did not quantify biomass, in plots with a parasite the average percent cover of the parasite was 7.25%, while the average percent cover for plots with and without a parasite were 52.4% and 39.7% respectively (including the parasite). The documentation by Spasojevic and Suding (2011) of increased plant growth as a result of parasitic-plant litter was in a setting where parasites made up nearly half of the biomass in plots where they were present.

Although community-level effects were absent, the presence of a parasite did significantly affect the N-fixer (but none of the other species present) (Fig 3). This further suggests that the parasites were actively attached to the N-fixers, although it doesn't preclude attachments to other species. That effects are observed only in the N-fixer suggests either

disproportionate parasitism on N-fixers or disproportionate vulnerability to parasitism in N-fixers (although the former seems more likely). The disproportionate parasitism on N-fixers could be caused by a difference in how likely the parasite is to attach to a particular host or a shift in the parasites' heterotrophy/autotrophy balance when attached to certain hosts.

In the N-fixer, the presence of a parasite was associated with a significant decrease in $\delta^{13}C$, signifying a lower WUE (Fig 3). Because parasitic plants penetrate host xylem and then pull water into their own xylem with transpiration rates high enough to maintain a favorable water potential gradient, the lower WUE may be a result of the N-fixer's increased transpiration due to competition with the parasite for its own xylem stream. The N-fixer's leaf N was unchanged in the presence of a parasite, further suggesting an upregulation of transpiration rather than downregulation of photosynthesis.

Interestingly, the presence of a parasite is also associated with a significant shift in the $\delta^{15}N$ signature of the N-fixer (Fig 3). The $\delta^{15}N$ of atmospheric N_2 is by definition 0‰, thus despite some discrimination within the fixation process biologically-fixed N is typically very close to 0‰, whereas soil N typically has a $\delta^{15}N$ signature distinct from the atmosphere (Dawson *et al*. 2002). Indeed when associated with an N-fixer the parasite's $\delta^{15}N$ signature shifts to being very close to 0‰. A wide range of factors, including different source pools, could cause the shift in $\delta^{15}N$ signature within the N-fixer. As mentioned above, a possible explanation is that N loss to the parasite leads to increased N fixation by the N-fixer, keeping the overall leaf N unchanged. However, here the $\delta^{15}N$ signature shifts further from the $\delta^{15}N$ signature of biologically fixed N. The shift in $\delta^{15}N$ signature may be a result of decreased N fixation, however, any conclusions here are premature. For example, it is not currently known whether nutrient transfer through haustoria results in N isotope fractionation. The observed shift could also be a result of discrimination as N leaves the individual plant towards the parasite, rather than simply reflecting the N-fixer's source N.

LEAF TRAITS FROM EXPERIMENTAL REMOVAL

In contrast to the observational work on *C. applegatei*, I did not find strong evidence for host-parasite interactions between the parasite and N-fixer in the experimental removal at Bodega. No leaf traits within the parasite were affected by the presence of an N-fixer (Fig 6). In particular, despite the N-fixer and parasite having distinct $\delta^{15}N$ signatures, there was no change in the $\delta^{15}N$ of the parasite when in the presence of an N-fixer. The N-fixer here, *L. arboreus*, has been shown to dramatically increase soil N availability in an otherwise sandy, nutrient-poor environment (Maron & Jefferies 1999). Thus, it was relatively surprising that the parasite did not appear to rely on the N-fixer here as a source of N, even indirectly from leaf litter. It is also possible that the distance (5m) set for whether a parasite was possibly attached to an N-fixer was too small and parasites were attached to N-fixers more than 5m away. Or similarly that deposit of N-rich litter by the N-fixer affected the soil more than 5m away.

The N-parasitism hypothesis would predict that low soil N at Bodega would make N rich hosts particularly important and drive high transpiration rates. However, the parasite was unaffected by the presence of an N-fixer and there was no correlation between leaf %N and $\delta^{13}C$, indicating that access to more N did not alter the WUE of the parasites (Fig 7). Compared to *C. applegatei*, the parasites at Bodega had somewhat lower leaf N with the majority of individuals below 3%. $\delta^{13}C$ values did not change from March to August in the parasite, while in the N-fixer $\delta^{13}C$ values increased significantly. This increased WUE likely reflects the increased water stress for plants at the end of California summer, when it typically does not rain, versus March at the

tail end of the rainy season. That the parasites' $\delta^{13}C$ remained unchanged suggests a disconnect from the water stress experienced by other plants, likely driven by parasites' access to water from the host xylem and consistent with a reputation as profligate water users. However, compared to *C. applegatei* the $\delta^{13}C$ signatures were heavier, indicating higher overall WUE, conceivably driven by the low-moisture, sandy soils at Bodega.

There was a significant decrease in leaf N and corresponding increase in leaf C/N from March to August, which likely reflects the growing season, as *C. wightii* begins to senesce in late summer/early fall. Similarly, the N-fixer also showed a decrease in leaf N and corresponding increase in leaf C/N from March to August, again likely reflecting the growing season trajectory. In addition, removing a parasite from the N-fixer (treatment group B) resulted in a significantly lower leaf N and higher C/N compared with the group where the parasites where not removed (treatment group A). Considering the theoretical N loss to a parasite, this is the opposite pattern than expected. However, neither groups A nor B were significantly different from group C, N-fixers with no parasites present, at either time point. This makes it difficult to draw many conclusions on the effect of parasites on the N-fixers here. Interestingly, when I returned to the site two years later the majority of the N-fixers in the experiment were dead. Large die-offs of *L. arboreus* are somewhat common and often attributed to root damage by subterranean ghost moth caterpillars, *Hepialus californicus* (Lepidoptera, Hepialidae) (Strong *et al*. 1995). It is possible that the *L. arboreus* individuals here were already infested at the time of the experiment.

Conclusions

The two systems here showed contrasting results on the importance of N-fixers to parasites. In one instance the availability of an N-fixing host significantly affected the parasite's leaf traits while in the other instance the N-fixer did not affect the parasite at all. The two ecosystems present may play an important role in altering the physiology of these species and mediating interactions. In addition, though the two parasites here were from the same genus, it is clear that there is considerable variation among species of *Castilleja*. The results here highlight the potential importance of N-fixers to parasitic plants, however, more work must be done to determine the drivers of N-parasitism in various contexts.

Tables

Table 1 Sampling locations at Sagehen Experimental Forest, 2015

Transect	Location (DM)	Altitude (m)	Leaf Samples
1	39°25.981', -120°14.758'	1940	N
2	39°26.050 ', -120°14.790'	1975	N
3	39°26.050 ', -120°14.791'	1975	N
4	39°26.147 ', -120°15.623'	1988	Y
5	39°26.201 ', -120°15.755'	2006	Y
6	39°25.913 ', -120°16.927'	2092	N
7	39°25.913 ', -120°16.928'	2092	N
8	39°26.488 ', -120°14.766'	2061	Y
9	39°26.512 ', -120°14.781'	2064	Y
10	39°26.763 ', -120°16.066'	2091	N
11	39°26.337 ', -120°15.806'	2022	Y
12	39°26.182', -120°15.937'	1999	Y

Table 2 Diversity and productivity in plots surveyed in Sagehen Experimental Forest, 2015.

Metric	*C. applegatei* present		*C. applegatei* absent		*C. prostratus* present		*C. prostratus* absent	
	Value	SE	Value	SE	Value	SE	Value	SE
Inverse Simpson (counts)	3.39	0.17	3.17	0.16	3.24	0.18	3.28	0.15
Shannon (counts)	1.34	0.05	1.27	0.05	1.27	0.05	1.33	0.05
Rarefied richness	2.28	0.05	2.22	0.05	2.20	0.05	2.25	0.04
Inverse Simpson (percent cover)	2.36	0.13	2.48	0.15	2.88	0.16	2.74	0.17
Shannon (percent cover)	1.02	0.05	1.03	0.06	1.17	0.05	1.09	0.07
Percent cover (actual)	52.50	3.22	39.58	3.06	56.15	3.06	35.59	2.85
Percent cover (w/o species of interest)	44.38	3.11	39.58	3.06	18.41	2.13	35.59	2.85

Table 3 Results of ANOVAs on diversity, productivity and soil moisture in 120 plots surveyed in Sagehen Experimental Forest, 2015.

Parameter	Metric		numDF	denDF	F-value	P-value
presence of parasite (*C. applegatei*)	Inverse simpsons (counts)		1	107	0.88	0.35
	Shannon-weaver (counts)		1	107	1.17	0.28
	Rarefied richness		1	107	0.76	0.38
	Inverse simpsons (percent cover)		1	107	0.41	0.52
	Shannon (percent cover)		1	107	0.06	0.81
	Percent cover (w/o *C. applegatei*)		1	107	1.26	0.26
presence of N-fixer (*C. applegatei*)	Inverse simpsons (counts)		1	107	0.00	0.95
	Shannon-weaver (counts)		1	107	0.30	0.59
	Rarefied richness		1	107	0.30	0.59
	Inverse simpsons (percent cover)		1	107	0.32	0.57
	Shannon (percent cover)		1	107	0.57	0.45
	Percent cover (w/o *C. prostratus*)		1	107	19.37	**<0.0001**
presence of parasite (*C. applegatei*) and N-fixer (*C. prostratus*)	Inverse simpson (counts)	*C. applegatei*	1	105	0.33	0.57
		C. applegatei	1	105	0.11	0.74
		interaction	1	105	0.02	0.88
	Shannon (counts)	*C. applegatei*	1	105	0.94	0.34
		C. prostratus	1	105	0.39	0.54
		interaction	1	105	0.32	0.57
	Rarefied richness	*C. applegatei*	1	105	0.41	0.53
		C. prostratus	1	105	0.59	0.44
		interaction	1	105	0.00	0.99
	Inverse simpson (percent cover)	*C. applegatei*	1	105	0.10	0.76
		C. prostratus	1	105	0.22	0.64
		interaction	1	105	0.15	0.70
	Shannon (percent cover)	*C. applegatei*	1	105	1.22	0.27
		C. prostratus	1	105	0.56	0.46
		interaction	1	105	0.15	0.70
	Percent cover (w/o *C. prostratus* or *C. applegatei*)	*C. applegatei*	1	105	0.11	0.74
		C. prostratus	1	105	8.47	**0.004**
		interaction	1	105	1.68	0.20
	Soil moisture (VWC%)	*C. applegatei*	1	97	3.88	**0.052**
		C. prostratus	1	97	1.81	0.18
		interaction	1	97	7.17	**0.009**

Table 4 Soil moisture (VWC%) in plots surveyed in Sagehen Experimental Forest, 2015.

***C. applegatei* present**	***C. prostratus* present**	**VWC %**	**SE**	**n**
No	No	5.86	0.49	35
No	Yes	5.61	0.49	25
Yes	No	5.46	0.66	24
Yes	Yes	5.93	0.50	36

Table 5 Results of Welchs two-sided t-test on four leaf traits on an N-fixer (*C. prostratus*) and a parasite (*C. applegatei*) depending on whether they were growing near a parasite and N-fixer, respectively. Samples collected in Sagehen Experimental Forest, 2015.

Species	**Parameter**	**Response variable**	**t**	**df**	**P-value**
Castilleja applegatei (parasite)	With *C. prostratus* (N-fixer)	$\delta^{15}N$	6.590	35.657	**<0.0001**
		$\delta^{13}C$	-7.343	36.770	**<0.0001**
		%N	-6.826	21.231	**<0.0001**
		C/N	9.579	36.899	**<0.0001**
Ceanothus prostratus (N-fixer)	With *C. applegatei* (parasite)	$\delta^{15}N$ *	Z = 3.0993		**0.0019**
		$\delta^{13}C$	2.884	14.421	**0.0117**
		%N	0.537	17.363	0.5981
		C/N	-0.378	17.685	0.7099

*Aysmpotic wilcoxon-mann-whitney test

Table 6 Results of two-way ANOVAs on four leaf traits on a two non-N-fixing, non-parasitic species (*A. tridentata*, *W. mollis*) depending on whether they were growing near an N-fixer (*C. prostratus*) and/or a parasite (*C. applegatei*). Samples collected in Sagehen Experimental Forest, 2015

Species	Response variable	Parameter	df	F	P-value
Artemisia tridentata	$\delta^{15}N$	w/ *C. prostratus*	1	1.957	0.181
		w/ *C. applegatei*	1	0.042	0.840
		Interaction	1	0.931	0.349
		Residuals	16		
	$\delta^{13}C$	w/ *C. prostratus*	1	0.046	0.833
		w/ *C. applegatei*	1	1.097	0.310
		Interaction	1	0.002	0.966
		Residuals	16		
	% N	w/ *C. prostratus*	1	6.116	**0.025**
		w/ *C. applegatei*	1	1.727	0.207
		Interaction	1	0.112	0.743
		Residuals	16		
	C/N	w/ *C. prostratus*	1	7.226	**0.016**
		w/ *C. applegatei*	1	1.478	0.242
		Interaction	1	0.036	0.852
		Residuals	16		
Wyethia mollis	$\delta^{15}N$	w/ *C. prostratus*	1	6.638	**0.020**
		w/ *C. applegatei*	1	0.004	0.953
		Interaction	1	0.842	0.372
		Residuals	16		
	$\delta^{13}C$	w/ *C. prostratus*	1	0.857	0.368
		w/ *C. applegatei*	1	0.882	0.362
		Interaction	1	1.029	0.326
		Residuals	16		
	% N	w/ *C. prostratus*	1	0.000	0.984
		w/ *C. applegatei*	1	1.331	0.266
		Interaction	1	0.907	0.355
		Residuals	16		
	C/N	w/ *C. prostratus*	1	0.029	0.867
		w/ *C. applegatei*	1	1.165	0.296
		Interaction	1	0.574	0.460
		Residuals	16		

Table 7 Results of ANCOVAs on the relationship between leaf traits within four species, including an N-fixer (*C. prostratus*), a parasite (*C. applegatei*), and two others (*A. tridentata, W. mollis*). Samples collected in Sagehen Experimental Forest, 2015.

Species	Response variable	Parameter	df	F value	P-value	Adjusted R2
Castilleja applegatei (parasite)	$\delta^{13}C$	%N	1	3.765	**0.0602**	0.610
		w/ *C. prostratus*	1	13.538	**0.0008**	
		Residuals	36			
	$\delta^{15}N$	$\delta^{13}C$	1	1.474	0.2329	0.560
		w/ *C. prostratus*	1	28.140	**<0.0001**	
		$\delta^{13}C$ * w/ *C. prostratus*	1	3.068	0.089	
		Residuals	35			
Ceanothus prostratus (N-fixer)	$\delta^{13}C$	%N	1	4.472	**0.044**	0.402
		w/ *C. applegatei*	1	8.238	**0.011**	
		Residuals	17			
	$\delta^{15}N$*	$\delta^{13}C$	1	0.443	0.515	~
		w/ *C. applegatei*	1	16.304	**0.009**	
		Residuals	17			
Artemisia tridentata	$\delta^{13}C$	%N	1	9.753	**0.007**	0.309
		w/ *C. prostratus*	1	4.505	**0.050**	
		%N * *w/ C. prostratus*	1	1.750	0.204	
		Residuals	17			
	$\delta^{15}N$	$\delta^{13}C$	1	0.353	0.562	0.147
		w/ *C. prostratus*	1	1.200	0.292	
		w/ *C. applegatei*	1	0.054	0.820	
		$\delta^{13}C$ * w/ *C. prostratus*	1	1.518	0.238	
		$\delta^{13}C$ * w/ *C. applegatei*	1	2.194	0.161	
		Residuals	14			
	$\delta^{13}C$**	%N	1	0.932	0.347	-0.004
		Residuals	18			
Wyethia mollis	$\delta^{15}N$	$\delta^{13}C$	1	8.103	**0.012**	0.504
		w/ *C. prostratus*	1	9.887	**0.006**	
		w/ *C. applegatei*	1	2.324	0.147	
		Residuals	17			

* Generalized least squares (gls) fit by restricted maximum likelihood with a variance structure that allows for different standard deviations in each stratum. R^2 not calculated for gls fit.
** linear regression rather than an ANCOVA because of elimination of non-significant parameters

Table 8 Results of ANOVAs on leaf traits of two species, a parasite (*C. wightii*) and an N-fixer (*L. arboreus*) from an experimental removal of the parasite at Bodega Marine Lab, 2016. Treatment groups are (A) N-fixer and parasite together, (B) N-fixer with parasite removed in March 2016 (parasite is present for leaf collection in March 2016 and removed shortly thereafter), (C) N-fixer with no parasite present and (D) parasite with no N-fixer present. For *C. wightii* analysis, treatment groups A and B were grouped into one treatment group: A.

Species	Response variable	Parameter	df1	df2	F-value	P-value
Castilleja wightii (parasite)	% N	Treatment Group	1	28	2.066	0.162
		Time	1	28	138.838	**<0.0001**
		Interaction	1	28	0.555	0.463
	C/N	Treatment Group	1	29	2.921	0.098
		Time	1	28	123.807	**<0.0001**
		Interaction	1	28	0.412	0.526
	$\delta^{13}C$	Treatment Group	1	20	0.830	0.373
		Time	1	21	1.930	0.179
		Interaction	1	21	2.060	0.166
	$\delta^{15}N$	Treatment Group	1	27	0.089	0.768
		Time	1	28	3.697	0.065
		Interaction	1	28	1.822	0.188
Lupinus arboreus (N-fixer)	% N	Treatment Group	2	58	6.166	**0.004**
		Time	1	58	130.515	**<0.0001**
		Interaction	2	58	2.981	0.059
	C/N	Treatment Group	2	58	6.488	**0.003**
		Time	1	58	154.581	**<0.0001**
		Interaction	2	58	2.714	0.075
	$\delta^{13}C$	Treatment Group	2	29	0.758	0.478
		Time	1	32	153.799	**<0.0001**
		Interaction	2	32	1.721	0.195
	$\delta^{15}N$	Treatment Group	2	58	0.157	0.855
		Time	1	58	7.774	**0.007**
		Interaction	2	58	0.218	0.805

Table 9 Results of ANCOVAs on the relationship between leaf traits within two species, a parasite (*C. wightii*) and an N-fixer (*L. arboreus*), from an experimental removal of the parasite at Bodega Marine Lab, 2016.

Response variable	Species	Parameter	Num df	Den df	F value	P-value
$\delta^{13}C$	*C. wightii* (parasite)	%N	1	45.689	0.115	0.736
		Treatment Group	1	20.325	1.161	0.294
		Time	1	39.414	0.590	0.447
	L. arboreus (N-fixer)	%N	1	50.422	1.524	0.223
		Treatment Group	2	51.134	3.680	**0.032**
		Time	1	44.745	31.765	**<0.0001**
		Treatment Group*Time	2	44.539	4.836	**0.013**
		%N*Treatment Group	2	50.194	4.176	**0.021**
$\delta^{15}N$	Both	$\delta^{13}C$	1	107.212	0.128	0.721
		Species	1	107.146	6.882	**0.010**
		Time	1	96.447	0.185	0.668
		Species*Time	1	96.447	11.210	**0.001**
		$\delta^{13}C$*Species	1	107.212	4.277	**0.041**

Figures

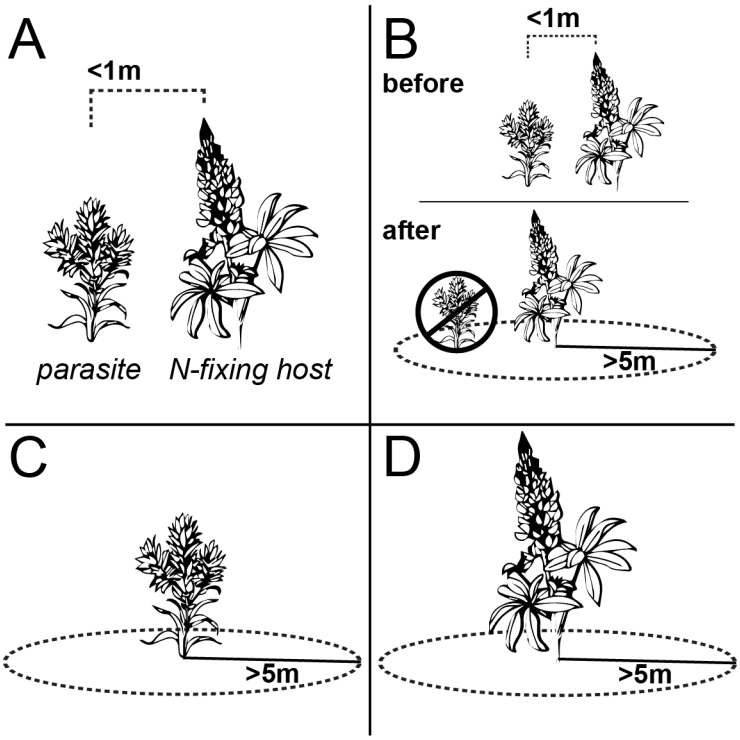

Figure 1 Treatment groups for parasite removal experiment at Bodega Marine Reserve, 2016.

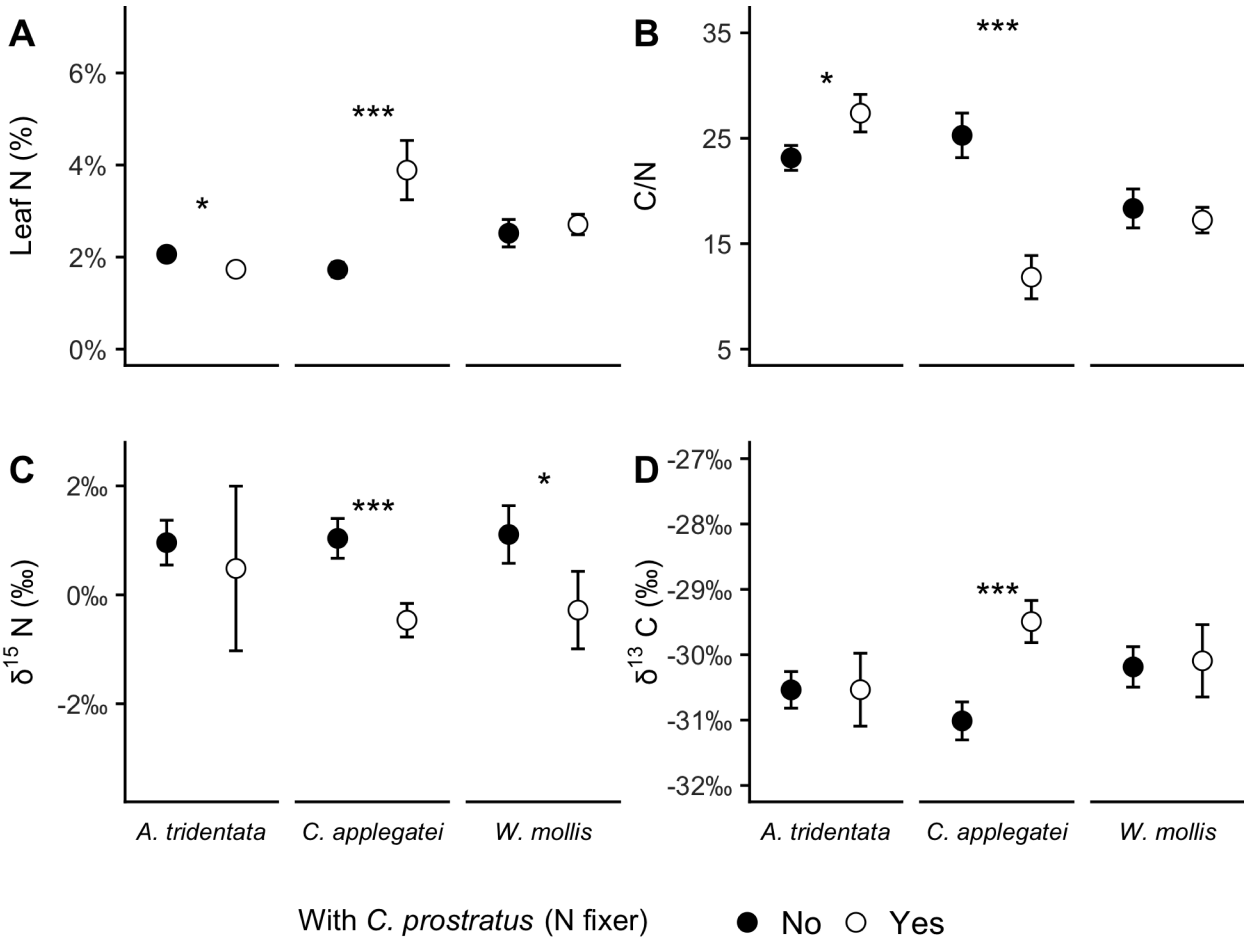

Figure 2 Differences in four leaf traits (A: foliar leaf %N, B: foliar C/N, C: $\delta^{15}N$, D: $\delta^{13}C$) among two non-N-fixer plant species (*A. tridentata, W. mollis*) and a root hemiparasite (*C. applegatei*) when in the presence of an N-fixer (*C. prostratus*). Leaves collected at Sagehen Experimental Forest in 2015. Asterisks denote significant differences within a species where: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. See Tables 3, 4 for statistics. Error bars are 95% confidence intervals.

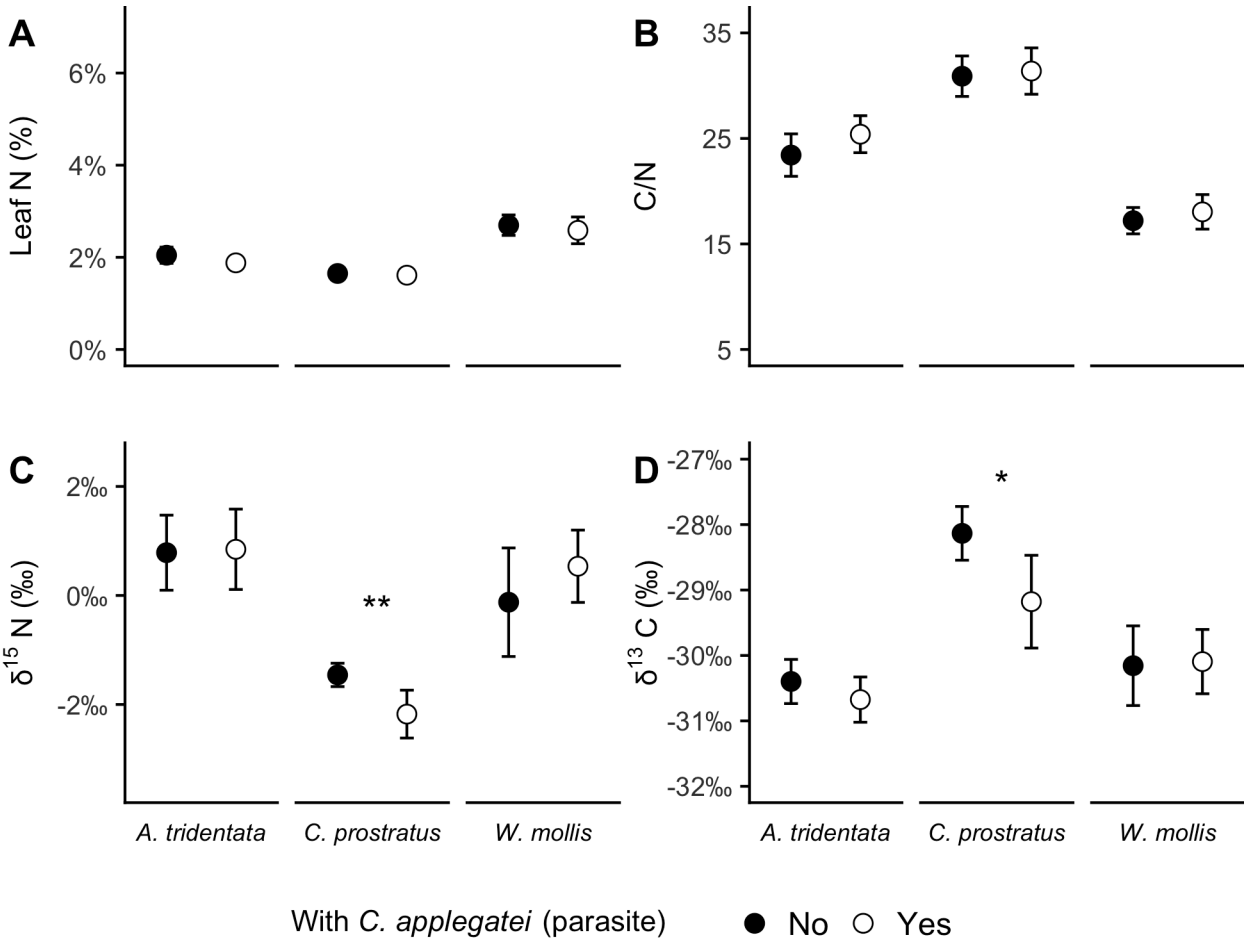

Figure 3 Differences in four leaf traits (A: foliar leaf %N, B: foliar C/N, C: $\delta^{15}N$, D: $\delta^{13}C$) among two non-N-fixer plant species (*A. tridentata, W. mollis*) and an N-fixer (*C. prostratus*) when in the presence of a parasite (*C. applegatei*). Leaves collected at Sagehen Experimental Forest in 2015. Asterisks denote significant differences within a species where: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. See Tables 3, 4 for statistics. Error bars are 95% confidence intervals.

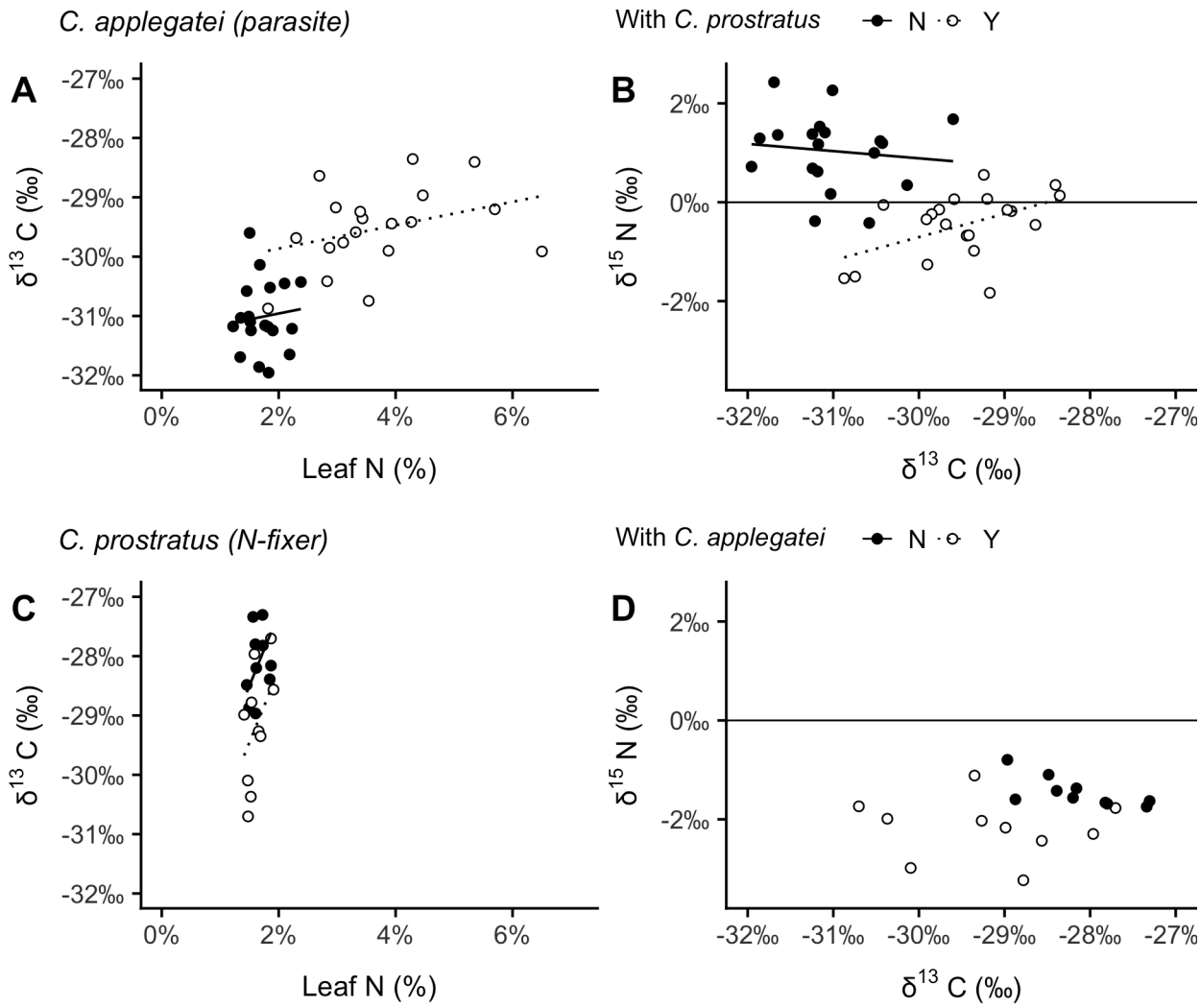

Figure 4 Leaf traits compared within a root hemiparasite (*C. applegatei*) when in the presence of an N-fixer (*C. prostratus*) (A-B) and within an N-fixer (*C. prostratus*) when in the presence of a root hemiparasite (*C. applegatei*) (C-D). Leaves collected at Sagehen Experimental Forest in 2015. Trendlines are shown where the continuous variable or interaction was at least marginally significant ($p < 0.1$) in ANCOVAS. See Table 5 for statistics.

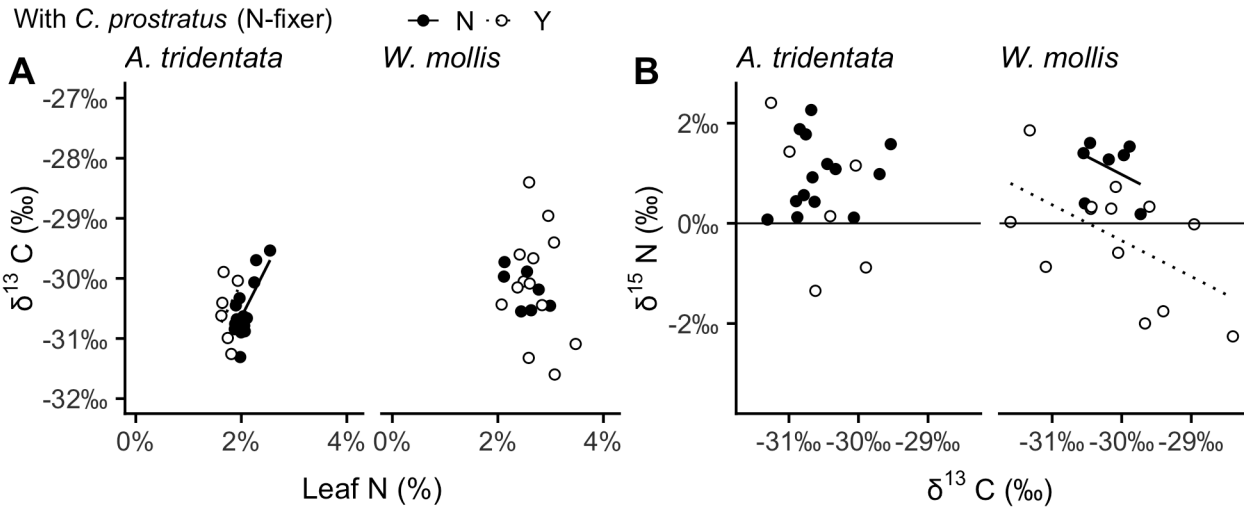

Figure 5 Leaf traits compared within two species, *A. tridentata* and *W. mollis*, when in the presence of an N-fixer (*C. prostratus)*. Leaves collected at Sagehen Experimental Forest in 2015. Trendlines are shown where the continuous variable or interaction was at least marginally significant ($p < 0.1$) in ANCOVAS. The presence of *C. applegatei* was not significant. See Table 5 for statistics.

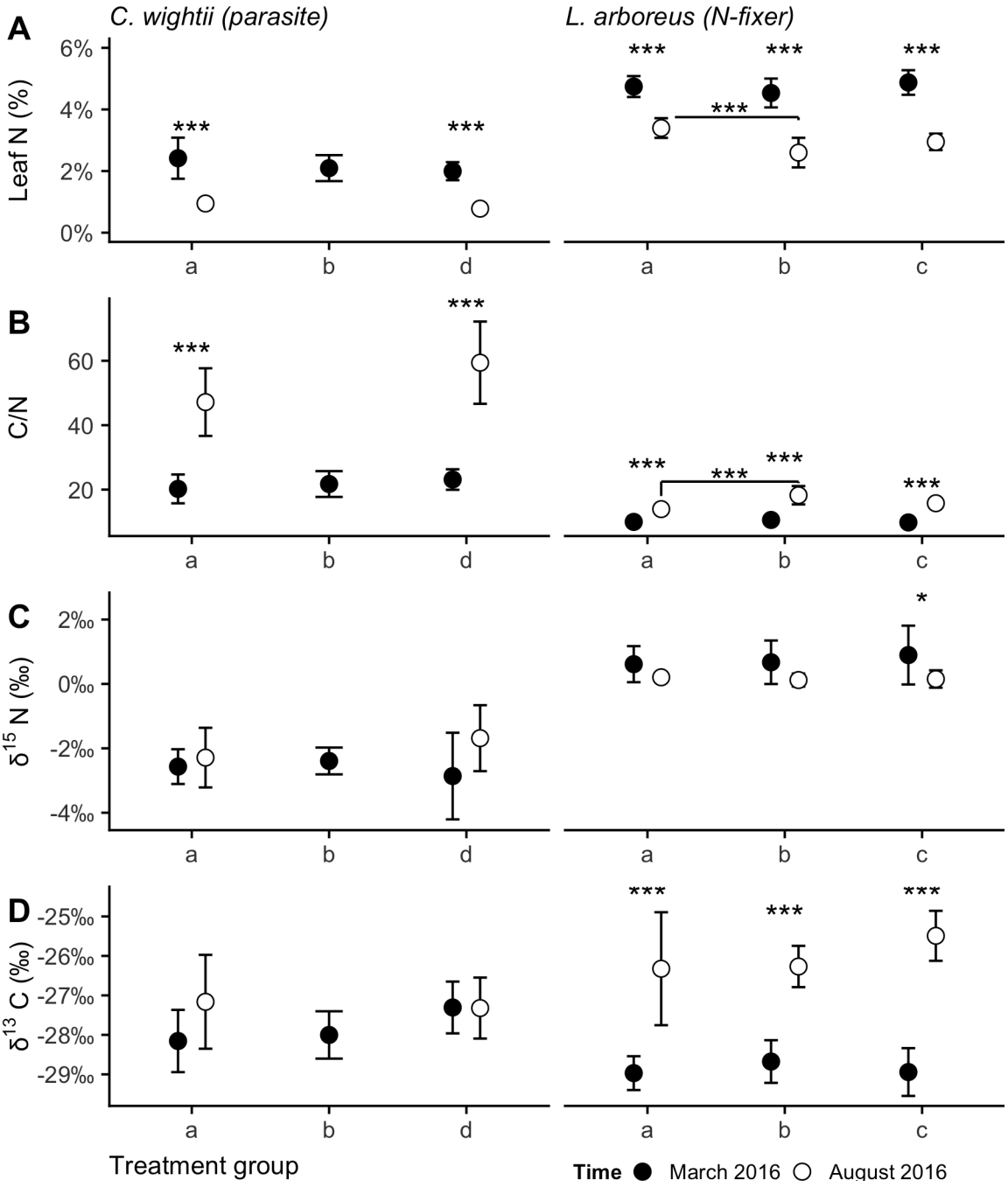

Figure 6 Differences in leaf traits: (A) foliar leaf %N, (B) foliar C/N, (C) $\delta^{15}N$, (D) $\delta^{13}C$) among an N-fixer (*L. arboreus*) and a root hemiparasite (*C. wightii*) compared between four treatment groups: (a) N-fixer and parasite together, (b) N-fixer with parasite removed in March 2016 (parasite is present for leaf collection in March 2016 and removed shortly thereafter), (c) N-fixer with no parasite present and (d) parasite with no N-fixer present. Leaves collected at Bodega Marine Lab in 2016. Asterisks denote significant differences between the two time points and asterisks with a bracket denote significant differences between two treatments groups within a time point, where: *p < 0.05, **p < 0.01, ***p < 0.001. See Tables 7, A1-1,A1-2 for statistics. Error bars are 95% confidence intervals.

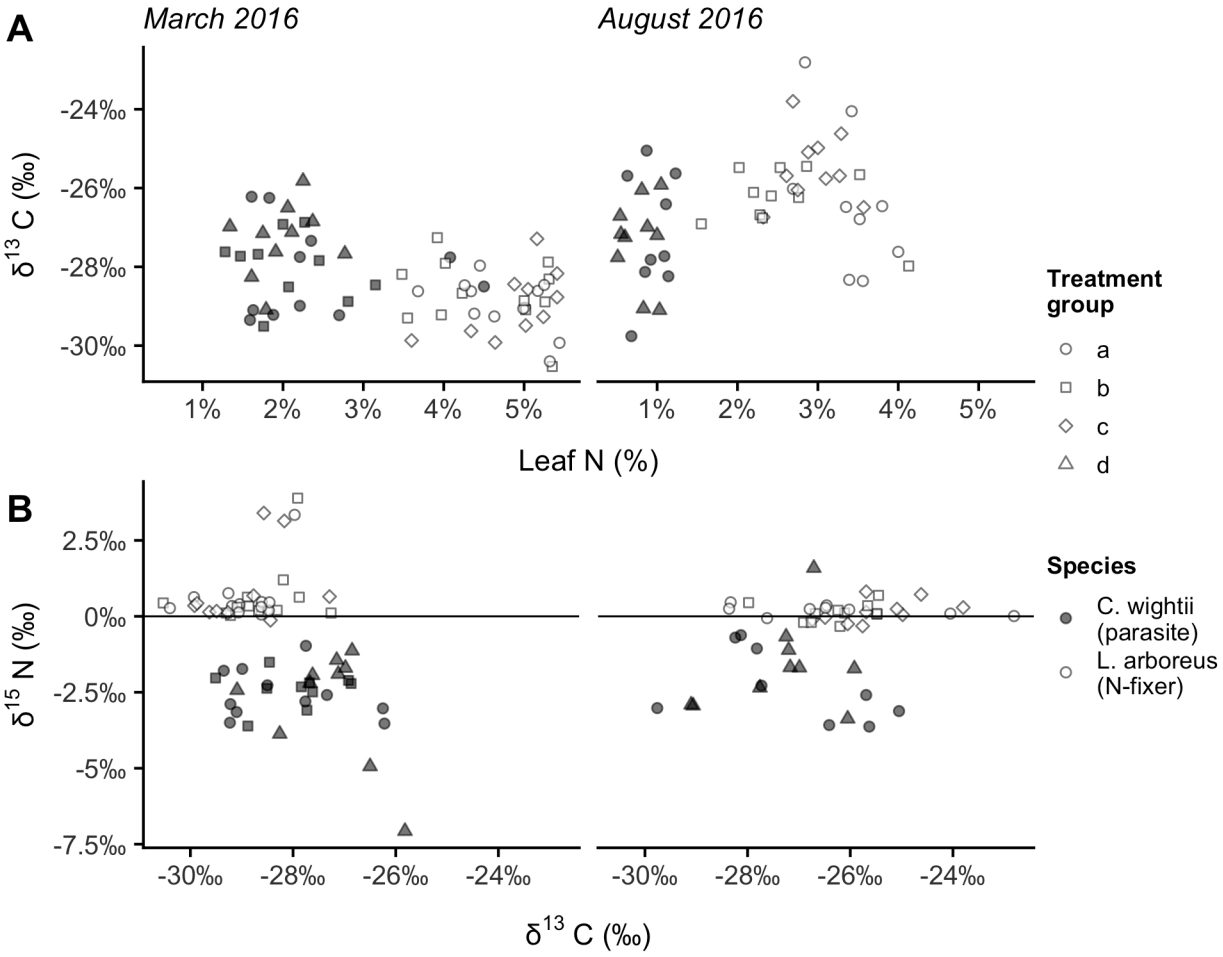

Figure 7 Leaf traits in an N-fixer (*L. arboreus*) and a root hemiparasite (*C. wightii*) compared between four treatment groups: (a) N-fixer and parasite together, (b) N-fixer with parasite removed in March 2016 (parasite is present for leaf collection in March 2016 and removed shortly thereafter), (c) N-fixer with no parasite present and (d) parasite with no N-fixer present. Leaves collected at Bodega Marine Lab in 2016. See Table 8 for statistics.

Chapter 2: Nighttime stomatal conductance in *Castilleja*, a genus of root hemiparasites

Abstract

Nighttime stomatal opening and consequent transpiration is typically considered rare because for most non-CAM plants stomatal closure at night limits water loss when there is no carbon to be gained from photosynthesis. Xylem parasites, however, also derive dilute carbon from their hosts' xylem. Because this source is unaffected by darkness, xylem parasites are theoretically released from one of the major drivers of nighttime stomatal closure. I measured nighttime stomatal conductance in eight *Castilleja* species, a widespread genus of root hemiparasites, across eight sites in California. Each *Castilleja* measurement was paired with one made on a neighboring individual of a non-parasitic plant species common at that site. On average parasites' nighttime stomatal conductance was 235% higher than the non-parasites, with values often in excess of 500 mmol H_2O m^{-2} s^{-1}, although all the plants displayed some nighttime stomatal conductance. Only one species of *Castilleja* had a lower average nighttime stomatal conductance than its non-parasitic neighbor. These data demonstrate that many *Castilleja* commonly transpire at night, adding them to the growing group of plants shown to open their stomata at night, and demonstrating a potential mechanism of benefit driving nighttime transpiration.

Introduction

It is generally assumed that plants do not transpire at night because stomata close in the dark. For non-CAM plants, darkness halts crucial aspects of photosynthesis and consequently stomatal closure limits water loss when there is no carbon to be gained (Farquhar & Sharkey 1982). Additionally, in the dark (night) the need for cooling is reduced. Although this is the default assumption, nighttime stomatal opening and consequent nighttime transpiration have been observed across many taxa and ecosystems (Bucci *et al*. 2004; Dawson *et al*. 2007; Zeppel *et al*. 2010). Various explanations have been proposed for why nighttime transpiration occurs, both adaptive and non-adaptive, including nutrient acquisition, leaky stomata, and delivery of O_2 to parenchyma (Caird, Richards & Donovan 2007; Dawson *et al*. 2007; Snyder *et al*. 2008; Rosado *et al*. 2012; Matimati *et al*. 2014b); however, nighttime transpiration remains poorly understood and often ignored (Zeppel *et al*. 2014). Many long-standing plant physiology methods and theories, as well as water flux models, assume nighttime transpiration is negligible (Green, Mcnaughton & Clothier 1989; Donovan *et al*. 1999; Bucci *et al*. 2004).

Parasitic plants may aid in expanding our understanding of this process because they are theoretically partially released from two of the major drivers of stomatal closure at night: the inability to gain carbon (C) in darkness and the need to conserve water. Instead of relying solely on photosynthesis, parasitic plants also derive some fraction of their C from their host, a source unaffected by darkness. Water can be relatively "cheap" for parasites because they have a way to divert, or steal, water directly from a host (see below) rather than build their own extensive root systems. In theory this unique C–water tradeoff could promote nighttime transpiration in parasitic plants.

Nutrient acquisition, a proposed explanation for nighttime transpiration in non-parasites, could operate similarly in hemiparasites (Scholz *et al*. 2007; Cernusak, Winter & Turner 2011; Matimati, Anthony Verboom & Cramer 2014a; Matimati *et al*. 2014b; Zeppel *et al*. 2014). Hemiparasites, which make up approximately 90% of parasitic plant species, typically only have access to host xylem while holoparasites also have access to the host phloem (Heide-Jørgensen 2008; Irving & Cameron 2009). Hemiparasites acquire water as well as dilute C and nutrients from the host xylem. The nitrogen (N) parasitism hypothesis suggests hemiparasites are N limited and N acquisition via the host xylem stream drives observed high transpiration rates and resulting low water-use-efficiency (WUE) (Schulze *et al*. 1984). It follows that N limitation would lead to nighttime transpiration because N supply via the host xylem stream is unaffected by daylight (as is N supply in a non-parasitic plant).

Understanding how parasitism affects transpiration patterns is important because parasites are common constituents of nearly all ecosystems. The parasitic strategy is successful and widespread; in angiosperms, parasitism has evolved independently 12-13 times and approximately 1% of angiosperms are parasitic (Westwood *et al.* 2010). In addition 10% of angiosperms are mycoheterotrophs, parasitizing mycorrhizal fungi at some point in their life cycle (distinct from the mutualistic mycorrhizal associations formed by the majority of plants) (Leake & Cameron 2010). Although the mechanisms and physiology of parasitism vary considerably among these groups, they all have altered C–water and/or nutrient–water tradeoffs, which may in turn affect transpiration patterns. Parasites also can play an outsize role in structuring communities in part because of their water use patterns (Pennings & Callaway 1996; Marvier 1998b; Smith 2000; Bardgett *et al.* 2006). For example, mistletoe infestations have been shown to alter host tree's hydraulic architecture, reduce their photosynthetic capacity and

sometimes kill host trees (Sala *et al.* 2001; Marias *et al.* 2014). Understanding whether or not parasitic plants engage in nighttime transpiration would enhance our understanding of this understudied, yet important group of plants and illuminate possible explanations for nighttime transpiration among all plants (Press & Phoenix 2005; Watson & Herring 2012).

Here I present evidence of nighttime transpiration in eight species or subspecies of *Castilleja* (paintbrushes), a common widespread genus in the Orobanchaceae family of root hemiparasites. I also compare each species to a common non-parasitic plant at each site. Orobanchaceae is the second largest family of parasites (after Santalales) representing 90 genera and approximately 2060 species and the only family with extant members representing the full range of host dependence (from facultative hemiparasites to obligate holoparasites) (Westwood *et al.* 2010; Mcneal *et al.* 2013). Orobanchaceae also has the dubious distinction of including some of the most agriculturally destructive and thus economically important genera of parasites: *Striga* spp. and *Orobanche* spp. (Fernández-Aparicio, Reboud & Gibot-Leclerc 2016; Runo & Kuria 2018). Nighttime transpiration has not been observed in *Castilleja* before but has been observed in other root hemiparasitic members of the Orobanchaceae family (Press *et al.* 1987, 1988; Jiang, Jeschke & Hartung 2003). Observations here address the questions a) do these root hemiparasites transpire at night? b) how does their nighttime transpiration compare to neighboring plants and c) how does ecosystem type and habit affect nighttime transpiration in *Castilleja* spp?

Materials and Methods

Species and Site Descriptions

I measured eight species or subspecies of *Castilleja*, each at a distinct site (Table 1). The species measured are found in a wide array of habitats from wet meadow to sagebrush scrub and are all perennials. Within the genus *Castilleja* perennials form a monophyletic group (~160 species) (Tank & Olmstead 2008). Each species and accompanying site is described below. All measurements were taken in July and August of 2019, with the exception of *C. applegatei* ssp. *pinetorum,* which was measured in July of 2015. In 2015, California was in the midst of a historic drought but by 2019 the vast majority of the state was considered drought-free (CADWR 2015; Hanak *et al*. 2016; National Drought Mitigation Center 2019). The sites are all in California east of the Sierra Nevada crest. Due to a rain shadow effect, the eastern Sierra Nevada and western Great Basin are considerably more arid than the western slopes of the Sierra Nevada but the sites are still broadly characterized by a Mediterranean-type climate with warm dry summers and cool, wet winters (Rundel & Millar 2016). In addition the California Great Basin sometimes receives monsoon influence driving generally minor summer precipitation (Millar 2015).

Castilleja applegatei ssp. *pallida* (Eastw.) T.I. Chuang & Heckard: *C. applegatei* (wavy-leafed paintbrush) is a highly variable group with many subspecies. Four subspecies are found in California across a diverse range of habitats, including chaparral, yellow pine forests, pinyon-juniper woodlands and alpine fell-fields. *C. applegatei* ssp. *pallida* is found only in montane environments in the Sierra Nevada including subalpine forests and alpine fell-fields (Jepson Flora Project 2019; The CalFlora DataBase 2019). I sampled *C. applegatei* ssp. *pallida* at a high elevation sagebrush scrub site. The site was dominated by *Artemisia tridentata*, with other shrubs such as *Symphoricarpos rotundifolius* and *Chrysothamnus viscidiflorus*.

Castilleja applegatei ssp. *pinetorum* (Fernald) T.I. Chuang & Heckard (wavy-leafed paintbrush) is typically found in open conifer forest and sagebrush scrub environments throughout the Sierra Nevada, the Southern Cascades, the High North coastal ranges and into the Modoc plateau. I measured *C. applegatei* ssp. *pinetorum* at Sagehen Creek Field Station, located in the central Sierra Nevada north of Truckee, CA. Vegetation types include mixed-conifer forest, meadow, shrub and conifer plantations. *C. applegatei* ssp. *pinetorum* can be found within the shrub type, which occurs on poor and/or shallow soils unable to support conifer forests and on more productive soils after disturbance (fire, logging). *Ceanothus velutinus*, *Arctostaphylos patula*, *Ceanothus prostratus*, *Ribes cereum*, *Ericameria bloomeri* and *Wyethia mollis* dominate the shrub vegetation (USFS 2008).

Castilleja chromosa A. Nelson (desert paintbrush) is generally found in arid habitats, including southern California deserts, pinyon-juniper woodlands and sagebrush scrub. I measured *C. chromosa* within mid-montane sagebrush scrub. The big sagebrush shrubland alliance, characterized by ubiquitous *Artemisia tridentata*, blankets much of the basins and lower slopes on the eastern Sierra Nevada where soil is well-drained, sandy and deep (Millar 2015). At this site *Purshia tridentata* was also common.

Castilleja lemmonii A. Gray (Lemmon's paintbrush) is a small, bright pink perennial species found in moist meadows throughout the Sierra Nevada and Southern Cascades. It is commonly found alongside *C. peirsonii* (below), however, it does not form the dense fields that *C. peirsonii* does. I measured *C. lemmonii* in a wet meadow adjacent the Gem Lakes in the John Muir Wilderness. Although *C. peirsonii* was growing nearby, there were only a few *C. peirsonii* individuals within the site. The meadow was characterized by sedges, grasses and small herbaceous flowers such as *Erythranthe primuloides, Erythranthe tilingii, Primula tetrandra, Potentilla gracilis* and *Oreostemma alpigenum,* and bordered by *Salix orestera* and *Senecio scorzonella*.

Castilleja linariifolia Benth. (Wyoming paintbrush) is found in arid environments such as sagebrush scrub, dry plains and rocky slopes. I measured *C. linariifolia* on a subalpine rocky slope by Minaret Summit. Although not above treeline the site was characterized by rocky substrate, low moisture and low-growing plants, such as *Eriogonum umbellatum*, *Linanthus pungens*, and *Astragalus whitneyi*. Sagebrush (*Artemisia tridentata* ssp. *vaseyana*) was present here as well but was more sparse and smaller than the sagebrush scrub communities lower in elevation. The *C. linariifolia* here was also relatively small in stature.

Castilleja miniata Douglas ex Hook. (giant red paintbrush) is found in moist areas in a wide variety of habitats throughout the Sierra Nevada. I measured a patch of *C. miniata* in a riparian forest along Lee Vining Creek. The site was characterized by a multi-layered canopy including a *Populus tremuloides* overstory, *Salix exigua*, and various herbs, shrubs and grasses in the understory, including *Phleum pratense*, *Maianthemum stellatum*, *Rosa woodsii*, *Potentilla gracilis* and *Verbascum thapsus* (Constantine 1993).

Castilleja nana Eastw. (dwarf alpine paintbrush) is a perennial species found in high-alpine, dry, rocky environments. I measured *C. nana* in the White Mountains of California, which sit in the rainshadow of the Sierra Nevada. This, in combination with their high elevation (White Mountain peak is 4344 m), contributes to a harsh environment with large temperature fluctuations, high solar irradiance and low moisture. Although not above treeline, the site was characterized by typical alpine flora of mats and cushion plants, such as *Stenotus acaulis*, *Phlox condensata*, and *Eriogonum gracilipes* growing on dolomitic soil (Rundel, Gibson & Sharifi 2008). Sagebrush scrub and bristlecone pine forest could be found nearby.

Castilleja peirsonii Eastw. (Peirson's paintbrush) is a small perennial species, which often forms dense stands that blanket montane and alpine meadows. I measured *C. peirsonii* in a wet subalpine meadow along a small tributary stream of the Gem Lakes in the John Muir Wilderness. The meadow was characterized by various sedges, *Phyllodoce breweri, Vaccinium cespitosum, Kalmia microphylla,* and bordered by *Salix orestera* and *Rhododendron columbianum*. Although *C. lemmonii* often co-occurs with *C. peirsonii* there was only one *C. lemmonii* individual within the site.

FIELD MEASUREMENTS

Before making measurements I first visually assessed which non-parasitic species were most abundant and commonly found neighboring *Castilleja*. Then, starting at one end of a transect traversing each sampling location, I flagged individuals which intersected the transect until I reached six *Castilleja*, six neighboring individuals of the common non-parasitic species, and six non-neighboring individuals of that same species. In some cases a second transect was laid parallel to the first in order to intersect enough individuals. A "neighbor" was defined as the nearest neighbor for that individual *Castilleja* (in nearly all cases the canopies overlapped with the *Castilleja* individual). Non-neighboring individuals were at least a designated distance from any *Castilleja* individuals. The designated distance depended on size of the *Castilleja* species. For the largest species (e.g. *C. miniata* or *C. applegatei ssp. pinetorum*) non-neighbors were at least five meters from any *Castilleja* individuals; for the smallest species (i.e. *C. nana*) the designated distance was one meter. This resulted in 12 plots per site (six *Castilleja* + neighbor plots, and six non-neighbor plots) and 18 individuals per site (six *Castilleja*, six neighbors and six non-neighbors, where the latter two are the same non-parasite species within a site). Volumetric soil water content was recorded at each plot using a FieldScout TDR 150 soil moisture meter with 12 cm rods. I took three measurements in an equilateral triangle at the edge of the canopy of the individual *Castilleja* and/or non-parasite. Where the soil was too rocky or compacted, I attempted to measure in a different location along the canopy edge and if that was not possible took only one to two measurements per plot. I averaged the soil moisture measurements for each plot.

C. applegatei ssp. *pinetorum* was measured in summer of 2015 with a porometer (see below) and soil moisture meter but slightly different sampling procedure. As part of a larger project I established twelve 50 m transects with ten 1x1m quadrats each (120 plots total) at Sagehen Creek Field Station. I established quadrats every five meters along each transect, alternating sides until I reached five plots with *C. applegatei* and five plots without. In order to reach 10 quadrats some transects were extended past 50 m (but none were greater than 75 m). Volumetric soil water content was recorded at three regular locations in the quadrat, or where the soil was sufficiently soft and rock-free to allow measurement. Along eight of the transects (a subset of the original 12) I took daytime conductance measurements. I measured each individual *C. applegatei* as well as three common neighboring species (*A. tridentata*, *W. mollis* and *C. prostratus*) within each quadrat (resulting in measurements of the neighbor species both in quadrats with and without a *C. applegatei* individual present). Following the same protocol, I also measured nighttime conductance along four of the quadrats (all four of which were included in the daytime measurements). Quadrats varied in their abundance of *C. applegatei* and neighbor species, resulting in 60 *C. applegatei* individuals measured in the day and 20 at night, while 85 non-parasite individuals were measured during the day and 28 at night, split roughly evenly among the three species and between quadrats with and without *C. applegatei*.

Using a METER Environment (Pullman, WA, USA) SC-1 Leaf Porometer, I measured the stomatal conductance (g_s) of each individual during daytime and nighttime (except at Sagehen where some individuals were only measured in the day). At each time, I measured three leaves per individual, selecting mature fully exposed leaves, recording the stomatal conductance, air temperature and time. Daytime and nighttime measurements for each site were made within 48 hours of each other. I calibrated the porometers at least every day, and also in between daytime and nighttime measurements. I took all measurements on the abaxial surface of the leaf in Auto mode. If no conductance was detected for two minutes, I recorded a 0. All measurements in 2019 were made with the desiccant chamber in place. Prior to that I used an older METER SC-1 model, which did not include a desiccant chamber. Measurements ranged from 0-1252 mmol m^{-2} s^{-1}, with >99% within the SC-1's recommended range of 0-1000 mmol m^{-2} s^{-1}. 89% of measurements fell in the ideal range of the instrument, 0-500 mmol m^{-2} s^{-1}, where accuracy is ±10% of measurement. Above 500 mmol m^{-2} s^{-1} absolute accuracy is unverifiable. The SC-1 can still detect relative change above 500 mmol m^{-2} s^{-1} and can measure values up to 6000 mmol m^{-2} s^{-1}.

Nighttime was defined as past astronomical twilight. Past this time the sun does not contribute to illumination of the sky. I used the NOAA Solar Calculator to obtain apparent sunrise, sunset and solar noon for each field site (NOAA Earth System Research Laboratory 2019).

Digital leaf area analysis

In an ideal measurement the leaf will entirely cover the porometer aperture, however, many of the plants measured had leaves smaller than the porometer aperture (diameter: 6.35 mm). For leaves that did not fill the aperture, I made a typical measurement and subsequently scaled the measurement up, proportionate to the leaf area within the aperture. To do this, I collected any leaves that didn't cover the entire aperture after their porometer measurement. I marked each leaf to show where it entered the porometer sensor head, and taped it to white paper. I then scanned each page using a flatbed scanner with color-charged coupled device technology and 600 dpi resolution. I imported the resulting images into Adobe Illustrator and cropped any sections of the leaf outside of the porometer aperture, using a template with the porometer aperture measurements. I then digitally analyzed the leaf area within the aperture using the *LeafArea* package in R (Katabuchi 2015).

Statistics

I did all my data analysis and graphing in R (Wickman 2009; R Core Team 2020). I analyzed the data from Sagehen Experimental Forest (*C. applegatei* ssp. *pinetorum* and three associated non-parasitic species) separately because the differing collection protocols resulted in a different data structure. For the measurements from Sagehen Experimental Forest, I used a type III ANOVA (i.e. marginal sums of squares) on a linear mixed effects model fit by restricted maximum likelihood estimation (Pinheiro *et al*. 2017). I log transformed the data to correct for heteroscedasticity. I used a backward model selection process, initially including all fixed effects and their interaction terms, then used single-term deletions (F-tests) and AIC/BIC scores to compare models (Zuur *et al.* 2007). The final model had PlantType, Temperature, DayVsNight, and the interaction between DayNight and PlantType as fixed effects and Location (i.e. transect), plot, and individual plant as nested random effects to account for spatial autocorrelation as well as repeated measures (Table 2). Soil moisture and all other interactions were dropped from the

model. PlantType is a factor combining species and presence/absence of a parasite (+/- P), resulting in seven levels (1: *A. tridentata - P*, 2: *A. tridentata* + P, 3: *W. mollis - P*, 4: *W. mollis* + P, 5: *C. prostratus - P*, 6: *C. prostratus* + P, and 7: *C. applegatei* ssp. *pinetorum* [P]). I then conducted pairwise comparisons on estimated marginal means using a Tukey adjustment for multiple comparisons of means (Lenth 2019a) (Table A2-1).

I analyzed the other seven species together. I again used a type III ANOVA on a linear mixed effects model, with the data log transformed. Using the same process of model selection as above I arrived at a model with DayVsNight, PlantType, Site and all interactions as fixed effects and Plot and Individual Plant as nested random effects to deal with spatial autocorrelation and repeated measures (Table 3). Soil moisture was not included in the model because of missing data from two sites. PlantType is a factor with three levels: 1) Parasite (P), 2) Non-Parasite - P, and 3) Non-Parasite + P. Each Site represents a different location and combination of species, identified in the results by the species of *Castilleja* at the site. I then conducted planned contrasts on estimated marginal means to compare the effects of DayVsNight and PlantType within each site, again using a Tukey adjustment (Tables 4, A2-2).

Results

All the plants measured here displayed some nighttime stomatal conductance (g_{night}). In general, g_{night} in parasitic plants was similar to or exceeded daytime stomatal conductance (g_{day}) while g_{night} in non-parasitic plants was generally lower than or similar to g_{day} (Figs 1-2).

In four of the eight species of parasite (*C. applegatei pallida*, *C. chromosa*, *C. linariifolia* and *C. nana*) g_{night} was significantly higher than g_{night} in the neighbor groups (i.e. non-parasite with or without a parasite neighbor). In seven of the eight parasitic species g_{night} was significantly higher than g_{night} in at least one group of neighbors. Only *C. miniata* was indistinguishable from its neighbors. On average the parasites' g_{night} was 235% higher than the non-parasites' and ranged from 24% lower in *C. miniata* to 877% higher in *C. chromosa*.

Parasitic g_{day} was more mixed. On average parasites' g_{day} was 104% higher than the non-parasites' g_{day}. But the parasites' g_{day} significantly exceeded both groups of neighbors' g_{day} in only half of the species (*C. chromosa*, *C. lemmonii*, *C. linariifolia*, and *C. miniata*), while in the other four species the parasites' g_{day} was in line with its neighbor's g_{day}.

There was little difference between the two groups of non-parasites: those neighboring a parasite (NP + P) and those without a parasite neighbor (NP - P). There were no significant differences between these groups' g_{day} at any of the sites. Only *Carex spectabilis* and *Wyethia mollis* (at sites with *C. lemmonii* and *C. applegatei pinetorum* respectively) had significantly increased g_{night} in the present of a parasite.

Neither temperature nor soil moisture appeared to play a large role in the g_s (Figs 3-4). Soil moisture was not significant and dropped from the analysis of *C. applegatei pinetorum* (Table 3). Temperature was significant in the analysis of *C. applegatei pinetorum*, but was dropped from the analysis with the other seven species.

Discussion

Although nighttime stomatal opening and consequent nighttime transpiration is increasingly recognized as common, in most plants g_{night} is typically relatively low, in the range

of 0-150 mmol H_2O m^{-2} s^{-1} (but see Donovan et al. 1999), especially compared to g_{day} for the same species (Caird *et al*. 2007; Dawson *et al*. 2007). Here g_{night} of the non-parasitic species were somewhat elevated compared to previous measurements, with means ranging from 63 to 497 mmol H_2O m^{-2} s^{-1}, but not out of the range of previous observations. The parasites, however, represented a significant departure from this pattern, with both high g_{night} relative to g_{day} and high g_{night} overall, frequently in excess of 500 mmol H_2O m^{-2} s^{-1}. This is consistent with previous observations that g_{day} in both root and stem parasites typically exceeds their non-parasitic hosts g_{day} by several fold, and is commonly above 500 mmol H_2O m^{-2} s^{-1}, while further extending this pattern into the dark (Ehleringer & Marshall 1995; Scalon & Wright 2017).

It should be noted that g_s is not directly equal to transpiration (E). Rather E is a function of g_s as well as the vapor pressure deficit (VPD) between the leaf and air, canopy structure and atmospheric mixing. VPD is in turn controlled by temperature and relative humidity and is therefore typically lower at night. Consequently given the same g_s, actual transpirational water loss will typically also be lower at night than during the day (Caird *et al*. 2007). The lower temperatures and higher relative humidity observed at night here support this general pattern.

In parasitic plants the ability to gain C, regardless of sunlight, fundamentally alters the C–water tradeoffs experienced by non-parasitic plants. The elevated g_{night} observed here in *Castilleja* supports the theory that this altered tradeoff in turn drives nighttime transpiration because it facilitates the acquisition water and dilute C from the host xylem stream. Similarly, and not mutually exclusive, acquisition of N or other nutrients may also drive nighttime transpiration. Nitrogen limitation in hemiparasites is thought to drive high transpiration rates, but importantly this process is not sunlight dependent (Schulze & Ehleringer 1984; Scalon & Wright 2015). Although phloem-tapping holoparasites' access to C and N is also detached from sunlight, they would not face the low nutrient concentrations in the xylem that are thought to partially drive high transpiration. Non-parasitic plants also do not rely on sunlight for nutrient acquisition (with the partial exception of N-fixing species). This is, in part, why nutrient limitation has been proposed as a driver of nighttime transpiration in non-parasitic plants as well (Zeppel *et al*. 2014). Teasing out the effects of C and N limitation would illuminate the ultimate drivers of nighttime transpiration in both parasites and non-parasites.

It is possible that nighttime transpiration is the result of a release of evolutionary pressure on certain aspects of physiology. For example, the negative impact of transpirational water loss via leaky stomata is likely muted in parasitic plants because water supply is buffered and/or enhanced by the host association. However, observations of nighttime transpiration in *Rhinanthus*, a genus of root hemiparasites also in the Orobanchaceae family, showed that *Rhinanthus* individuals display typical stomatal regulation when unattached to a host and then keep their stomata open continuously when attached to a host (Jiang *et al*. 2003). Given that, elevated g_{night} is more likely regulated within individual plants and a direct result of their attachment to hosts.

Parasitic plants are thought to generally be profligate water users. The high g_s in both the day and night supports this while adding the additional wrinkle that high water use occurs at all hours. The resulting transpirational water loss from high g_{night} is likely muted compared to high g_{day}, but potentially still has significant impacts on the surrounding community. If stomata always remain open, overall transpirational water loss via a parasite is of course higher, possibly exacerbating host water stress. In addition, nighttime transpiration could impact host hydraulic lift. In previous experiments with *Artemisia tridentata*, one of the neighbor species here and a common host for *Castilleja*, nighttime transpiration (induced by shining lights 24 hours a day)

decreased hydraulic redistribution and lowered daytime transpiration rates in subsequent days (Caldwell & Richards 1989; Howard *et al*. 2009). Nighttime transpiration via a parasite could induce the same reaction wherein the groundwater, which normally would be redistributed to upper soil layers and available to *Artemisia* and other plants the next day, would instead be lost to the atmosphere via transpiration by the parasite. The magnitude of impact would depend in part on the leaf area of the parasite relative to a host.

Perhaps counterintuitively, in some cases it appears that higher g_{night} may actually accompany lower overall water use. In *C. applegatei pinetorum,* for example, g_{day} is indistinguishable from the other species but g_{night} is slightly elevated, raising the possibility that *C. applegatei pinetorum* is only actively parasitizing at night, leading to overall less transpirational water loss. At night when a host typically has low g_s, the parasite can achieve a favorable water potential gradient by only slightly exceeding the host g_s, and dropping its leaf water potential below that of the neighbor. However, other species do not display this pattern. In *C. chromosa* for example both g_{night} and g_{night} generously exceed the neighbors' g_s.

It is worth noting that the ecosystem water availability does not appear to play a large role. *C. peirsonii* and *C. lemmonii* were measured in wet meadows, while most of the other species were measured in more arid environs, such as *C. chromosa* in sagebrush scrub where the soil volumetric water content was frequently below the detection limit of the soil moisture probe. Yet qualitatively the diurnal g_s patterns do not appear to differ based on soil moisture. Furthermore, *C. miniata*, the only *Castilleja* species indistinguishable from it's neighbor, is also more distantly related than the other species (Tank & Olmstead 2008). This may tentatively suggest that phylogenetic relatedness rather than other aspects of physiology or ecology drive similarities in g_s.

Conclusion

Data presented here show that *Castilleja* commonly transpires at night, adding this group of root hemiparasites to the growing group of plants understood to open their stomata at night. It is clear though that *Castilleja* remain separated from typical plants by the high magnitude of their stomatal conductance. It remains an open question, however, whether this is a difference of degree or kind. Future work should seek to further understand the underlying mechanisms driving nighttime transpiration and whether the pattern extends to other families and types of parasites.

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Tables

Table 1 Study Sites

Site	*Castilleja* species	Neighbor species	Habitat type	Location (latitude, longitude)	Altitude (m)
1	*C. applegatei* ssp. *pallida*	*Symphoricarpos rotundifolius* A. Gray	High elevation sagebrush scrub	37.65493, -119.06082	2803
2	*C. applegatei* ssp. *pinetorum*	*Ceanothus prostratus* Benth. *Wyethia mollis* A. Gray *Artemisia tridentata* Nutt.	Mixed-conifer and shrubland	39.43343, -120.24721	1958
3	*C. chromosa*	*Artemisia tridentata* Nutt ssp. *tridentata*	Mid-montane sagebrush scrub	37.86321, -119.12807	2284
4	*C. lemmonii*	*Carex spectabilis* Dewey	Wet sub-alpine meadow	37.38967, -118.75757	3335
5	*C. linariifolia*	*Artemisia tridentata* ssp. *vaseyana* (Rydb.) Beetle	Sub-alpine rocky slope	37.65382, -119.06113	2800
6	*C. miniata*	*Phleum pratense* L.	Riparian forest	37.92929, -119.15144	2221
7	*C. nana*	*Stenotus acaulis* (Nutt.) Nutt	Alpine plateau	37.39636, -118.17847	3133
8	*C. peirsonii*	*Vaccinium cespitosum* Michx.	Wet sub-alpine meadow	37.38995, -118.75712	3335

Table 2 Results of a Type III ANOVA of a linear mixed effects model on stomatal conductance measurements of the root hemiparasite *C. applegatei pinetorum* and three associated non-parasitic species, *A. tridentata*, *C. prostratus*, and *W. mollis* in Sagehen Experimental Forest, 2015.

Parameter	df (num/den)	F-value	P-value
Day vs. Night	1 / 118	9.99	**0.0020**
Plant Type	6 / 118	1.37	0.2339
Temperature	1 / 332	11.76	**0.0007**
Day vs. Night * Plant Type	6 / 118	19.29	**<0.0001**

Table 3 Results of a Type III ANOVA based on a linear mixed effects model on stomatal conductance measurements of seven species of *Castilleja*, a genus of root hemiparasites, and associated non-parasitic plants.

Parameter	df (num/den)	F-value	P-value
Day vs. Night	1 / 556	1.36	0.2442
Plant Type	2 / 28.	8.45	**0.0013**
Site	6 / 77.	41.10	**<0.001**
Day vs. Night * Plant Type	2 / 556	9.10	**0.0001**
Day vs. Night * Site	6 / 556	18.12	**<0.0001**
Plant Type * Site	12 / 28.	8.26	**<0.0001**
Day vs. Night * Plant Type * Site	12 / 556	7.38	**<0.0001**

Table 4 Results of planned contrasts (Plant Type within the two levels of Day vs. Night) of estimated marginal means based on a linear mixed effects model on stomatal seven species of Castilleja, a genus of root hemiparasites, and associated non-parasitic plants. Results are averaged over levels of Site. NP - P indicates a non-parasite with no neighboring Castilleja individuals, NP + P indicates a non-parasitic species with a neighboring Castilleja individual(s), and P indicates a parasite of the genus Castilleja. P-values use Tukey adjustment for multiple comparisons.

Day vs. Night	Contrast	Ratio	SE	t-ratio	P-value
Day	NP-P — NP+P	1.079	0.071	1.157	0.488
	NP-P — P	0.518	0.034	-10.026	**<0.0001**
	NP+P — P	0.480	0.030	-11.735	**<0.0001**
Night	NP-P — NP+P	0.800	0.058	-3.075	**0.013**
	NP-P — P	0.367	0.026	-13.992	**<0.0001**
	NP+P — P	0.458	0.031	-11.378	**<0.0001**

Figures

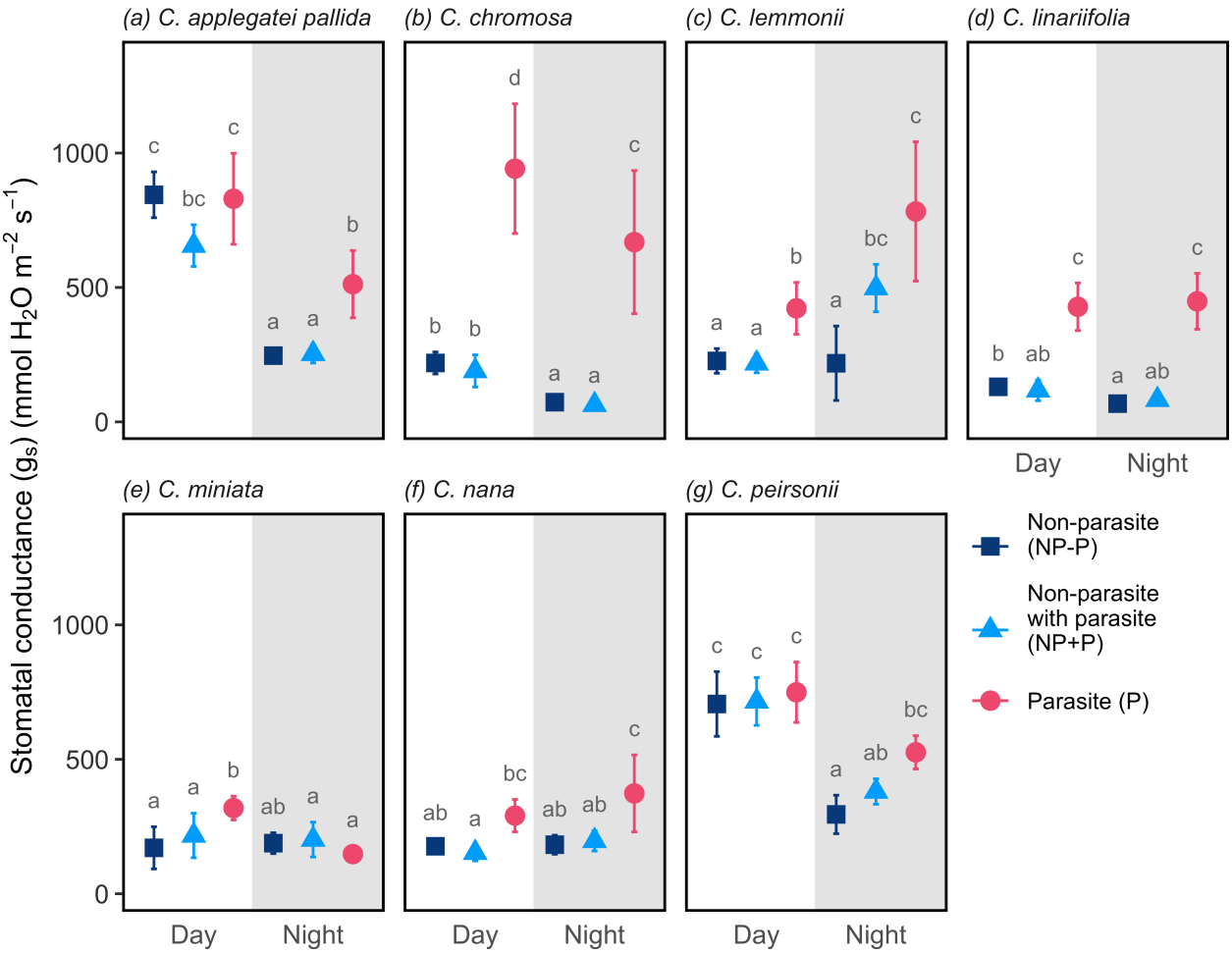

Figure 1 Nighttime and daytime stomatal conductance among parasites and associated plants for seven different species of root hemiparasites in the genus *Castilleja*. Each panel represents a different site with a unique parasite and non-parasite pair. The *Castilleja* species is shown at the top of each panel. Error bars are 95% confidence intervals. Letters represent significant differences (alpha = .05) from post-hoc Tukey adjusted pairwise comparisons within each site. Pairwise comparisons were run on a mixed effects model including all the sites. See Table 3, 4 and A2-2 for statistics

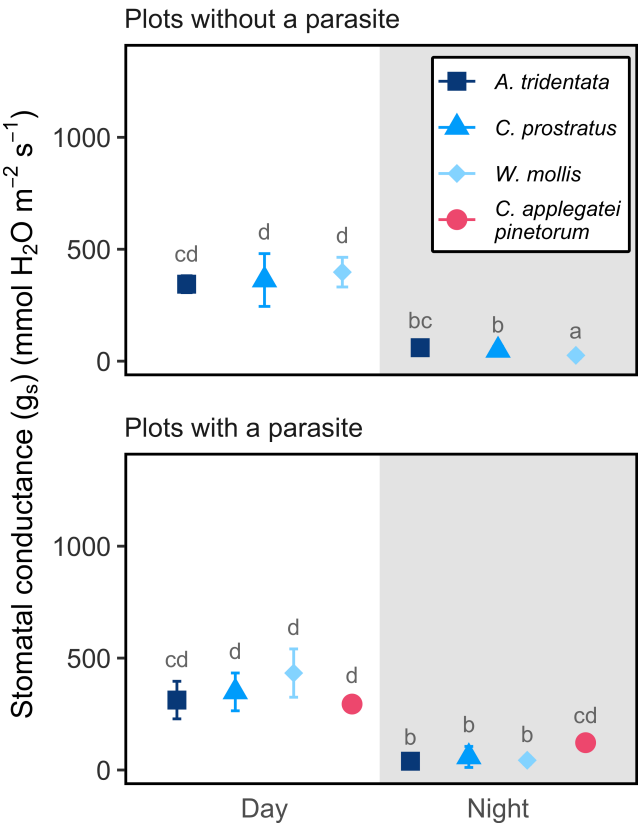

Figure 2 Nighttime and daytime stomatal conductance among the root hemiparasite, *C. applegatei pinetorum* and three associated plants in Sagehen Experimental Forest. The top panel represents non-parasitic plants with no parasitic neighbors, while the bottom represents parasitic individuals and neighboring non-parasitic individuals. Error bars are 95% confidence intervals. Letters represent significant differences (alpha = .05) from post-hoc Tukey adjusted pairwise comparisons. Pairwise comparisons were run on a mixed effects model. See Table 2 and A2-1 for statistics

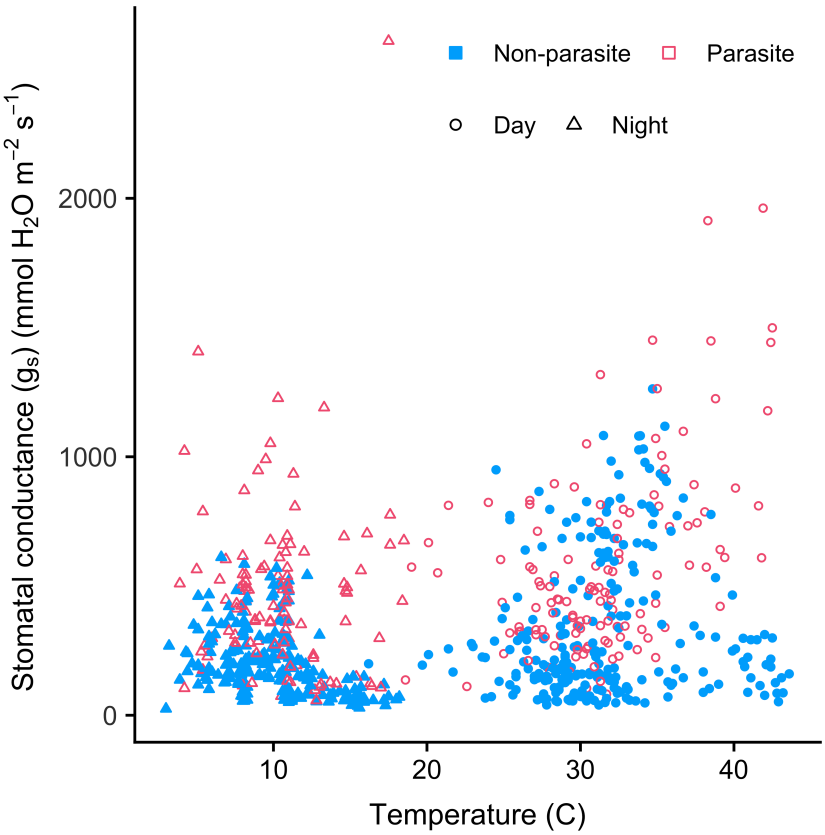

Figure 3 Air temperature at the time of measurement compared to stomatal conductance. Filled symbols represent *Castilleja* species (root hemiparasites) and open symbols represent non-parasitic species

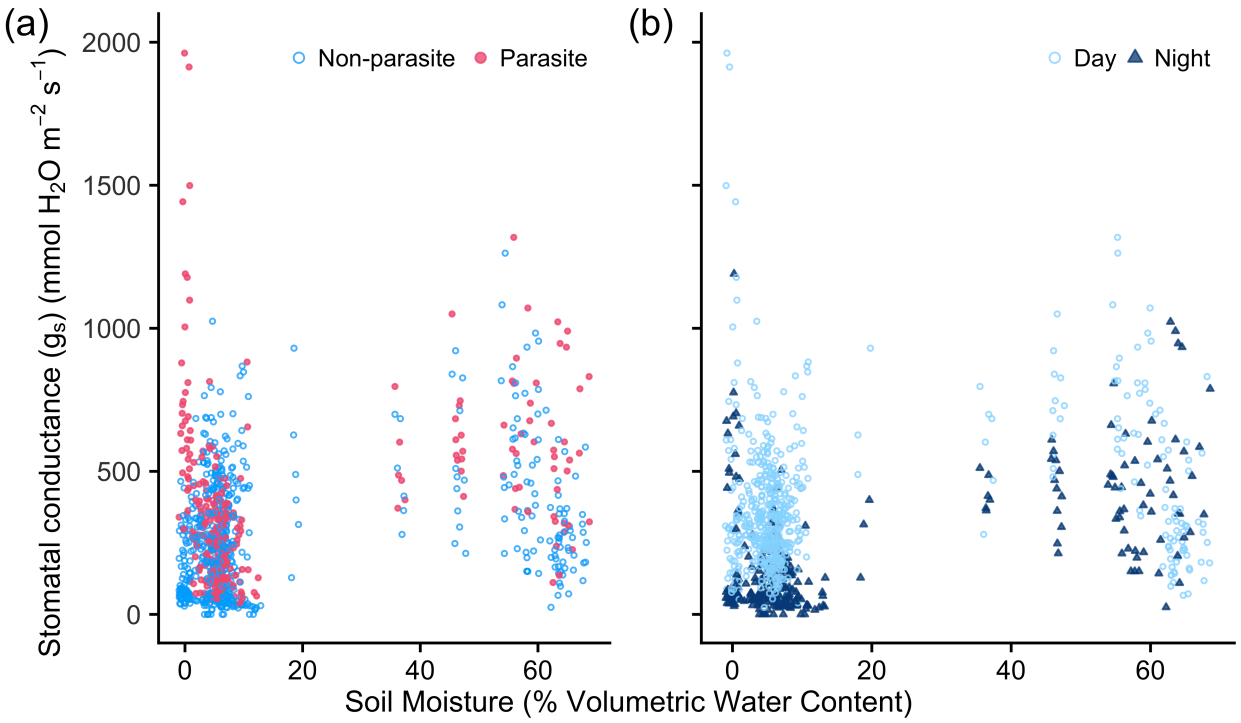

Figure 4 Plot level soil moisture compared to stomatal conductance by parasite status and time of day (i.e. daytime versus nighttime). Measurements are shown for five of the total sites: (listed by the *Castilleja* species present) *C. applegatei pinetorum*, *C. chromosa*, *C. lemmonii*, *C. miniata*, and *C. peirsonii*

Chapter 3: Where do parasitic plants fit on the leaf economic spectrum?

Abstract

The leaf economic spectrum (LES) quantifies correlations between key leaf traits across vascular plants, and distills much of the variation in these traits to a single axis. The LES is, in part, driven by physiological tradeoffs in the acquisition of carbon (C). Here I ask how one functional group, parasitic plants, fit into the LES. Heterotrophy in parasitic plants supplants some of the essential functions of leaves and in theory could lead to departures from the LES. Using global leaf trait data from the TRY database I compare the LES suite of leaf traits in parasitic plants to their non-parasitic counterparts, and additionally look at leaf traits within parasitic types. Despite their unique physiology, parasitic plants do not deviate dramatically from the LES, although there are examples of differences in position on the LES and relationships among traits. Further research on the physiology of parasitic plants will improve our understanding of patterns in resource acquisition and utilization.

Introduction

Tradeoffs in the evolution of leaf traits have long been known to be ecologically significant and provide a useful framework for understanding species' ecological strategies (Reich, Walters & Ellsworth 1992; Westoby *et al*. 2002). In 2004, Wright *et al*. formally described a suite of co-varying leaf traits, representing key chemical, structural and physiological properties of leaves, which collectively form the global leaf economic spectrum (LES) (Table 1). The LES, remarkable in its universality, embodies differing plant strategies for investment of resources while maximizing photosynthetic return (analogous to an economic return on investment [ROI]). At one end of the spectrum are 'fast-return' leaves, which have high photosynthetic rates (per second or day) and are cheaper resource-wise but tend to have shorter life spans. These fast-return leaves also tend to have high nutrient concentrations, low leaf mass per area, and high respiration rates. On the opposite end are 'slow-return' leaves, which are more expensive resource-wise and have lower short-term yields but longer lives.

Importantly, the LES is thought to reflect not just ecological strategies but also underlying physiological constraints and evolutionary tradeoffs (Reich 2001; Shipley *et al*. 2006; Onoda *et al*. 2017). Although originally framed in reference to just two crucial resources, carbon (C) and nutrients (especially nitrogen), the LES traits also co-vary with traits related to water acquisition (Reich 2014). Plants tend to have a uniformly fast, medium or slow strategy with respect to all three resources and across the entire plant body (i.e. beyond their leaves) (Reich 2014).

The LES framework has proved useful in exploring and predicting ecological processes: leaf traits correlate with plant productivity (Shipley *et al*. 2005), litter decomposition (Kazakou *et al*. 2006; Santiago 2007; de la Riva, Prieto & Villar 2019), mycorrhizal associations (Shi *et al*. 2020), herbivory and plant defense (Kempel *et al*. 2011; Armani *et al*. 2020), and ecosystem structure and function (Díaz *et al*. 2004; Musavi *et al*. 2015). Climate, soil conditions, phylogeny, and plant growth form all affect where on the spectrum plants reside, but relationships between traits are relatively stable (Ackerly & Reich 1999; Wright *et al*. 2005b; a; Cornwell & Ackerly 2009; Ordoñez *et al*. 2009).

Several works have explored how the LES performs at local scales (Wright & Sutton-Grier 2012; Wigley *et al*. 2016; Messier *et al*. 2017) and within functional groups (Wright *et al*. 2005a; Shiklomanov *et al*. 2020), however, none have explored the LES across the functional group comprised of parasitic plants. Parasitic plants make up ~1% of angiosperms and are found in nearly all ecosystems (Westwood *et al*. 2010), but have not been explored in the context of the LES.

Parasitic plants are of particular interest because their unique physiology alters some of physiological constraints driving the LES tradeoffs. Defined functionally by the presence of haustoria, a specialized organ that penetrates the tissue of other plants, parasitic plants can acquire C, water, and/or nutrients from their hosts. Parasites are diverse in form, although a basic distinction can be drawn between those that infect hosts' roots versus stems. Further, holoparasites are entirely heterotrophic, acquiring C from their host's phloem without photosynthesizing themselves. Hemiparasites are capable of photosynthesis but are not strict autotrophs, acquiring dilute C and nutrients along with the water from their host's xylem stream (Ehleringer *et al*. 1985; Těšitel, Plavcová & Cameron 2010). Although the mechanisms and efficiency of uptake vary across species and types of parasitic plants, they all have alternative sources of C, water, and nutrients not available to typical plants, either via their hosts' xylem,

phloem or both. Maximizing C gain, while minimizing water loss is a major leaf function, which underlies many of the LES tradeoffs. However, these alternative sources of C, water and nutrients complicate the role of leaves in parasitic plants and could lead to departures from the LES.

There are three broad though not mutually exclusive options for how parasitic plants, as a group, would fit into or deviate from the LES (Fig 1). The decoupling of photosynthesis from other traits could lead to novel trait combinations, such as low photosynthetic assimilation rates (A_{mass}) and low leaf mass per area (LMA). Related, but not mutually exclusive, this decoupling could also lead to a change in the correlations between traits, such as less tight correlation between A_{mass} and leaf Nitrogen (N). Lastly parasitic plants could occupy a subset of the LES, rather than span the entirety.

Research on parasitic plant physiology points to several examples of possible deviations. For example, many parasites, particularly mistletoes, are known to have unusually high N and other minerals in their leaf tissue (Glatzel 1983; Lamont 1983; Pate, True & Kuo. J. 1991; Tennakoon & Pate 1996; Quested *et al*. 2002; Tennakoon *et al*. 2014). Globally the LES shows strong positive relationships between leaf N, leaf phosphorus (P) and photosynthetic capacity (Wright *et al*. 2004), in part because N is a key component of RuBisCo (Chapin *et al.* 1987; Evans 1989). But would we expect the observed high leaf N and P to correspond to high photosynthetic capacity in parasites when they have an alternative source of C?

The departure of parasitic plants from the LES is readily apparent in holoparasites, which do not photosynthesize at all. Necessarily, photosynthetic capacity traits (A_{mass} and A_{area}) would be zero in these species. Perhaps more obviously, many holoparasites also lack leaves entirely (e.g. *Rafflesia* which, lacking leaves, stems and roots, are composed of just a flower and haustoria) or have highly reduced leaves (e.g. *Cuscuta*, of which the vegetative portion is largely stems).

Approximately 90% of parasitic plants, however, are hemiparasites (Heide-Jørgensen 2008), which conform more to typical plant physiology (although many lack root systems). Able to photosynthesize, hemiparasites typically only access the host xylem (Irving & Cameron 2009). It is unclear to what extent, if at all, heterotrophy in hemiparasites will result in deviations from the LES, or if other fundamental tradeoffs will maintain the LES relationships. For example, the LES shows a strong positive relationship between leaf lifespan (LL) and LMA, in part because a higher LMA corresponds to increased durability, protecting against wear and tear and herbivory, which allows for a longer lifespan (Coley 1983; Westoby *et al*. 2002). The LL-LMA relationship is driven by structural tradeoffs, which should operate largely independently of an autotrophy-heterotrophy axis.

Understanding leaf traits in parasitic plants is of particular interest because their unique physiology is thought to underlie their ecological interactions, particularly ecological interactions beyond negative impacts on their hosts (Smith 2000; Phoenix & Press 2005). However, it's not clear how generalizable these traits and therefore these ecological interactions are. For example, some parasitic plants have been shown to accelerate rates of decomposition and alter the biomass and nutrient status of co-occurring plants through the deposition of N-rich litter (Quested *et al.* 2003; Spasojevic & Suding 2011; Fisher *et al.* 2013; Demey *et al.* 2014). Understanding the prevalence of high leaf N in parasitic plants would aid in estimating the importance of parasitic plant litter in ecosystems more broadly. To this end, I also include leaf $\delta^{13}C$ and leaf $\delta^{15}N$ in the leaf traits, although these are not strictly speaking LES traits, because stable isotopes have commonly been used to elucidate species interactions in parasitic plants (Dawson *et al*. 2002).

Here, I explore parasitic plant leaf traits in the LES framework, asking the following questions: What is the scope of data that exist on LES leaf traits within parasitic plants? Do leaf trait relationships in parasites depart from global leaf trait relationships? What position do parasitic plants occupy on the LES? Does parasitic type (stem vs. root and holo- vs. hemi-) affect these relationships?

Methods

Data acquisition and cleaning

I focused on the suite of traits originally described in the LES (Wright *et al*. 2004): 1) leaf mass per area (LMA), 2) leaf N on a mass and area basis (N_{mass} and N_{area}), 3) leaf P on a mass and area basis (P_{mass} and P_{area}), 4) leaf lifespan (LL), 5) photosynthetic assimilation rates on a mass and area basis (A_{mass} and A_{area}), and 6) dark respiration rate on a mass and area basis (R_{mass} and R_{area}). In addition, I looked at leaf $\delta^{13}C$ and leaf $\delta^{15}N$ (Table 1). I extracted pulled these traits, along with species characteristics (more on these below) and site data from the TRY Database version 5 (Kattge *et al*. 2020). Site data included location, mean annual temperature (MAT), mean annual rainfall (MAR), and Koeppen-Geiger climate classification. First initiated in 2007, the TRY Database integrates plant trait data from author-contributed plant trait datasets into a consistent format, including standardizing taxonomy, trait units and trait names. Datasets include both published and unpublished work. Trait names within the TRY Database conform to the standards set in the Thesaurus of Plant Characteristics (TOP) (Garnier *et al*. 2017). Managed and curated by Jens Kattge and Gerhard Boenisch at the Max Planck Institute for Biogeochemistry, the database is periodically updated to include more datasets. TRY version 5 includes over 400 datasets, 2100 traits, 160000 plant taxa, and 11 million trait records (www.try-db.org). The database includes both public datasets and restricted datasets, which require author approval to access. I requested data from all available species for the traits listed above. For restricted datasets, which included these traits, I reviewed the species in each dataset to see if parasitic plants were included. If so, I requested access to the dataset. In all cases, requests were approved.

The TRY Database also includes several species characteristics, including growth form and photosynthetic pathway, but many species are missing data. For non-parasitic species I restricted my analysis to individuals with complete records for plant growth form. For the parasitic species, I added growth form data where it was missing, relying primarily on local floras, the Encyclopedia of Life (Parr *et al*. 2014) and the PLANTS database (USDA & NRCS 2020). For photosynthetic pathway, I designated all parasitic species as C_3. The C_4 photosynthetic pathway occurs in 19 families (although it has evolved independently ~66 times), none of which include parasitic plants (Westwood *et al*. 2010; Sage 2016).

The TRY species characteristics data include parasite status but many known parasitic species are not categorized as such and instead have blanks in that column. Instead, I compiled a list of parasitic genera primarily from the Parasitic Plant Connection (Nickrent 2018), cross-referencing the plant names on TROPICOS (Missouri Botanical Garden 2020) and The Plant List ("The Plant List" 2013). The parasitic plants are further designated as obligate or facultative, holoparasites or hemiparasites, and by haustoria attachment position (at the stem versus root). This list of parasitic genera, including associated characteristics is available via Figshare (Haynes 2020).

LMA can be measured several ways, depending on the inclusion of the petiole and other leaf parts. Here I use LMA measurements that include the petiole. However, to increase sample

size within parasites I extrapolated LMA-with-petiole values from LMA-without-petiole values. In cases of parasitic species for which both measurements have been made, LMA-with-petiole and LMA-without-petiole were highly correlated (Pearson's $r = 0.988$, $p<0.001$). Using the resulting linear regression model ($y=1.055x+0.036$ on the log transformed data), I translated the rest of the parasitic LMA-without-petiole measurements to LMA-with-petiole. For non-parasitic plants I only included LMA-with-petiole measurements.

I excluded all duplicate entries and outliers. Outliers were detected in the non-parasitic plants based on the reported error risk (termed "z-score") within the TRY database. Observations with a z-score > 4 may indicate problems with the data and were excluded (e.g. Kattge *et al*. 2011). I kept all parasitic entries regardless of z-score. This is because a priori I expected that the parasitic species may deviate from other species and for species without multiple entries the score is calculated at higher taxonomic levels (e.g. genus, family or the mean of all data). Since the parasitic plants are somewhat rare in the TRY database, we would expect the z-score to be less precise and a less effective indicator of whether deviation from the mean is real or a measurement error.

'Possibly parasitic' plants were also excluded from all statistical analysis for two reasons: first, they typically had small numbers of observations and were not represented among all plant growth forms and second, the designation of 'possibly parasitic' reflects a lack of knowledge about the plants, not necessarily a biological difference that we would expect to see reflected in leaf traits.

I averaged multiple measurements made on a single individual. Following previous work, I log-transformed the trait values (not including isotope measurements) to account for right skewness (Wright *et al*. 2004; Onoda *et al*. 2017; Shiklomanov *et al*. 2020). Datasets were stored in a PostgresSQL database and imported into R, where all data cleaning and analysis was conducted (R Core Team 2020).

COMPARISONS OF INDIVIDUAL LEAF TRAITS BY PARASITIC STATUS

To understand what position parasitic plants occupy in the leaf economic spectrum, I compared parasites and non-parasites, and root and stem parasites for each leaf trait. Plant growth form has a large bearing on leaf traits; however, parasitic plants are only found in certain growth forms (herbs, shrubs, trees). To address this, I restricted the dataset to herbs, shrubs and trees (excluding ferns, graminoids, lichens, and mosses), and compared leaf traits within each plant growth form. Similarly, I restricted comparison of $\delta^{13}C$ to just plants with the C_3 photosynthetic pathway. Using the *nlme* package, I constructed a linear mixed effects model for each leaf trait with plant growth form and parasite status as fixed effects and species as a random effect (Pinheiro *et al*. 2017). I then ran a type III ANOVA with 'sum to zero' contrasts to deal with unbalanced sampling (package: *car*) (Fox & Weisberg 2019). For N_{area} and LL there were not enough observations within each growth form, so comparisons for these two traits excluded trees and excluded trees and shrubs respectively. Using the *emmeans* package, I conducted pairwise comparisons of means comparing the effect of parasite-status within each growth form using a Tukey adjustment (Lenth 2019b).

I also aimed to compare leaf traits among different parasite types. However, the dataset only included two genera of holoparasites, thus I did not make any comparisons between holoparasites and hemiparasites, and only compared parasites by haustoria position. I again built a linear mixed effects model for each leaf trait with position (stem versus root) as a fixed effect

and species as a random effect, and then ran a type III Anova with 'sum to zero' contrasts. A_{area}, A_{mass}, LL, R_{mass}, N_{area}, and $\delta^{15}N$ were excluded because they had too few observations.

COMPARISONS OF PAIRWISE TRAIT RELATIONSHIPS

To address whether parasitic plants depart from patterns of trait covariance I both compared correlation coefficients and fitted the pairwise relationships using a standardized major axis. I was unable to run a multi-trait analysis because there were no complete cases of parasitic plants with the LES traits. Successively dropping traits did not produce more than 20 observations until there were only three traits included. Therefore, I opted for pairwise comparisons.

I compared the strength of correlation coefficients among leaf traits between parasites and non-parasites, using both Fisher's z and Zou's confidence intervals (package: *cocor*) (Fisher 1925; Zou 2007; Diedenhofen & Musch 2015). I accounted for the multiple comparisons of correlation coefficients by using the Dunn-Šidák correction (Šidák 1967; Quinn & Keough 2002). Given 36 pairwise trait comparisons, $\alpha = 0.05$ and a 95% confidence interval, the Dunn-Šidák correction yields an adjusted α of 0.00142, and an adjusted confidence interval of 99.86%.

I also used a standardized major axis to further compare pairwise trait relationships between parasites and non-parasites (package: *smatr*) (Warton *et al*. 2012). I first tested for whether the two groups shared a common slope. If the two groups did share a common slope (i.e. $p > \alpha$) then I also tested for differences in elevation and shifts along the common major axis. Again, to account for multiple comparisons I used the adjusted α of 0.00142.

Results

In the TRY database, the total number of species and observations varied among leaf traits, with LMA having the most in both categories and R_{mass} having the least (Table 2). The vast majority of parasitic plants with leaf traits were hemiparasites (only two genera of holoparasites were represented), while the split between root and stem parasites was more even (Table 3). There were 11 families represented and 53 genera (including 1 possibly parasitic genus: *Heisteria*). Parasitic species were sampled on all continents except Antarctica from a wide array of climates (Fig 2, Fig 3).

Within a plant growth type, parasitic plants did not generally differ dramatically from their non-parasitic counterparts. Parasitic status or the interaction between parasitic status and plant growth form had a significant impact on several leaf traits, including A_{mass}, LL, LMA, N_{area}, N_{mass}, R_{area}, R_{mass} and $\delta^{15}N$ (Table 4, Fig 4, 5). Posthoc comparisons only showed significant differences between parasites and non-parasites within shrubs and in only three traits: A_{mass}, LMA, and N_{area}. Parasitic shrubs had significantly higher LMA and N_{area} and significantly lower A_{mass} than their non-parasitic shrub counterparts (Table 5, Fig 4).

Just four leaf traits (LMA, P_{mass}, N_{mass}, $\delta^{13}C$) had enough observations to compare between stem and root parasites. Of these, LMA was significantly higher in stem parasites than root parasites ($P < 0.001$) (Table 6, Fig 6). No comparisons could be made between hemiparasites and holoparasites. Correlation coefficients between leaf traits differed significantly between parasite and non-parasites in only one of the pairwise trait comparisons: P_{area} and N_{mass} had a Pearson's r of -0.96 in parasites and 0.07 in non-parasites (Table 7). Similarly just one pairwise relationship differed significantly in slope: the slope of the major axis between LMA and N_{area} was significantly steeper in non-parasites than parasites (Table 8, Fig 7-

11). Eight of the 36 pairwise trait relationships had significantly different elevations in the major axis between parasites and non-parasites and 12 had significant shifts along the major axis. Each trait had at least one relationship with a significant difference in slope, elevation or shift along the axis, but LMA had the most by far, with 11 significant differences (next closest was R_{area} with seven) (Table 8, Fig 7-11).

Discussion

HOW DO PARASITIC PLANTS FIT INTO THE LEAF ECONOMIC SPECTRUM?

For the most part, parasitic plants appear to operate within the bounds of the LES, insomuch as they do not have novel trait combinations (i.e. Fig 1 A). None of the pairwise trait relationships showed parasitic plants outside of the trait-space occupied by non-parasitic plants (Figs 7-9).

There is little evidence that parasitic plants may have different pairwise leaf trait relationships than non-parasitic plants (i.e. Fig 1 B). None of the pairwise leaf trait relationships had significantly different correlation coefficients between parasitic plants and non-parasitic plants. However, these comparisons generally had small sample sizes and thus must be interpreted with caution. Further, there were not enough parasitic observations to look at multiple traits at once.

There is some evidence that parasitism affects position on the LES (i.e. Fig 1 C) because there are 12 instances where parasitism was associated with a shift along the major axis (Table 8). In some of the pairwise trait relationships, particularly where there are the most data points, parasitic plants clearly occupy the fast-return subset of the LES. For example, parasitic plants have a low LMA and high N_{mass} (Table 8, Fig 9 D), and high P_{mass} and N_{mass} (Fig 10 F), consistent with the fast-return strategy. However, this is not uniformly the case: in the A_{mass}-LMA relationship, parasites fell on the slow-return end of the spectrum, for example (Table 8, Fig 8 B). Of course overall strategy is affected by plant growth form, with herbs more likely to have fast-return strategies (e.g. Fig 12) (Wright *et al*. 2005a). Within parasitic plants 85.5%, 9.5% and 5% of observations for all traits were from herbs, shrubs and trees respectively. The general trend toward the fast end of the spectrum is perhaps simply a byproduct of parasitic plants mostly being herbs. But underlying that explanation is the question: why are most parasites herbs?

Parasite's position on the LES is somewhat complicated by the individual traits. Even when plant growth form was accounted for, there was still an effect of parasitism on the leaf traits A_{mass}, LMA, N_{area}, and R_{mass} and an interaction effect of parasitism and plant growth form on the leaf traits LMA, N_{area}, N_{mass} and $\delta^{15}N$ (Table 4). This suggests that parasitism itself affects a plant's position on the LES. However, in contrast to overall patterns, the significant differences between groups showed that parasitic plants were more on the slow-return side than the non-parasites (e.g. within the shrub growth form the parasites had a higher LMA and lower A_{mass} than non-parasites). However, this may be driven by differences parasite type by growth form in shrubs and parasite type, as is discussed further below.

Position on the LES does not appear to be dramatically affected by parasite type (i.e. root versus stem). The exception was that LMA was significantly higher in stem parasites than root parasites (Fig 6, Table 6). This may reflect changes in whole plant biomass investment driven by a lack of root systems in stem parasites. The accumulation of nonstructural carbohydrates in roots serve a critical role in regrowth following herbivory, fire or other damage (Coyne & Cook

1970; Heilmeier, Schulze & Whale 1986). Without roots to allocate biomass to, stem parasites may 'invest' more heavily in wear-resistant, higher LMA leaves.

Parasitic shrubs also had a significantly higher LMA than non-parasitic shrubs (Fig 4, Tables 4, 5). This may also be driven by the difference between stem and root parasites because stem parasites make up a larger share of parasitic shrubs than other growth forms. For LMA measurements, root parasites accounted for 100% of observations in trees, >99% of observations in herbs, and 25% of observations in shrubs.

$\delta^{13}C$ and leaf $\delta^{15}N$ did not differ significantly between parasites and non-parasites within each plant growth form. This is unsurprising as the isotopic signature of a parasite is derived in part from their host (Bannister & Strong 2001). However, there is also evidence that many parasites have low water use efficiency (Schulze *et al.* 1984; Press *et al.* 1987, 1988; Scalon & Wright 2017), for which $\delta^{13}C$ is often used as a proxy for (Farquhar *et al.* 1982). Given that, one would expect low $\delta^{13}C$ across parasites, but that is not the case here.

I focused on heterotrophic plants which parasitize other plants, however, a full 10% of angiosperms are mycoheterotrophs, which instead parasitize mycorrhizal fungi at some point in their life cycle (distinct from mutualistic mycorrhizal associations) (Leake & Cameron 2010). Although structurally and phylogenetically distinct, mycoheterotrophs and the haustorial parasites explored here are functionally similar as they both exhibit heterotrophy (Nickrent 2020). This group may serve to further illuminate any effects, or lack thereof, of heterotrophy on leaf traits.

How well represented are parasitic plants in a global plant trait database?

There is an ever-expanding amount of genetic, location and functional trait data available to researchers, however, there are large gaps and biases in the coverage of these data for certain parts of the world and groups of plants (Cornwell *et al*. 2019). Parasitic plants illustrate one facet of this problem. Parasitic plants are neither exceedingly rare nor very abundant. Although they are common in many ecosystems, parasitic plants rarely represent a large portion of community biomass (Press & Phoenix 2005). So, what would adequate representation in databases such as TRY look like? It's difficult to estimate what proportional representation by biomass or number of individuals would be. By number of species, parasitic plants likely account for ~0.9% of all plant species, because ~90% of all plant species are angiosperms (Crepet & Niklas 2009; RBG Kew 2016) of which around 1% are parasitic (Westwood *et al.* 2010), and there is only one parasitic gymnosperm species, *Parasitaxus ustus* (Feild & Brodribb 2005). In this analysis, across all leaf traits, parasitic plants account for 0.74% of species and 0.72% of observations (excluding possibly parasitic species, Table 2). This is likely a slight overestimate of the true proportion in TRY because I only requested access to restricted datasets that included parasitic species. Even so, parasitic plants are slightly underrepresented, although this is probably relatively good representation compared to many other plant groups. Indeed, in a global tally of plant data and diversity, three parasitic plant families, Loranthancaceae, Orobanchaceae, and Santalaceae, are listed as 'broadly covered' in the TRY database (Cornwell *et al*. 2019). Despite this, there are such low sample sizes in the current dataset that many comparisons are impossible to make and the ones that are, are not very robust. In particular the lack of leaf traits across the same individuals is limiting. For example, only pairwise comparisons were made here instead of a multi-trait analysis.

Similarly, holoparasites are scarcely represented, as there are only two genera with LES leaf traits (Table 3*)*. However, their representation is still largely proportional. Many

holoparasites have highly reduced or absent leaves, making their appearance more unlikely. Further, ~90% of parasites are hemiparasites (Heide-Jørgensen 2008).

Although parasitic plants may be relatively well represented proportionately, their small numbers overall still make their representation inadequate. This is likely not an unusual problem given the vast number of plant species in the world and that ~36.5% of those species are rare (Enquist *et al*. 2019).

Conclusions

There is small, but growing database on parasitic plant leaf traits available in TRY. In addition, here I compiled a freely available dataset of all global parasitic plant genera (Haynes 2020). In the existing TRY dataset, there is some evidence that relationships among traits and LES strategy (i.e. fast versus slow return) may differ from non-parasitic plants and parasites fall generally towards the fast end, but these differences may also be affected by parasite type and growth form. Surprisingly though, parasitic plants seem to operate within the general bounds of the LES tradeoffs even though they do not depend solely on direct photosynthesis: there are no examples of novel trait combinations wherein parasitic plants are completely outside the global spectrum of traits and pairwise trait relationships did not differ dramatically between non-parasites and parasites. Despite being heterotrophs, a characteristic which usurps an essential function of leaves (namely C acquisition), parasitic plants appear largely constrained by the same structural, chemical and physiological tradeoffs that determine the scope of leaf traits in all plants.

Tables

Table 1 The core leaf economic spectrum traits and two isotope traits

Abbreviation	Definition	Units
A_{area}	Light-saturated photosynthetic rate per unit leaf area (often shorthanded as photosynthetic capacity per unit leaf area)	μmol m^{-2} s^{-1}
A_{mass}	Light saturated photosynthetic rate per unit leaf dry mass (often shorthanded as photosynthetic capacity per unit leaf mass)	μmol g^{-1} s^{-1}
LL	Leaf lifespan	years
LMA	Leaf mass per area. Equal to 1/specific leaf area (SLA)	mg mm^{-2}
N_{area}	Leaf nitrogen content per unit leaf area	g m^{-2}
N_{mass}	Leaf nitrogen content per unit leaf dry mass	mg g^{-1}
P_{area}	Leaf phosphorus content per unit leaf area	g m^{-2}
P_{mass}	Leaf phosphorus content per unit leaf dry mass	mg g^{-1}
R_{area}	Leaf dark respiration rate per unit leaf area	μmol m^{-2} s^{-1}
R_{mass}	Leaf dark respiration rate per unit leaf dry mass	μmol g^{-1} s^{-1}
$\delta^{13}C$	Leaf carbon isotopic composition	‰
$\delta^{15}N$	Leaf nitrogen isotopic composition	‰

Table 2 Observation and species counts for each leaf trait

Leaf trait	Species			Observations		
	Parasitic	*Possibly parasitic*	*Non-parasitic*	*Parasitic*	*Possibly parasitic*	*Non-parasitic*
A_{area}	9	1	2067	23	2	16332
A_{mass}	9	2	1524	26	2	5520
LL	8	1	980	14	1	1661
LMA	60	4	4236	625	81	33087
N_{area}	19	1	2382	31	1	7172
N_{mass}	49	5	6553	254	26	45536
P_{mass}	25	4	4169	67	5	19839
P_{area}	6	1	1900	8	1	3900
R_{mass}	5	1	933	12	1	5087
R_{area}	6	1	1386	12	1	5515
$\delta^{13}C$	19	1	2405	22	1	8360
$\delta^{15}N$	14	1	1890	88	1	10550

Table 3 Parasitic genera included in the TRY database. Blank indicates unknown Ob/Fac status

Family	Genus	Species	Obs	Mistletoe	Position	Holo/Hemi	Ob/Fac
Convolvulaceae	*Cuscuta*	1	1	No	Stem	Holo	Obligate
Krameriaceae	*Krameria*	4	70	No	Root	Hemi	
Lauraceae	*Cassytha*	1-2	3	No	Stem	Hemi	Obligate
Loranthaceae	*Agelanthus*	1	1	Yes	Stem	Hemi	Obligate
	Alepis	1	1	Yes	Stem	Hemi	Obligate
	Amyema	6-7	11	Yes	Stem	Hemi	Obligate
	Englerina	1	1	Yes	Stem	Hemi	Obligate
	Erianthemum	1	1	Yes	Stem	Hemi	Obligate
	Gaiadendron	1	14	Yes	Stem	Hemi	Obligate
	Helixanthera	1	1	Yes	Stem	Hemi	Obligate
	Ligaria	1	1	Yes	Stem	Hemi	Obligate
	Loranthus	1-2	2	Yes	Stem	Hemi	Obligate
	Lysiana	2	4	Yes	Stem	Hemi	Obligate
	Macrosolen	1	1	Yes	Stem	Hemi	Obligate
	Nuytsia	1	8	Yes	Stem	Hemi	Obligate
	Peraxilla	2-3	7	Yes	Stem	Hemi	Obligate
	Psittacanthus	1	1	Yes	Stem	Hemi	Obligate
	Tapinanthus	1	1	Yes	Stem	Hemi	Obligate
	Taxillus	1	1	Yes	Stem	Hemi	Obligate
	Tupeia	1	1	Yes	Stem	Hemi	Obligate
Olacaceae	*Aptandra*	2	3	No	Root	Hemi	
	Cathedra	1	5	No	Root	Hemi	
	Chaunochiton	1-2	10	No	Root	Hemi	
	Dulacia	1	5	No	Root	Hemi	
	*Heisteria**	9-10	102	No	Root	Hemi	
	Olax	2	4	No	Root	Hemi	
	Ongokea	1	8	No	Root	Hemi	
	Ptychopetalum	1	34	No	Root	Hemi	
Opiliaceae	*Agonandra*	2-3	11	No	Root	Hemi	
Orobanchaceae	*Agalinis*	2-3	4	No	Root	Hemi	Facultative
	Bartsia	3	46	No	Root	Hemi	
	Buchnera	1	52	No	Root	Hemi	
	Castilleja	4-7	93	No	Root	Hemi	Facultative
	Cymbaria	1	6	No	Root	Hemi	
	Euphrasia	5-7	55	No	Root	Hemi	
	Melampyrum	5	91	No	Root	Hemi	
	Odontites	3	30	No	Root	Hemi	
	Orobanche	1	1	No	Root	Holo	Obligate
	Parentucellia	2	5	No	Root	Hemi	
	Pedicularis	17-22	204	No	Root	Hemi	
	Rhinanthus	4-5	79	No	Root	Hemi	
	Siphonostegia	1	2	No	Root	Hemi	
Santalaceae	*Acanthosyris*	1	1	No	Root	Hemi	
	Comandra	1-2	104	No	Root	Hemi	
	Dendrotrophe	1	2	No	Stem	Hemi	Obligate
	Osyris	1	1	No	Root	Hemi	
	Santalum	5	25	No	Root	Hemi	
	Thesium	4-5	51	No	Root	Hemi	
Schoepfiaceae	*Schoepfia*	3	5	No	Root	Hemi	
Viscaceae	*Korthalsella*	2-3	3	Yes	Stem	Hemi	Obligate
	Phoradendron	2-3	4	Yes	Stem	Hemi	Obligate
	Viscum	1	5	Yes	Stem	Hemi	Obligate
Ximeniaceae	*Ximenia*	2	13	No	Root	Hemi	

*possibly parasitic; Obs: Observations, Holo: holoparasites, hemi: hemiparasite, Ob: obligate, Fac: facultative

Table 4 Type III ANOVAs on each leaf trait based on linear models with parasite status and plant growth form as explanatory variables and species as a random effect. See Table 5 for post hoc comparisons

Leaf trait	Parameter	num df	den df	F	p-value
A_{area}	Parasitic	1	2070	0.330	0.566
	Growth form	2	2070	1.739	0.176
	Parasitic * Growth form	2	2070	0.426	0.653
A_{mass}	Parasitic	1	1527	5.455	**0.020**
	Growth form	2	1527	14.317	**<0.001**
	Parasitic * Growth form	2	1527	2.592	0.075
LL*	Parasitic	1	302	5.827	**0.016**
LMA	Parasitic	1	4290	33.559	**<0.001**
	Growth form	2	4290	50.422	**<0.001**
	Parasitic * Growth form	2	4290	14.432	**<0.001**
N_{area}*	Parasitic	1	1285	6.271	**0.012**
	Growth form	1	1285	12.286	**<0.001**
	Parasitic * Growth form	1	1285	5.981	**0.015**
N_{mass}	Parasitic	1	6596	1.489	0.222
	Growth form	2	6596	10.903	**<0.001**
	Parasitic * Growth form	2	6596	2.977	**0.051**
P_{area}*	Parasitic	1	799	0.503	0.478
	Growth form	1	799	0.323	0.570
	Parasitic * Growth form	1	799	0.317	0.574
P_{mass}	Parasitic	1	4188	1.006	0.316
	Growth form	2	4188	8.794	**<0.001**
	Parasitic * Growth form	2	4188	0.280	0.756
R_{area}*	Parasitic	1	774	3.876	**0.049**
	Growth form	1	774	0.422	0.516
	Parasitic * Growth form	1	774	0.008	0.927
R_{mass}	Parasitic	1	932	4.127	**0.042**
	Growth form	2	932	4.508	**0.011**
	Parasitic * Growth form	2	932	0.772	0.462
$\delta^{13}C$	Parasitic	1	2183	0.484	0.487
	Growth form	2	2183	2.093	0.124
	Parasitic * Growth form	2	2183	1.227	0.293
$\delta^{15}N$	Parasitic	1	1898	0.066	0.797
	Growth form	2	1898	5.875	**0.003**
	Parasitic * Growth form	2	1898	3.272	**0.038**

LL: leaf lifespan, LMA: leaf mass per area; *indicates not all growth forms included due to lack of data: LL included only the herbs; N_{area} excluded trees; P_{area} excluded shrubs; R_{area} excluded herbs

Table 5 Planned contrasts (parasite status within each level of plant growth form) of estimated marginal means based on linear models of each leaf trait. P-values use Tukey adjustment for multiple comparisons. See Table 4 for ANOVAs

Leaf trait	Growth form	Estimate	SE	t-ratio	p-value
A_{mass}	Herb	0.04	0.133	0.289	0.772
	Shrub	-0.37	0.176	-2.130	**0.033**
	Tree	-0.50	0.284	-1.773	0.076
LMA	Herb	0.03	0.030	1.106	0.269
	Shrub	0.53	0.088	6.046	**<0.001**
	Tree	0.11	0.071	1.587	0.112
N_{area}	Herb	0.00	0.058	0.068	0.946
	Shrub	0.33	0.122	2.741	**0.006**
R_{area}	Shrub	0.24	0.216	1.119	0.263
	Tree	0.26	0.140	1.892	0.059
R_{mass}	Herb	-0.08	0.185	-0.422	0.673
	Shrub	-0.36	0.284	-1.252	0.211
	Tree	-0.46	0.281	-1.641	0.101

LMA: leaf mass per area, SE: standard error

Table 6 Type III ANOVAs on each leaf trait based on linear mixed effects models with parasite position (stem vs. root) as the explanatory variable and species as a random effect. Missing leaf traits were excluded because of too few observations.

Leaf trait	Parameter	num df	den df	F	p-value
LMA	Position	1	93	90.597	**<0.001**
N_{mass}	Position	1	70	0.511	0.477
P_{mass}	Position	1	38	0.000	0.995
$\delta^{13}C$	Position	1	44	0.035	0.853

LMA: leaf mass per area, df: degrees of freedom

Table 7 Comparison of pairwise leaf trait correlations between parasites and non-parasites using Fishers z-score and Zou's confidence intervals. Adjusted $\alpha = 0.0014$ and confidence interval is 99.85% using Dunn-Šidák method. Bold signifies significant differences at the adjusted significance level. For Zou's method, if the confidence interval does not include 0 the null hypothesis is rejected.

Leaf traits		Pearson's r							Fisher		Zou	
		Parasites			*Non-parasites*			rP - rNP	z	p-value	CI (rP - rNP)	
		n	r	p-value	n	r	p-value				lower	upper
A_{area}	LL	4	-0.72	0.277	756	-0.39	**<0.0001**	-0.34	-0.51	0.612	-0.63	1.37
	LMA	20	-0.01	0.977	3031	0.15	**<0.0001**	-0.15	-0.63	0.526	-0.80	0.50
	N_{area}	15	-0.31	0.261	4419	0.22	**<0.0001**	-0.53	-1.89	0.059	-1.07	0.32
	N_{mass}	14	0.17	0.560	5192	0.19	**<0.0001**	-0.02	-0.07	0.940	-0.85	0.62
	P_{area}	6	-0.20	0.702	1761	0.14	**<0.0001**	-0.34	-0.59	0.553	-1.11	0.79
	P_{mass}	6	0.59	0.219	1894	0.20	**<0.0001**	0.39	0.82	0.414	-1.02	0.79
	R_{area}	6	-0.26	0.613	2416	0.21	**<0.0001**	-0.47	-0.84	0.402	-1.18	0.71
	R_{mass}	6	-0.70	0.124	1927	0.32	**<0.0001**	-1.02	-2.07	0.039	-1.32	0.43
A_{mass}	LL	4	-0.97	0.028	916	-0.76	**<0.0001**	-0.21	-1.13	0.257	-0.27	1.55
	LMA	22	-0.43	0.044	859	-0.44	**<0.0001**	0.01	0.03	0.976	-0.40	0.71
	N_{area}	13	-0.82	**0.0005**	3023	-0.20	**<0.0001**	-0.62	-3.05	0.002	-0.78	0.04
	N_{mass}	19	0.71	**0.0006**	4801	0.56	**<0.0001**	0.15	1.02	0.309	-0.47	0.37
	P_{area}	6	-0.76	0.082	1462	-0.10	**0.0001**	-0.66	-1.53	0.125	-0.91	0.80
	P_{mass}	9	0.85	0.004	1744	0.48	**<0.0001**	0.37	1.80	0.071	-0.52	0.52
	R_{area}	8	0.79	0.020	1852	-0.01	0.607	0.80	2.41	0.016	-0.33	1.01
	R_{mass}	10	0.82	0.003	1930	0.56	**<0.0001**	0.27	1.43	0.152	-0.59	0.43
LL	LMA	2			233	0.25	**0.0001**					
	N_{area}	5	0.97	0.006	695	0.21	**<0.0001**	0.76	2.66	0.008	-0.37	0.89
	N_{mass}	6	-0.95	0.003	1266	-0.58	**<0.0001**	-0.38	-2.07	0.039	-0.46	0.57
	P_{area}	1			198	0.16	0.028					
	P_{mass}	1			453	-0.65	**<0.0001**					
	R_{area}	2			243	0.09	0.172					
	R_{mass}	2			223	-0.76	**<0.0001**					
LMA	N_{area}	11	0.86	**0.0007**	1885	0.71	**<0.0001**	0.15	1.17	0.243	-0.54	0.28
	N_{mass}	79	-0.70	**<0.0001**	10044	-0.57	**<0.0001**	-0.13	-1.94	0.053	-0.28	0.11
	P_{area}	4	-0.65	0.346	1213	0.60	**<0.0001**	-1.26	-1.48	0.139	-1.61	0.38
	P_{mass}	21	-0.76	**<0.0001**	2832	-0.36	**<0.0001**	-0.40	-2.60	0.009	-0.59	0.13
	R_{area}	5	-0.63	0.255	655	-0.10	0.009	-0.53	-0.90	0.366	-0.91	1.01
	R_{mass}	5	-0.93	0.023	610	-0.59	**<0.0001**	-0.34	-1.38	0.169	-0.46	1.13
N_{area}	P_{area}	7	-0.12	0.805	3667	0.32	**<0.0001**	-0.43	-0.89	0.372	-1.26	0.58
	P_{mass}	6	-0.91	0.012	2851	-0.11	**<0.0001**	-0.80	-2.45	0.014	-0.91	0.42
	R_{area}	6	-0.04	0.943	1839	0.35	**<0.0001**	-0.38	-0.69	0.491	-1.30	0.60
	R_{mass}	7	-0.65	0.112	1859	-0.21	**<0.0001**	-0.44	-1.12	0.262	-0.78	0.89
N_{mass}	P_{area}	7	-0.96	0.001	2652	0.07	**0.0003**	-1.03	-3.96	**0.00007**	**-1.10**	**-0.37**
	P_{mass}	81	0.66	**<0.0001**	24394	0.53	**<0.0001**	0.13	1.80	0.072	-0.12	0.29
	R_{area}	9	0.37	0.326	2938	0.13	**<0.0001**	0.24	0.64	0.525	-0.85	0.81
	R_{mass}	9	0.76	0.017	3488	0.65	**<0.0001**	0.11	0.55	0.584	-0.94	0.33
P_{area}	R_{area}	5	-0.96	0.011	1259	0.23	**<0.0001**	-1.18	-3.03	0.002	-1.28	0.11
	R_{mass}	5	-0.97	0.007	1254	-0.04	0.127	-0.92	-2.82	0.005	-1.02	0.26
P_{mass}	R_{area}	5	0.91	0.030	1333	0.07	0.007	0.84	2.08	0.037	-0.69	0.96
	R_{mass}	5	0.95	0.015	1268	0.43	**<0.0001**	0.52	1.89	0.059	-0.86	0.61

CI: confidence interval, LL: leaf lifespan, LMA: leaf mass per area, NP: non-parasites, P: parasites

Table 8 Comparisons of pairwise leaf trait relationship by parasite status using a standardized major axis (SMA). Adjusted $\alpha = 0.0014$ and confidence interval is 99.85% using Dunn-Šidák method. Bold signifies significant differences at the adjusted significance level.

Leaf traits		*H_0: slopes are equal*			*H_0: elevations are equal*			*H_0: no shift along common axis*		
		Likelihood ratio	df	p-value	Wald	df	p-value	Wald	df	p-value
A_{area}	LL	0.10	1	0.751	0.75	1	0.386	0.01	1	0.939
	LMA	0.14	1	0.712	17.14	1	**<0.0001**	12.92	1	0.0003
	N_{area}	0.85	1	0.357	6.19	1	0.013	29.35	1	**<0.0001**
	N_{mass}	4.97	1	0.026	4.81	1	0.028	0.27	1	0.600
	P_{area}	0.49	1	0.485	1.15	1	0.283	2.80	1	0.094
	P_{mass}	0.15	1	0.697	0.05	1	0.821	0.01	1	0.924
	R_{area}	1.05	1	0.306	6.09	1	0.014	10.55	1	**0.0012**
	R_{mass}	0.91	1	0.339	0.58	1	0.446	4.25	1	0.039
A_{mass}	LL	1.01	1	0.314	2.20	1	0.138	0.43	1	0.513
	LMA	0.24	1	0.623	1.20	1	0.274	27.12	1	**<0.0001**
	N_{area}	3.17	1	0.075	2.41	1	0.121	8.68	1	0.003
	N_{mass}	0.01	1	0.924	1.06	1	0.303	4.09	1	0.043
	P_{area}	5.03	1	0.025	1.60	1	0.206	0.42	1	0.516
	P_{mass}	5.30	1	0.021	8.56	1	0.003	0.06	1	0.809
	R_{area}	0.83	1	0.362	1.57	1	0.210	465.10	1	**<0.0001**
	R_{mass}	4.11	1	0.043	9.27	1	0.002	3.40	1	0.065
LL	N_{area}	4.34	1	0.037	13.54	1	**0.00023**	0.00	1	0.955
	N_{mass}	1.01	1	0.315	6.22	1	0.013	0.57	1	0.451
LMA	N_{area}	15.47	1	**<0.0001**						
	N_{mass}	0.53	1	0.466	10.91	1	**0.001**	22.35	1	**<0.0001**
	P_{area}	0.74	1	0.389	1.20	1	0.273	14.18	1	**0.00017**
	P_{mass}	0.09	1	0.768	13.38	1	**0.00025**	10.45	1	**0.0012**
	R_{area}	1.82	1	0.177	6.44	1	0.011	42.78	1	**<0.0001**
	R_{mass}	0.17	1	0.684	36.00	1	**<0.0001**	11.45	1	**0.0007**
N_{area}	P_{area}	0.07	1	0.797	0.001	1	0.977	9.16	1	0.002
	P_{mass}	0.03	1	0.854	21.57	1	**<0.0001**	0.52	1	0.472
	R_{area}	0.01	1	0.938	0.05	1	0.816	67.42	1	**<0.0001**
	R_{mass}	4.99	1	0.025	2.49	1	0.115	9.11	1	0.003
N_{mass}	P_{area}	9.20	1	0.002	1.31	1	0.252	3.23	1	0.072
	P_{mass}	0.99	1	0.321	2.01	1	0.156	5.49	1	0.019
	R_{area}	1.50	1	0.221	58.04	1	**<0.0001**	3.00	1	0.083
	R_{mass}	0.12	1	0.724	8.29	1	0.004	14.70	1	**0.0001**
P_{area}	R_{area}	4.06	1	0.044	0.04	1	0.838	107.30	1	**<0.0001**
	R_{mass}	9.95	1	0.002	0.38	1	0.537	1.99	1	0.158
P_{mass}	R_{area}	1.93	1	0.165	50.54	1	**<0.0001**	0.99	1	0.321
	R_{mass}	7.82	1	0.005	0.02	1	0.880	0.86	1	0.355

LL: leaf lifespan, LMA: leaf mass per area; df: degrees of freedom

Figures

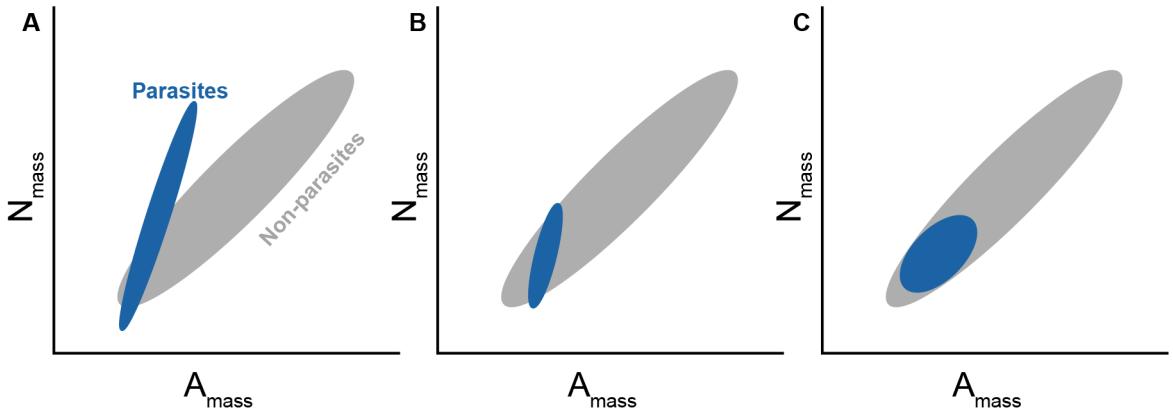

Figure 1 Conceptual diagram of how parasitic plants might fit within, or deviate from, the leaf economic spectrum. (A) shows a deviation from the LES wherein parasitic plants have a different relationship between traits, resulting in novel trait combinations such as simultaneously high N_{mass} and low A_{mass}; (B) shows a more cryptic deviation where the slope of relationship between A_{mass} and N_{mass} differs but parasitic plants stay within the LES trait space; (C) shows parasitic plants occupying a particular position on the LES but not deviating from the relationships or falling outside of the trait space.

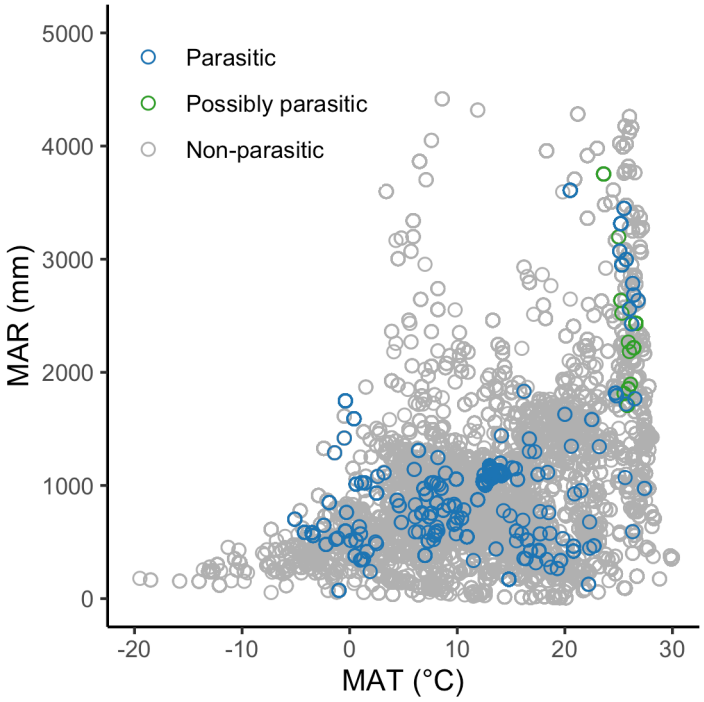

Figure 2 Mean annual temperature (MAT) and mean annual rainfall (MAR) at the sampling location of 1061 parasitic or possibly parasitic individuals and 132970 non-parasitic individuals

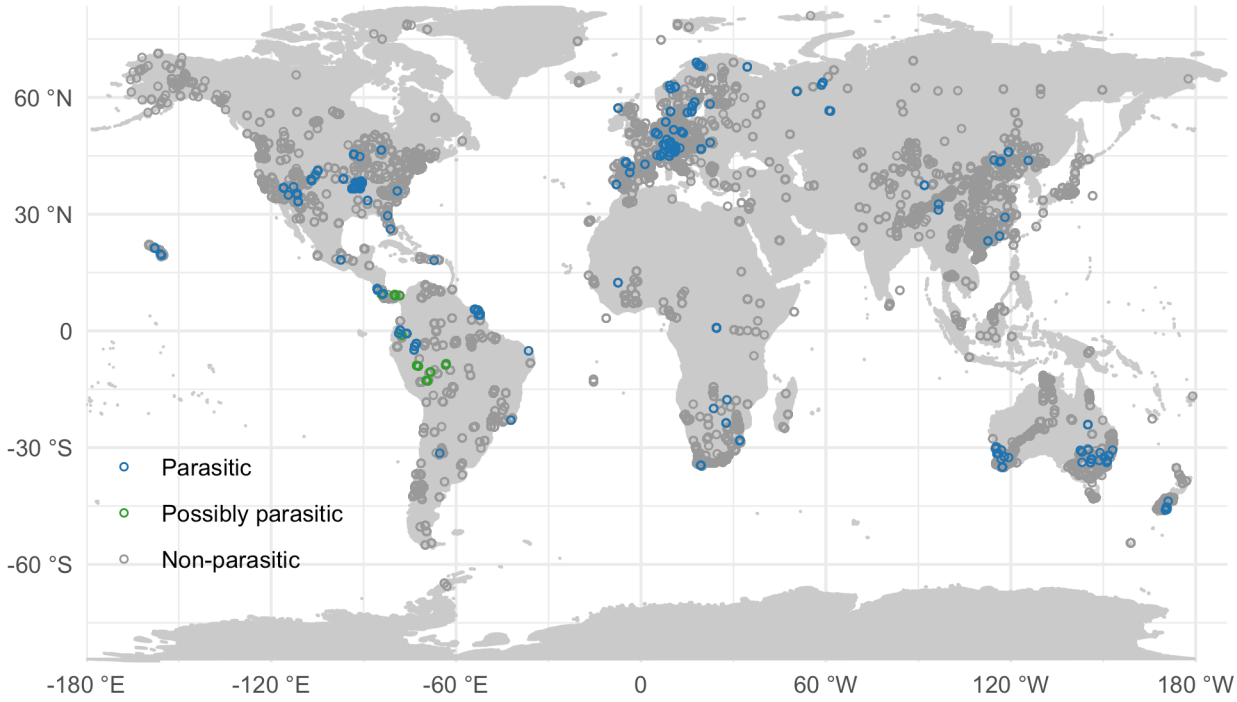

Figure 3 Sampling locations for parasitic or possibly parasitic individuals (249 unique sites) and non-parasitic individuals (6352 unique sites).

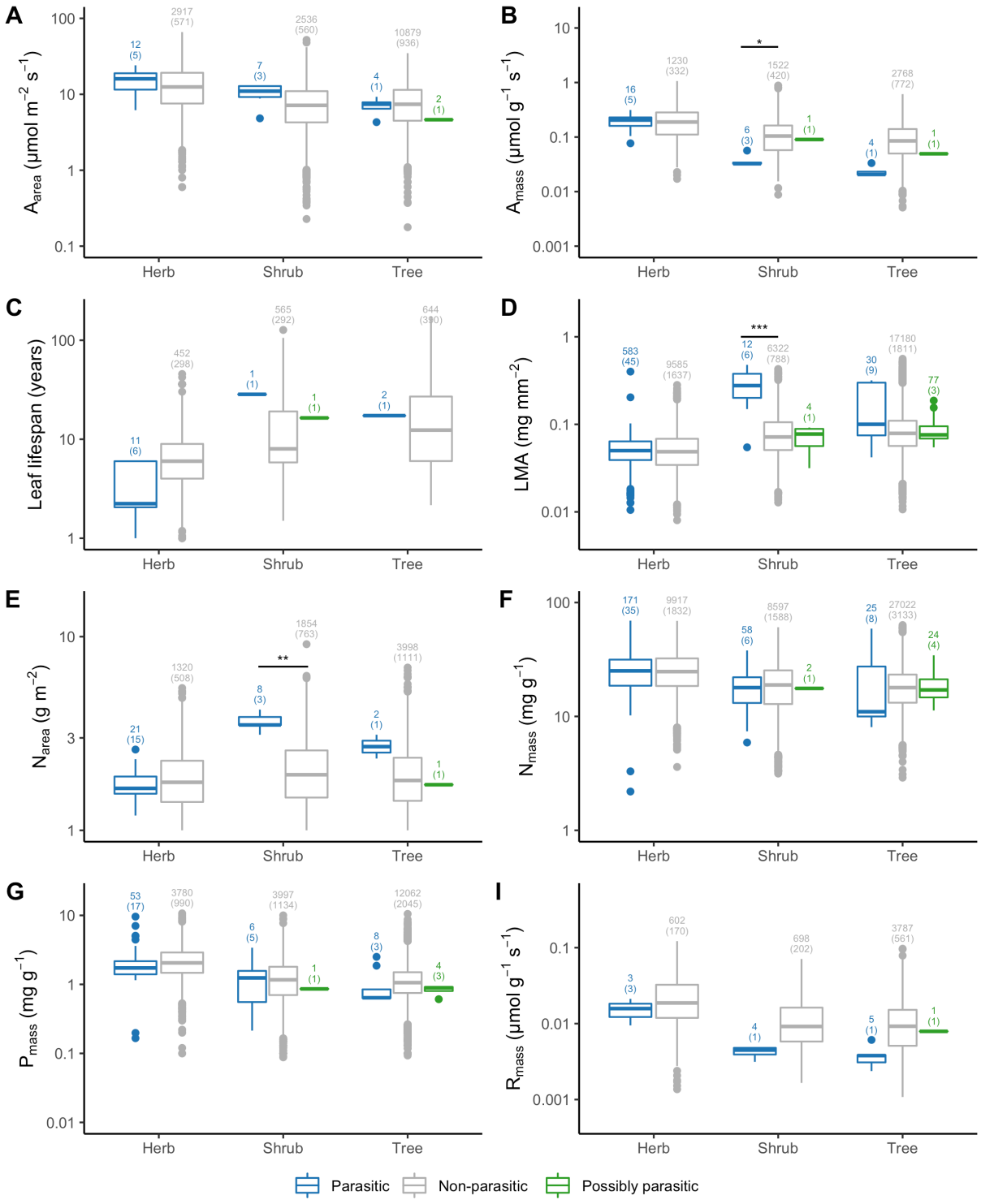

Figure 4 Leaf traits compared between parasitic and non-parasitic individuals within plant growth form. Asterisks denote significance where * p<0.05, ** p<0.01, *** p <0.001. Possibly parasitic plants were not included in ANOVAs. See Table 4 for ANOVAs and Table 5 for posthoc comparisons. Boxplot hinges correspond to first and third quartiles for the lower and upper hinges, while the whiskers extend to the most extreme value no further than 1.5*inter-quartile range

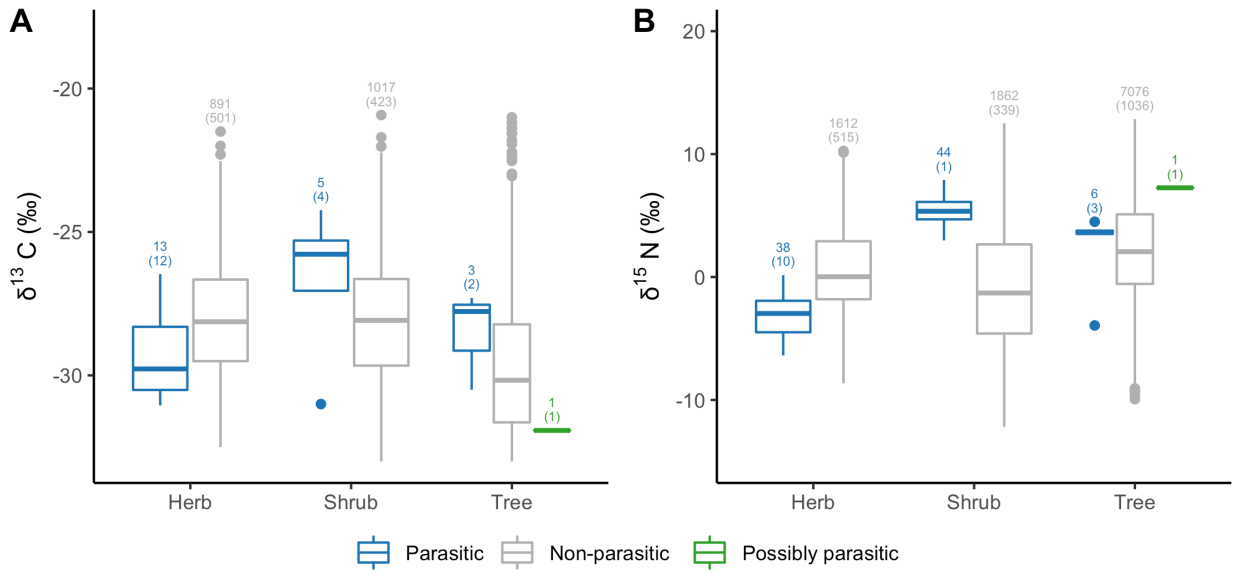

Figure 5 Leaf isotope values compared between parasitic and non-parasitic individuals within plant growth form. Asterisks denote significance where * p<0.05, ** p<0.01, *** p <0.001. Possibly parasitic plants were not included in ANOVAs. See Table 4 for ANOVAs and Table 5 for posthoc comparisons. Boxplot hinges correspond to first and third quartiles for the lower and upper hinges, while the whiskers extend to the most extreme value no further than 1.5*inter-quartile range

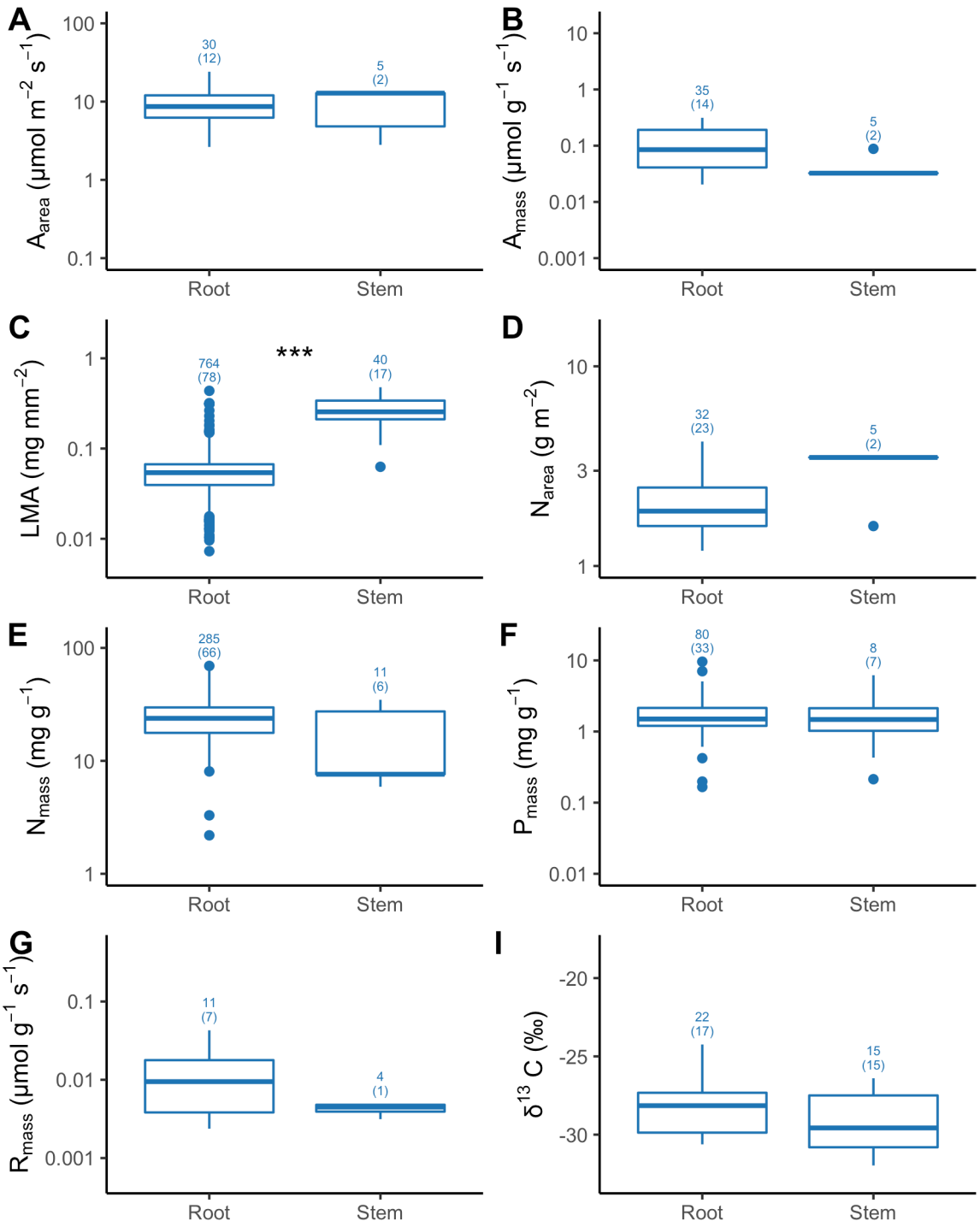

Figure 6 Leaf traits within parasitic plants compared among root versus stem parasites. Asterisks denote significance where * p<0.05, ** p<0.01, *** p <0.001. Possibly parasitic plants were not included in ANOVAs. LL and $\delta^{15}N$ are not graphed due to lack of observations. A_{area}, A_{mass}, R_{mass}, N_{area} do not include statistics due to lack of observations. See Table 6 for ANOVAs. Boxplot hinges correspond to first and third quartiles for the lower and upper hinges, while the whiskers extend to the most extreme value no further than 1.5*inter-quartile range

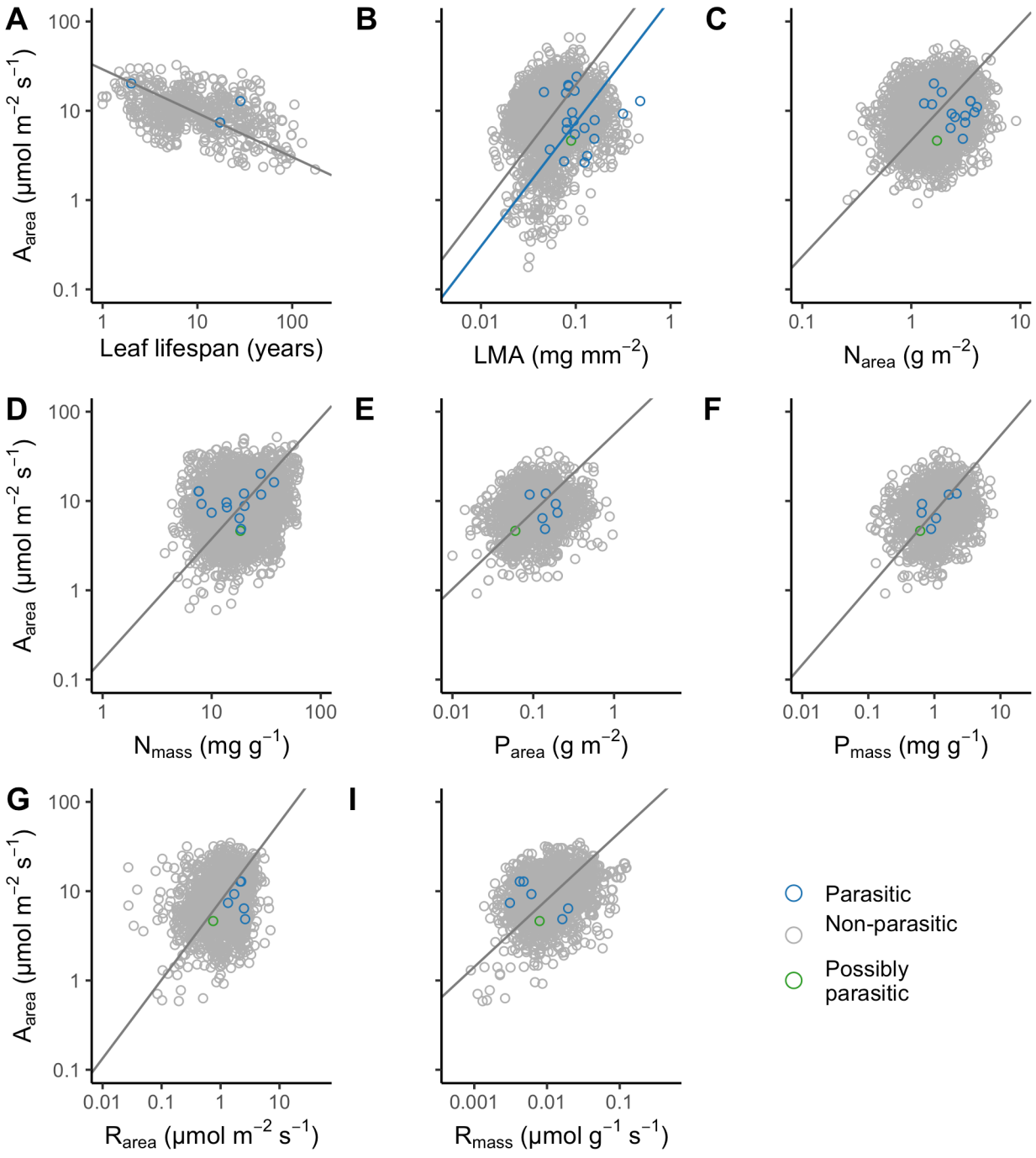

Figure 7 Pairwise leaf trait comparisons. Trendlines derived from SMA fits. See Table 7 for statistics

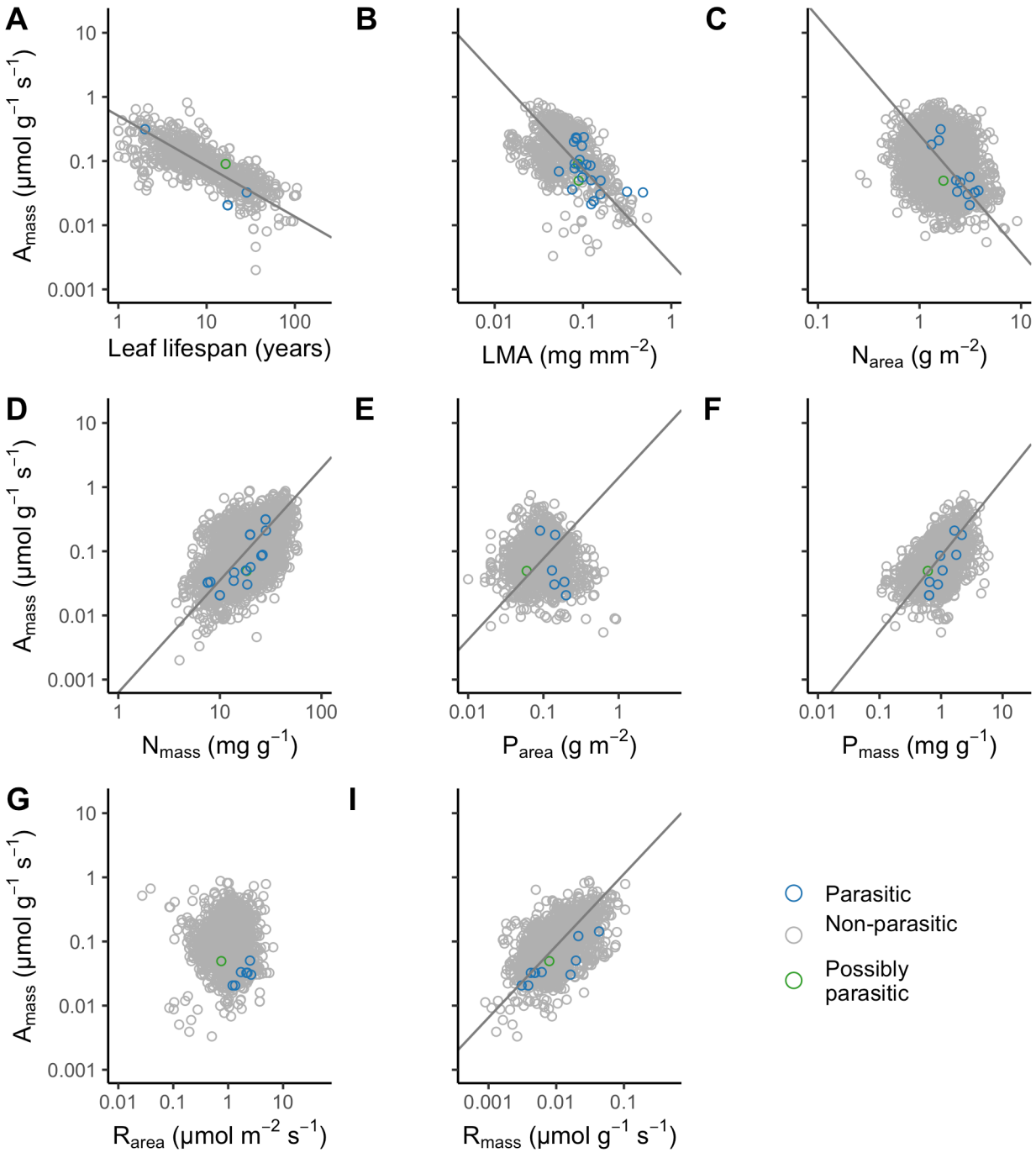

Figure 8 Pairwise leaf trait comparisons. Trendlines derived from SMA fits. See Table 7 for statistics

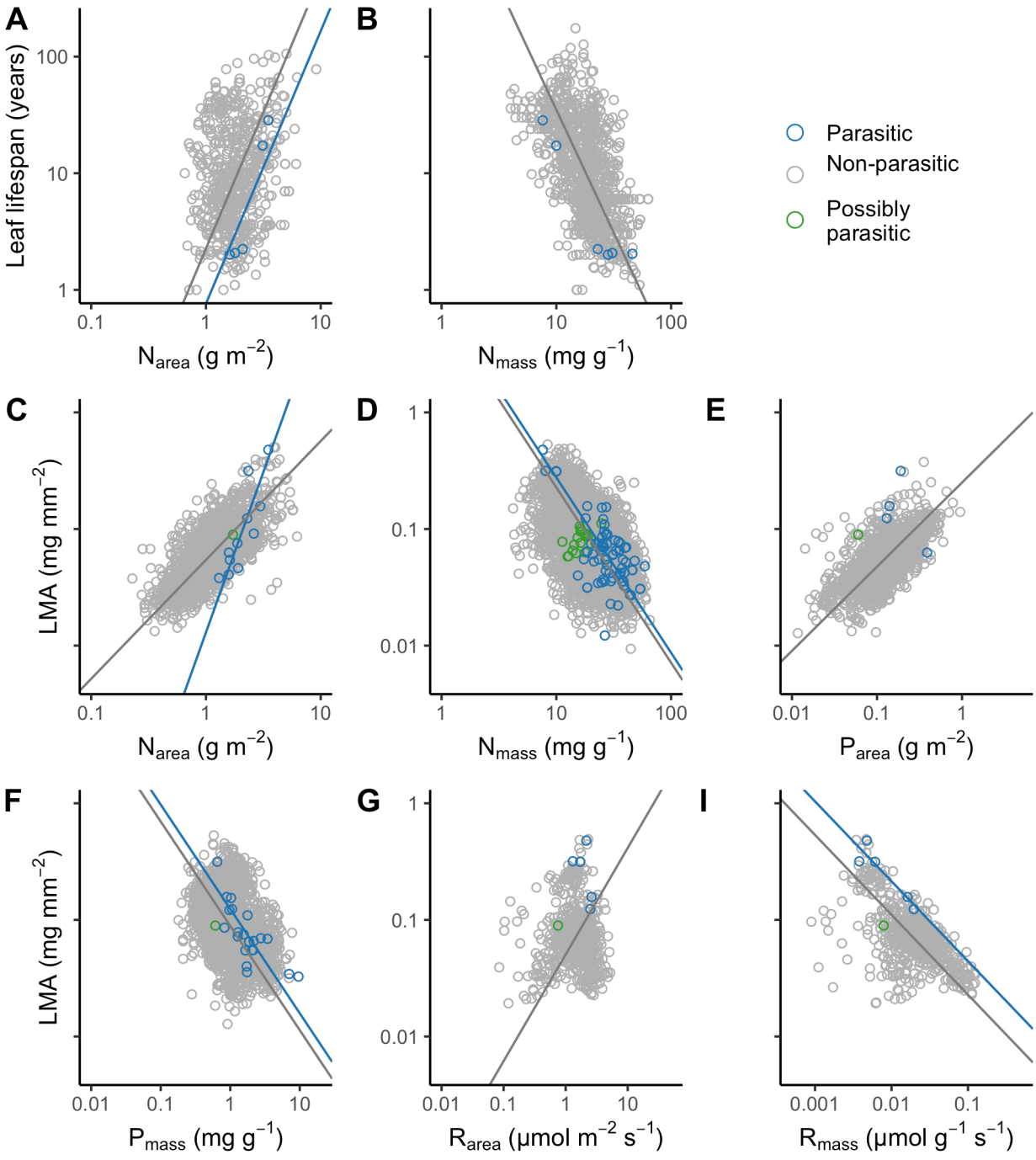

Figure 9 Pairwise leaf trait comparisons. Trendlines derived from SMA fits. See Table 7 for statistics

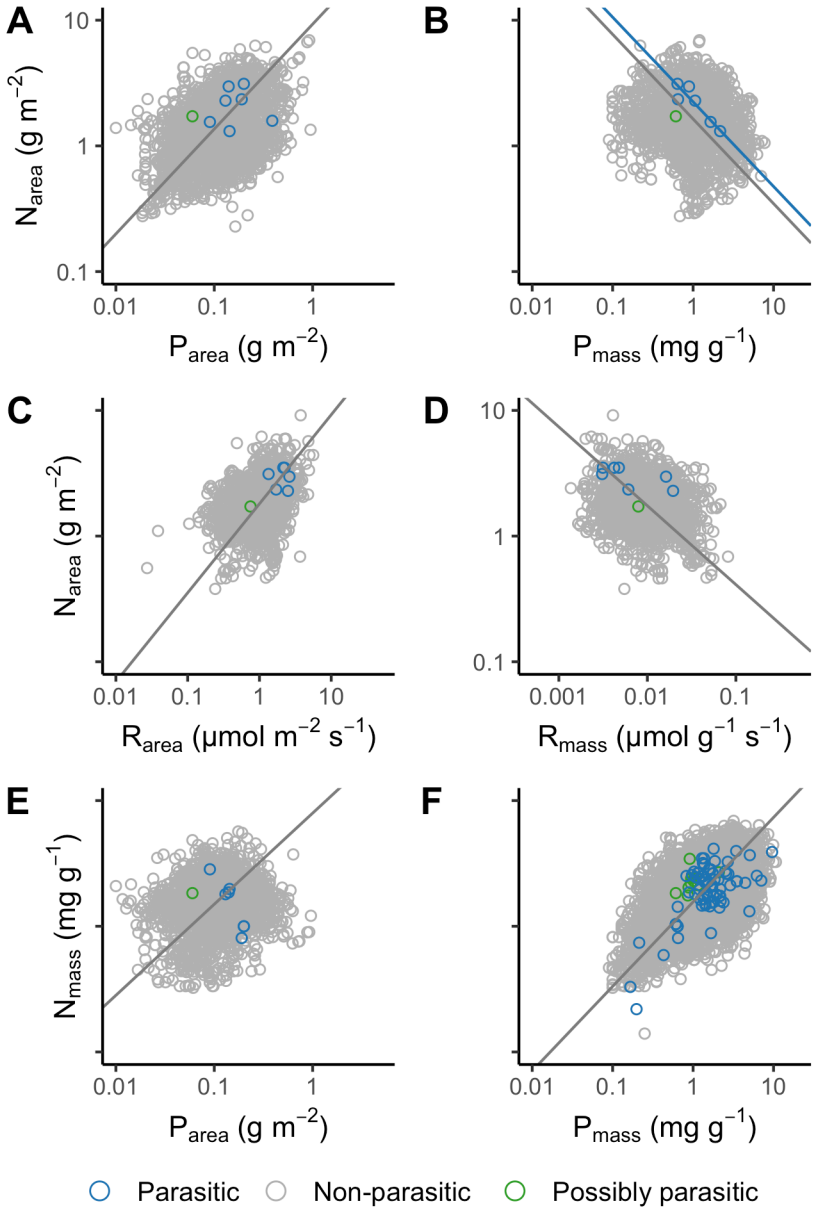

Figure 10 Pairwise leaf trait comparisons. Trendlines derived from SMA fits. See Table 7 for statistics

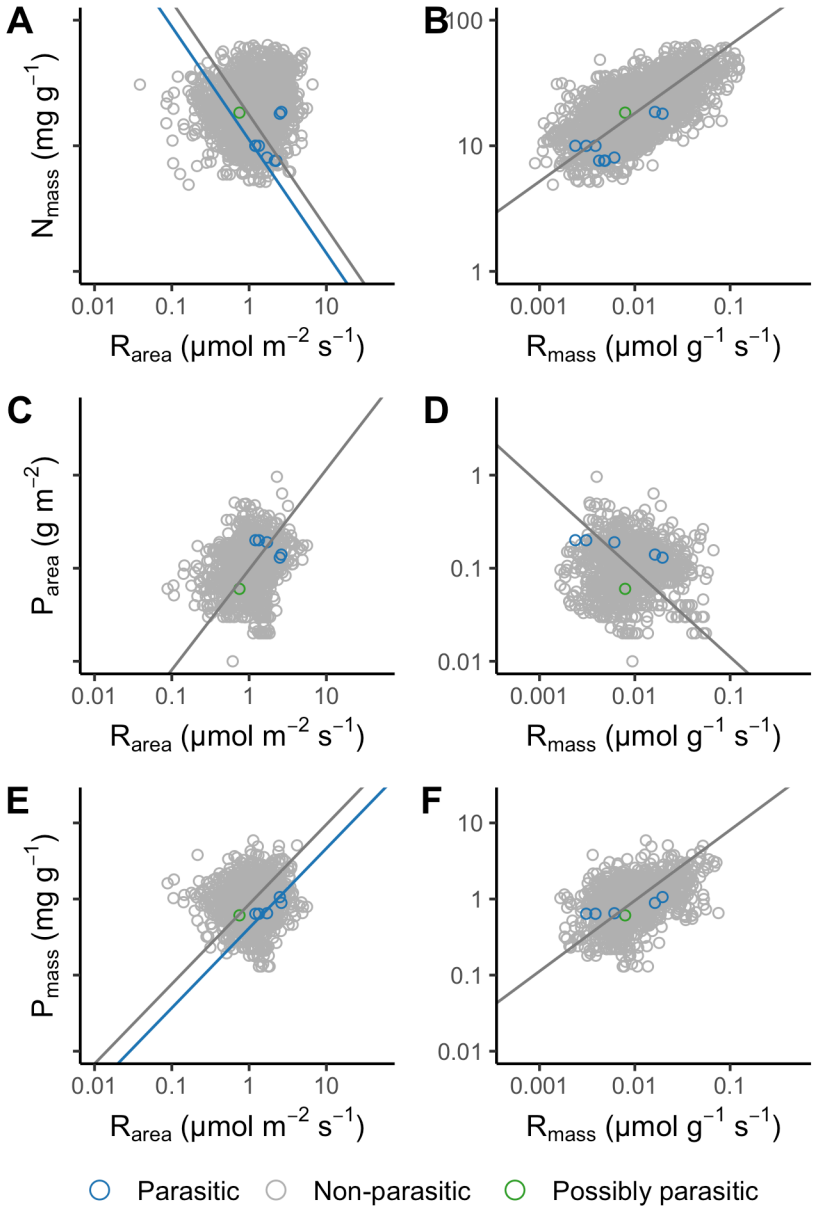

Figure 11 Pairwise leaf trait comparisons. Trendlines derived from SMA fits. See Table 7 for statistics

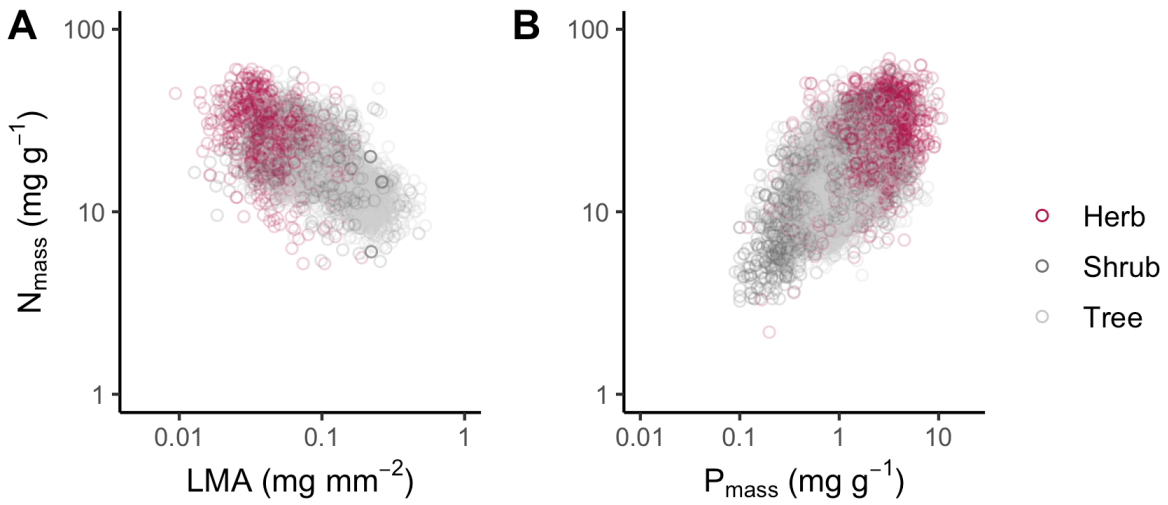

Figure 12 Two examples of pairwise leaf trait relationships by plant growth form.

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Appendix 1

Table A1-1 Results of Tukey pairwise comparisons on soil moisture (VWC%) in plots in Sagehen Experimental Forest. See Chapter 1 Table 3 for ANOVA.

Pairwise comparison		Estimate	Std. error	df	t	P-value
C. applegatei or *C. prostratus* present	*C. applegatei* or *C. prostratus* present					
N,N	N,Y	0.79	0.59	97	1.35	0.54
N,N	Y,N	1.10	0.56	97	1.97	0.21
N,N	Y,Y	-0.19	0.55	97	-0.34	0.99
N,Y	Y,N	0.31	0.67	97	0.46	0.97
N,Y	Y,Y	-0.97	0.52	97	-1.89	0.24
Y,N	Y,Y	-1.29	0.65	97	-1.96	0.21

Table A1-2 Results of Tukey pairwise comparisons on leaf traits of the parasite, *C. wightii*. See Chapter 1 Table 7 for ANOVAs. Treatment groups are (A) N -fixer and parasite together, (B) N -fixer with parasite removed in March 2016 (parasite is present for leaf collection in March 2016 and removed shortly thereafter), (C) N -fixer with no parasite present and (D) parasite with no N -fixer present. For analysis, treatment groups A and B were grouped into one treatment group: A. $\delta^{13}C$ and $\delta^{15}N$ are not included because of non-significant initial ANOVAs.

Response variable	Pairwise comparison			df	Estimate	Std. error	t	P-value
N%	Time:	March	A-D	43.133	0.076	0.111	0.685	0.497
		August	A-D	45.736	0.191	0.132	1.447	0.155
	Treatment group:	A	March-August	31.140	0.854	0.108	7.930	**<0.001**
		D	March -August	20.005	0.969	0.113	8.565	**<0.001**
C/N	Time:	March	A-D	43.167	-0.110	0.112	-0.982	0.331
		August	A-D	45.742	-0.210	0.133	-1.577	0.122
	Treatment group:	A	March -August	31.168	-0.819	0.109	-7.535	**<0.001**
		D	March-August	20.019	-0.919	0.114	-8.044	**<0.001**

Table A1-3 Results of Tukey pairwise comparisons on leaf traits of the N -fixer, *L. arboreus*. See Chapter 1 Table 7 for ANOVAs. Treatment groups are (A) N -fixer and parasite together, (B) N -fixer with parasite removed in March 2016 (parasite is present for leaf collection in March 2016 and removed shortly thereafter), (C) N -fixer with no parasite present and (D) parasite with no N -fixer present.

Response variable	Pairwise comparison			df	Estimate	Std. error	t	P-value
% N	Time:	March	A-B	56.280	0.054	0.070	0.765	0.726
			A-C	56.557	-0.028	0.073	-0.385	0.922
			B-C	57.995	-0.082	0.073	-1.120	0.506
	Time:	August	A-B	57.531	0.302	0.077	3.906	**0.001**
			A-C	57.995	0.147	0.079	1.868	0.157
			B-C	57.481	-0.155	0.075	-2.068	0.106
	Treatment group:	A	March-August	32.298	0.349	0.076	4.611	**<0.001**
		B	March-August	39.809	0.597	0.072	8.273	**<0.001**
		C	March-August	29.382	0.524	0.076	6.866	**<0.001**
C/N	Time:	March	A-B	56.280	-0.054	0.059	-0.921	0.629
			A-C	56.557	0.019	0.061	0.310	0.949
			B-C	57.995	0.073	0.061	1.195	0.461
	Time:	August	A-B	57.531	-0.251	0.065	-3.891	**0.001**
			A-C	57.995	-0.123	0.066	-1.877	0.155
			B-C	57.481	0.128	0.063	2.043	0.111
	Treatment group:	A	March-August	32.298	-0.333	0.063	-5.257	**<0.001**
		B	March-August	39.809	-0.530	0.060	-8.782	**<0.001**
		C	March-August	29.382	-0.475	0.064	-7.445	**<0.001**
$\delta^{13}C$	Time:	March	A-B	52.796	-0.286	0.424	-0.674	0.780
			A-C	52.438	-0.034	0.444	-0.077	0.997
			B-C	55.680	0.252	0.437	0.576	0.834
	Time:	August	A-B	55.404	-0.107	0.464	-0.231	0.971
			A-C	55.718	-0.840	0.469	-1.791	0.182
			B-C	54.835	-0.733	0.451	-1.627	0.243
	Treatment group:	A	March-August	30.789	-2.645	0.404	-6.551	**<0.001**
		B	March-August	37.182	-2.466	0.394	-6.258	**<0.001**
		C	March-August	28.738	-3.451	0.404	-8.537	**<0.001**
$\delta^{15}N$	Time:	March	A-B	56.280	-0.059	0.333	-0.177	0.983
			A-C	56.557	-0.283	0.349	-0.811	0.698
			B-C	57.995	-0.224	0.348	-0.643	0.797
	Time:	August	A-B	57.531	0.086	0.367	0.233	0.970
			A-C	57.995	0.053	0.373	0.141	0.989
			B-C	57.481	-0.033	0.356	-0.093	0.995
	Treatment group:	A	March-August	32.298	0.408	0.359	1.135	0.265
		B	March-August	39.809	0.553	0.343	1.612	0.115
		C	March-August	29.382	0.743	0.362	2.051	**0.049**

Appendix 2

Table A2-1 Results of planned contrasts (Day vs. Night and Plant Type) of estimated marginal means based on a linear mixed effects model on stomatal conductance measurements of *C. applegatei* ssp. *pinetorum* and three associated non-parasitic species *A. tridentata*, *C. prostratus*, and *W. mollis* in Sagehen Experimental Forest, 2015. P-values use Tukey adjustment for multiple comparisons.

Contrast	Ratio	SE	t-ratio	P-value
Day:*A. tridentata*-P— Night:*A. tridentata*-P	2.995	1.040	3.160	0.103
Day:*A. tridentata*-P — Day:*A. tridentata*+P	1.102	0.252	0.424	1.000
Day:*A. tridentata*-P — Night:*A. tridentata*+P	5.060	1.551	5.290	**<0.001**
Day:*A. tridentata*-P — Day:*C. applegatei pinetorum*-P	0.871	0.171	-0.700	1.000
Day:*A. tridentata*-P — Night:*C. applegatei pinetorum*-P	1.365	0.366	1.158	0.996
Day:*A. tridentata*-P — Day: *C. prostratus*-P	0.748	0.165	-1.314	0.989
Day:*A. tridentata*-P — Night:*C. prostratus*-P	3.799	1.299	3.904	**0.011**
Day:*A. tridentata*-P — Day:*C. prostratus*+P	0.845	0.179	-0.795	1.000
Day:*A. tridentata*-P — Night:*C. prostratus*+P	5.514	1.634	5.763	**<0.001**
Day:*A. tridentata*-P — Day:*W. mollis*-P	0.752	0.157	-1.369	0.984
Day:*A. tridentata*-P — Night:*W. mollis*-P	12.745	3.783	8.574	**<0.001**
Day:*A. tridentata*-P — Day:*W. mollis*+P	0.697	0.152	-1.652	0.928
Day:*A. tridentata*-P — Night:*W. mollis*+P	3.537	1.088	4.108	**0.005**
Night:*A. tridentata*-P — Day:*A. tridentata*+P	0.368	0.122	-3.009	0.150
Night:*A. tridentata*-P — Night:*A. tridentata*+P	1.689	0.519	1.706	0.910
Night:*A. tridentata*-P — Day:*C. applegatei pinetorum*-P	0.291	0.090	-3.986	**0.008**
Night:*A. tridentata*-P — Night:*C. applegatei pinetorum*-P	0.456	0.125	-2.872	0.205
Night:*A. tridentata*-P — Day:*C. prostratus*-P	0.250	0.081	-4.270	**0.003**
Night:*A. tridentata*-P — Night:*C. prostratus*-P	1.268	0.439	0.688	1.000
Night:*A. tridentata*-P — Day:*C. prostratus*+P	0.282	0.090	-3.980	**0.009**
Night:*A. tridentata*-P — Night:*C. prostratus*+P	1.841	0.556	2.019	0.752
Night:*A. tridentata*-P — Day:*W. mollis*-P	0.251	0.079	-4.399	0.002
Night:*A. tridentata*-P — Night:*W. mollis*-P	4.255	1.266	4.866	**<0.001**
Night:*A. tridentata*-P — Day:*W. mollis*+P	0.233	0.076	-4.472	0.001
Night:*A. tridentata*-P — Night:*W. mollis*+P	1.181	0.371	0.530	1.000
Day:*A. tridentata*+P — Night:*A. tridentata*+P	4.592	1.296	5.403	**<0.001**
Day:*A. tridentata*+P — Day:*C. applegatei pinetorum*-P	0.791	0.136	-1.362	0.984
Day:*A. tridentata*+P — Night:*C. applegatei pinetorum*-P	1.239	0.299	0.885	1.000
Day:*A. tridentata*+P — Day:*C. prostratus*-P	0.679	0.137	-1.918	0.813
Day:*A. tridentata*+P — Night:*C. prostratus*-P	3.448	1.118	3.816	**0.015**
Day:*A. tridentata*+P — Day:*C. prostratus*+P	0.767	0.145	-1.400	0.980
Day:*A. tridentata*+P — Night:*C. prostratus*+P	5.005	1.364	5.909	**<0.001**
Day:*A. tridentata*+P — Day:*W. mollis*-P	0.682	0.129	-2.022	0.751
Day:*A. tridentata*+P — Night:*W. mollis*-P	11.567	3.195	8.863	**<0.001**
Day:*A. tridentata*+P — Day:*W. mollis*+P	0.632	0.125	-2.324	0.541

Contrast	Ratio	SE	t-ratio	P-value
Day:*A. tridentata*+P — Night:*W. mollis*+P	3.210	0.913	4.099	**0.006**
Night:*A. tridentata*+P — Day:*C. applegatei pinetorum*-P	0.172	0.044	-6.888	**<0.001**
Night:*A. tridentata*+P — Night:*C. applegatei pinetorum*-P	0.270	0.059	-6.027	**<0.001**
Night:*A. tridentata*+P — Day:*C. prostratus*-P	0.148	0.041	-6.945	**<0.001**
Night:*A. tridentata*+P — Night:*C. prostratus*-P	0.751	0.233	-0.923	1.000
Night:A. tridentata+P — Day:*C. prostratus*+P	0.167	0.044	-6.742	**<0.001**
Night:A. tridentata+P — Night:*C. prostratus*+P	1.090	0.278	0.337	1.000
Night:A. tridentata+P — Day:*W. mollis*-P	0.149	0.039	-7.219	**<0.001**
Night:A. tridentata+P — Night:*W. mollis*-P	2.519	0.640	3.634	**0.027**
Night:A. tridentata+P — Day:*W. mollis*+P	0.138	0.038	-7.218	**<0.001**
Night:A. tridentata+P — Night:*W. mollis*+P	0.699	0.185	-1.349	0.986
Day:*C. applegatei pinetorum*-P — Night:*C. applegatei pinetorum*-P	1.566	0.315	2.229	0.609
Day:*C. applegatei pinetorum*-P — Day:*C. prostratus*-P	0.858	0.119	-1.101	0.998
Day: *C. applegatei pinetorum*-P — Night:*C. prostratus*-P	4.360	1.276	5.033	**<0.001**
Day:*C. applegatei pinetorum*-P — Day:*C. prostratus*+P	0.970	0.116	-0.256	1.000
Day:*C. applegatei pinetorum*-P — Night:*C. prostratus*+P	6.328	1.492	7.827	**<0.001**
Day:*C. applegatei pinetorum*-P — Day:*W. mollis*-P	0.863	0.110	-1.153	0.997
Day:*C. applegatei pinetorum*-P — Night:*W. mollis*-P	14.627	3.543	11.074	**<0.001**
Day:*C. applegatei pinetorum*-P — Day:*W. mollis*+P	0.800	0.112	-1.601	0.943
Day:*C. applegatei pinetorum*-P — Night:*W. mollis*+P	4.060	1.026	5.545	**<0.001**
Night:*C. applegatei pinetorum*-P — Day:*C. prostratus*-P	0.548	0.123	-2.677	0.305
Night:*C. applegatei pinetorum*-P — Night:*C. prostratus*-P	2.784	0.750	3.801	**0.016**
Night:*C. applegatei pinetorum*-P — Day:*C. prostratus*+P	0.619	0.131	-2.258	0.589
Night:*C. applegatei pinetorum*-P — Night:*C. prostratus*+P	4.041	0.814	6.930	**<0.001**
Night:*C. applegatei pinetorum*-P — Day:*W. mollis*-P	0.551	0.117	-2.796	0.241
Night:*C. applegatei pinetorum*-P — Night:*W. mollis*-P	9.339	1.903	10.963	**<0.001**
Night:*C. applegatei pinetorum*-P — Day:*W. mollis*+P	0.511	0.115	-2.984	0.159
Night:*C. applegatei pinetorum*-P — Night:*W. mollis*+P	2.592	0.556	4.440	**0.002**
Day:*C. prostratus*-P — Night:*C. prostratus*-P	5.080	1.550	5.326	**<0.001**
Day:*C. prostratus*-P — Day:*C. prostratus*+P	1.130	0.176	0.786	1.000
Day:*C. prostratus*-P — Night:*C. prostratus*+P	7.373	1.885	7.815	**<0.001**
Day:*C. prostratus*-P — Day:*W. mollis*-P	1.005	0.160	0.033	1.000
Day:*C. prostratus*-P — Night:*W. mollis*-P	17.040	4.426	10.918	**<0.001**
Day:*C. prostratus*-P — Day:*W. mollis*+P	0.932	0.160	-0.412	1.000
Day:*C. prostratus*-P — Night:*W. mollis*+P	4.729	1.287	5.712	**<0.001**
Night:*C. prostratus*-P — Day:*C. prostratus*+P	0.222	0.067	-5.017	**<0.001**
Night:*C. prostratus*-P — Night:*C. prostratus*+P	1.451	0.430	1.257	0.992
Night:*C. prostratus*-P — Day:*W. mollis*-P	0.198	0.059	-5.433	**<0.001**
Night:*C. prostratus*-P — Night:*W. mollis*-P	3.354	0.988	4.107	**0.005**
Night:*C. prostratus*-P — Day:*W. mollis*+P	0.183	0.057	-5.481	**<0.001**
Night:*C. prostratus*-P — Night:*W. mollis*+P	0.931	0.290	-0.229	1.000

Contrast	Ratio	SE	t-ratio	P-value
Day:*C. prostratus*+P — Night:*C. prostratus*+P	6.525	1.595	7.676	**<0.001**
Day:*C. prostratus*+P — Day:*W. mollis*-P	0.890	0.131	-0.792	1.000
Day:*C. prostratus*+P — Night:*W. mollis*-P	15.082	3.794	10.787	**<0.001**
Day:*C. prostratus*+P — Day:*W. mollis*+P	0.825	0.130	-1.224	0.994
Day:*C. prostratus*+P — Night:*W. mollis*+P	4.186	1.097	5.462	**<0.001**
Night:*C. prostratus*+P — Day:*W. mollis*-P	0.136	0.034	-8.094	**<0.001**
Night:*C. prostratus*+P — Night:*W. mollis*-P	2.311	0.558	3.468	**0.044**
Night:*C. prostratus*+P — Day:*W. mollis*+P	0.126	0.032	-8.076	**<0.001**
Night:*C. prostratus*+P — Night:*W. mollis*+P	0.641	0.163	-1.746	0.895
Day:*W. mollis*-P — Night:W. *mollis*-P	16.951	4.219	11.371	**<0.001**
Day:*W. mollis*-P — Day:*W. mollis*+P	0.927	0.148	-0.476	1.000
Day:*W. mollis*-P — Night:*W. mollis*+P	4.705	1.234	5.905	**<0.001**
Night:*W. mollis* -P — Day:*W. mollis*+P	0.055	0.014	-11.072	**<0.001**
Night:*W. mollis*-P — Night:*W. mollis*+P	0.278	0.071	-5.019	**<0.001**
Day:*W. mollis*+P — Night:*W. mollis*+P	5.076	1.378	5.983	**<0.001**

Table A2-2 Results of planned contrasts (Day vs. Night and Plant Type) of estimated marginal means within each Site (indicated here by the associated *Castilleja* species) based on a linear mixed effects model on stomatal conductance measurements of seven *Castilleja* species and associated non-parasitic species. NP indicates a non-parasite with no neighboring *Castilleja* individuals, NP + P indicates a non-parasitic species with a neighboring *Castilleja* individual(s), and P indicates a parasite of the genus *Castilleja*. P-values use Tukey adjustment for multiple comparisons.

Site	Contrast	Ratio	SE	t-ratio	P-value
C. applegatei ssp. *pallida*	NP Day — NP+P Day	1.302	0.225	1.524	0.652
	NP Day — P Day	1.086	0.188	0.478	0.997
	NP Day — NP Night	3.460	0.483	8.887	**<0.001**
	NP Day — NP+P Night	3.402	0.589	7.072	**<0.001**
	NP Day — P Night	1.768	0.306	3.290	**0.029**
	NP+P Day — P Day	0.834	0.138	-1.094	0.879
	NP+P Day — NP Night	2.657	0.460	5.646	**<0.001**
	NP+P Day — NP+P Night	2.613	0.365	6.877	**<0.001**
	NP+P Day — P Night	1.358	0.225	1.848	0.453
	P Day — NP Night	3.185	0.551	6.692	**<0.001**
	P Day — NP+P Night	3.132	0.518	6.900	**<0.001**
	P Day — P Night	1.627	0.227	3.486	**0.007**
	NP Night — NP+P Night	0.983	0.170	-0.097	1.000
	NP Night — P Night	0.511	0.088	-3.880	**0.007**
	NP+P Night — P Night	0.520	0.086	-3.957	**0.006**
C. chromosa	NP Day — NP+P Day	1.268	0.220	1.372	0.743
	NP Day — P Day	0.241	0.042	-8.212	**<0.001**
	NP Day — NP Night	2.868	0.401	7.545	**<0.001**
	NP Day — NP+P Night	3.403	0.589	7.074	**<0.001**
	NP Day — P Night	0.366	0.063	-5.800	**<0.001**
	NP+P Day — P Day	0.190	0.031	-10.028	**<0.001**
	NP+P Day — NP Night	2.262	0.392	4.714	**0.001**
	NP+P Day — NP+P Night	2.683	0.375	7.067	**<0.001**
	NP+P Day — P Night	0.289	0.048	-7.504	**<0.001**
	P Day — NP Night	11.886	2.058	14.299	**<0.001**
	P Day — NP+P Night	14.100	2.333	15.994	**<0.001**
	P Day — P Night	1.518	0.212	2.990	**0.034**
	NP Night — NP+P Night	1.186	0.205	0.987	0.918
	NP Night — P Night	0.128	0.022	-11.887	**<0.001**
	NP+P Night — P Night	0.108	0.018	-13.470	**<0.001**
C. lemmonii	NP,Day — NP+P,Day	0.987	0.171	-0.075	1.000
	NP,Day — P,Day	0.546	0.095	-3.492	**0.018**
	NP,Day — NP,Night	1.259	0.249	1.166	0.853
	NP,Day — NP+P,Night	0.415	0.089	-4.120	**0.004**
	NP,Day — P,Night	0.279	0.060	-5.977	**<0.001**

Site	Contrast	Ratio	SE	t-ratio	P-value
	NP+P,Day — P,Day	0.553	0.092	-3.576	**0.015**
	NP+P,Day — NP,Night	1.275	0.284	1.094	0.880
	NP+P,Day — NP+P,Night	0.420	0.079	-4.625	**<0.001**
	NP+P,Day — P,Night	0.283	0.059	-6.092	**<0.001**
	P,Day — NP,Night	2.304	0.513	3.753	**0.009**
	P,Day — NP+P,Night	0.760	0.157	-1.326	0.768
	P,Day — P,Night	0.511	0.096	-3.583	**0.005**
	NP,Night — NP+P,Night	0.330	0.084	-4.350	**0.002**
	NP,Night — P,Night	0.222	0.057	-5.904	**<0.001**
	NP+P,Night — P,Night	0.673	0.163	-1.639	0.581
	NP Day — NP+P Day	1.195	0.209	1.017	0.908
	NP Day — P Day	0.302	0.053	-6.845	**<0.001**
	NP Day — NP Night	1.784	0.282	3.662	**0.004**
	NP Day — NP+P Night	1.479	0.278	2.076	0.328
	NP Day — P Night	0.294	0.053	-6.850	**<0.001**
	NP+P Day — P Day	0.253	0.042	-8.311	**<0.001**
	NP+P Day — NP Night	1.494	0.279	2.150	0.292
C. linariifolia	NP+P Day — NP+P Night	1.238	0.193	1.365	0.748
	NP+P Day — P Night	0.246	0.042	-8.274	**<0.001**
	P Day — NP Night	5.909	1.103	9.516	**<0.001**
	P Day — NP+P Night	4.896	0.879	8.845	**<0.001**
	P Day — P Night	0.974	0.141	-0.181	1.000
	NP Night — NP+P Night	0.829	0.165	-0.944	0.931
	NP Night — P Night	0.165	0.031	-9.481	**<0.001**
	NP+P Night — P Night	0.199	0.036	-8.813	**<0.001**
	NP Day — NP+P Day	0.767	0.134	-1.515	0.658
	NP Day — P Day	0.390	0.068	-5.387	**<0.001**
	NP Day — NP Night	0.663	0.096	-2.853	0.051
	NP Day — NP+P Night	0.665	0.119	-2.278	0.236
	NP Day — P Night	0.873	0.153	-0.779	0.969
	NP+P Day — P Day	0.508	0.084	-4.093	**0.004**
	NP+P Day — NP Night	0.864	0.151	-0.835	0.958
C. miniata	NP+P Day — NP+P Night	0.867	0.125	-0.986	0.922
	NP+P Day — P Night	1.137	0.188	0.779	0.969
	P Day — NP Night	1.701	0.297	3.037	**0.052**
	P Day — NP+P Night	1.707	0.290	3.148	**0.041**
	P Day — P Night	2.239	0.313	5.771	**<0.001**
	NP Night — NP+P Night	1.003	0.180	0.019	1.000
	NP Night — P Night	1.316	0.230	1.572	0.623
	NP+P Night — P Night	1.312	0.223	1.599	0.606
C. nana	NP Day — NP+P Day	1.177	0.204	0.940	0.932

Site	Contrast	Ratio	SE	t-ratio	P-value
	NP Day — P Day	0.640	0.111	-2.579	0.136
	NP Day — NP Night	0.980	0.137	-0.147	1.000
	NP Day — NP+P Night	0.902	0.156	-0.598	0.990
	NP Day — P Night	0.543	0.094	-3.525	**0.017**
	NP+P Day — P Day	0.544	0.090	-3.682	**0.011**
	NP+P Day — NP Night	0.833	0.144	-1.058	0.893
	NP+P Day — NP+P Night	0.766	0.107	-1.907	0.399
	NP+P Day — P Night	0.462	0.076	-4.671	**0.001**
	P Day — NP Night	1.531	0.265	2.461	0.170
	P Day — NP+P Night	1.409	0.233	2.073	0.330
	P Day — P Night	0.849	0.119	-1.172	0.850
	NP Night — NP+P Night	0.920	0.159	-0.480	0.997
	NP Night — P Night	0.555	0.096	-3.406	**0.022**
	NP+P Night — P Night	0.603	0.100	-3.062	**0.049**
	NP Day — NP+P Day	0.968	0.168	-0.188	1.000
	NP Day — P Day	0.927	0.160	-0.440	0.998
	NP Day — NP Night	2.535	0.354	6.661	**<0.001**
	NP Day — NP+P Night	1.814	0.314	3.441	**0.020**
	NP Day — P Night	1.302	0.225	1.523	0.653
	NP+P Day — P Day	0.957	0.158	-0.263	1.000
	NP+P Day — NP Night	2.619	0.453	5.562	**<0.001**
C. peirsonii	NP+P Day — NP+P Night	1.874	0.262	4.498	**<0.001**
	NP+P Day — P Night	1.345	0.223	1.791	0.488
	P Day — NP Night	2.736	0.474	5.813	**<0.001**
	P Day — NP+P Night	1.958	0.324	4.061	**0.004**
	P Day — P Night	1.405	0.196	2.433	0.147
	NP Night — NP+P Night	0.716	0.124	-1.933	0.404
	NP Night — P Night	0.513	0.089	-3.850	**0.007**
	NP+P Night — P Night	0.718	0.119	-2.007	0.364