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A novel form and aspects of plant facilitation

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A Novel Mechanism and Aspects of Plant Facilitation

DISSERTATION

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in Ecology & Evolutionary Biology

by

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ABSTRACT OF THE DISSERTATION

A Novel Mechanism and Aspects of Plant Facilitation

by

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Professor Travis E. Huxman, Chair

Coexistence theory has largely focused on negative interactions (i.e. competition) between plant species despite growing evidence that positive plant-plant interactions, generally known as plant facilitation, are equally or more important to structuring biological communities. Facilitative plant species alleviate stressors for neighboring heterospecifics and can increase biodiversity. The literature on plant facilitation has largely focused on alleviation of stressors through shading, hydraulic lift, pollinator attraction, or other means that require well-developed canopies, roots, flowers, and other traits typically found only during late growth stages. Consequently, facilitation by seedlings of long-lived perennials has not been generally tested – if at all. Moreover, positive species interactions in plant facilitation systems are highly context dependent. Plant facilitation's Stress Gradient Hypothesis (SGH) predicts that as ameliorated stressors become less severe, interactions become competitive. This includes temporal gradients in which once long-lived beneficiaries establish and are no longer vulnerable seedlings, they will kill the plant that once nursed them. However, facilitation has not been considered in combination with other mechanisms of coexistence, such as resource partitioning or complimentary competitive effects, that may make these systems more stable. Here, a series of

greenhouse and field experiments test a hypothesis that the long-lived, competitive shrub *Eriogonum fasciculatum* acidifies soils beginning at the seedling stage and facilitates its co-dominant shrub *Artemisia californica* through alleviation of alkalinity stress in their native, calcareous soils.

In Chapter 1, a greenhouse experiment shows *E. fasciculatum* seedlings increased *A. californica* seedling shoot growth. In concurrence with the SGH and the facilitation via soil acidification hypothesis, a field study found association rates of *A. californica* with *E. fasciculatum* had a positive linear relationship with increasing soil pH. Chapter 1 highlights the importance of investigating facilitation during early growth stages, particularly if the means of stress alleviation is physiological manipulation of soil chemistry, which may begin soon after germination.

Despite plant facilitation's ecological significance, few – if any - studies investigate how tertiary invasive species affect interactions between native species in these systems. Chapter 2 asks the question: Can *A. californica* protect *E. fasciculatum* from facilitating the harmful invasive *B. nigra*? As expected in an additive field experiment, adult *A. californica* prevented *E. fasciculatum* from facilitating recruitment of *B. nigra*. In a subsequent greenhouse experiment, *A. californica* seedlings outcompeted *E. fasciculatum* for light as *E. fasciculatum* continued to facilitate *A. californica.* Surprisingly, the presence of vertical growth dominant *B. nigra* elicited *A. californica* to grow taller, mitigating *A. californica* negative effects of light competition on *E. fasciculatum* while *A. californica* reduced *E. fasciculatum* facilitation of *B. nigra.* Thus, Chapter 2 demonstrates the importance of considering facilitative systems in their biological contexts of intensely invaded ecosystems. This second round of greenhouse experiments also further supported the hypothesis that *E. fasciculatum* facilitates via acidification of alkaline soils.

Lowering soil pH mimicked *E. fasciculatum's* facilitative effects on *A. californica.* This dissertation suggests positive facilitatory interactions between long-lived competitive natives can begin in the seedlings stage, result in stable coexistence, and potentially increase invasion resistance for the larger biological community.

INTRODUCTION

The importance of understanding how interactions between plant species affect biodiversity has moved beyond foundational ecology – or even conservation biology – and into existential questions for life on earth as human activities continue to synergistically degrade, disrupt, restrict, shift, and destroy what is left of the world's biological communities (Brooker et al. 2009). However, coexistence theory has been largely preoccupied with competition despite growing evidence that positive plant-plant interactions are equally or more important to structuring biological communities (Hacker & Gaines 1997, Brooker 2007). It is generally understood that plant species tend to compete for limited resources along abiotic gradients, resulting in a few top-competitors suppressing overall diversity (Whittaker 1951, Whittaker and colleagues 1978). Whittaker's rigorous fieldwork and statistical methods empirically reinforced the Principle of Competitive Exclusion, which had already been so readily accepted by 20th Century ecologists in Western culture that theories on coexistence and mechanisms that increase diversity largely focused on population regulation of top-competitors (Brooker 2006).

Alternatively, a lesser but significant body of work with a history almost as old as *On the Origin of the Species* (Darwin 1859) attempts to explain coexistence and increased diversity through mutual aid between animal species in the extreme cold of a Siberian winter (Kropotkin 1890). Positive plant-plant species interactions are now understood to include facilitated succession, by which ecosystem engineers improve growing conditions for successional biological communities (Clements 1916), as well as positive interactions between nurse plants and facilitated recruitment of seedlings beneath nurse canopies, the more rarely known facilitation between adult plants (Bertness & Callaway 1994), or the undocumented facilitation by seedlings. Despite less attention, facilitation and other positive species interactions already appear to be of equal or greater importance to structuring biological communities compared to competition (Hacker & Gaines 1997). This dearth of representation on positive plant-plant interactions in the literature highlights that the role of facilitation to promoting coexistence and diversity is greatly underappreciated in the literature (Brooker et al. 2007). But like all things biological, facilitation is context dependent and facilitatory interactions can shift from positive commensalisms to competition. Here, however, no coexistence theory on its own could explain preliminary observations of two long-lived, competitive plants coexisting as adults at microhabitat spatial scales in their limiting, semi-arid ecosystem.

Coexistence may be inherently interesting to those of us concerned with foundational ecology, but as anthropogenic habitat degradation continues to accelerate, investigations into how interactions between species and their environment inhibit or promote coexistence, and thus affect biodiversity, are now existential questions for our species (Sandifer et al. 2015). Biodiversity is the gold standard metric of ecosystem health (Sandifer et al. 2015). Not only is biodiversity relatively easy to quantify, biodiversity correlates strongly to an ecosystem's ability to provide ecosystem services on which all life depends (Sandifer et al. 2015). Increasing diversity improves productivity and atmospheric carbon sequestration (Isabell et al. 2015) as well as resistance and resilience (Yachi et al. 1999) to disturbance – including resistance to accelerating rates of species invasions (Kennedy et al. 2002). Biodiversity provides means of population regulation of human pathogen vectors, such as mosquitos, hanta-virus carrying rodents, and deer ticks infected with lime disease (Pongsiri et al. 2009). Native plant diversity in watersheds improves water quality (Cardinale 2011) and scenic vistas to fuel tourism jobs in the post-industrial service economy (Queiroz 2014). While conservation efforts in the United States have placed approximately 25% of its lands under some form of protection (Bigelow & Allison 2012) and brought back a handful of iconic species from the brink of extinction (*e.g.* grey wolves (*Canis lupus*) and the nation's animal totem, the bald eagle (*Haliaeetus leucocephalus*), habitat loss, habitat degradation, and habitat fragmentation due to urban sprawl continues a steady pace of overall biodiversity loss (Wilcove et al. 1998), especially for less-relatable plant species (Small 2011). More disconcerting is growing evidence that climate change is already beginning to synergistically interact with habitat loss to negatively affect relative species abundances (Giam et al. 2007). For example, rising mean temperatures and shorter winters are disrupting the phenologies of angiosperms and their pollinators (Inouye 2019) and beginning to decouple when these obligate mutualists are ready to serve their pollinator-syndromes.

Like the ecosystem nutrient and water budgets they seek to preserve, efforts to preserve and restore biodiversity have limited resources. Public opinion polls show wide support for conservation (Bruskotter et al. 2018), but the budget resources of the industries driving habitat loss and degradation are almost invariably greater than those of conservation efforts. However, if we can better understand how species interactions affect biodiversity, we may enhance our limited efforts to mitigate the 6th Mass Extinction in the Anthropocene (Gilman et al. 2010).

Despite the abundance of species diversity so vast it is yet to be catalogued in its entirety (Wilson 2003), a vast majority of research focuses on how competition structures biological communities (Brooker 2006) even though the costs of competition theoretically decrease diversity (see the collected works of Whittaker). Competition was so readily accepted by 20th century ecologists in Western Europe and the Americas that it seemed intuitive to free-market culture and is evident in the nomenclature. The Principle of Competitive Exclusion, which proclaims no two species can exploit the same resource indefinitely, is called a ‘principle,’ and not considered a ‘hypothesis’ or even a ‘theory.’ Rather competition is the only supposed law in ecology (and evolution), and thus nature. Indeed, it is supported by an abundance of empirical studies. In particular to flora, the rigorous fieldwork of Whittaker and colleagues demonstrates that plant species tend to sort independently as they compete for limited resources along abiotic gradients, from the temperate forests of Appalachia (1967) to semi-arid Mediterranean-type scrublands (1972). This intense asymmetrical competition has an overall effect of a handful of top-competitors dominating landscapes with reverberations throughout food webs. Diversity is thus determined by less competitive, less common species occupying niches at the extremes of stress gradients. Instances of coexistence thus continued to challenge ecological theory.

Unsurprisingly, a vast majority of theory on coexistence continues to focus on biotic and abiotic factors regulating the abundance of top-competitors (Brooker 2006). While Neutral Theory has its proponents (*e.g.* Hubbell 1971), most research shows coexistence and increased diversity is only possible if competitive populations are regulated by reoccurring intermediate disturbance (Hutchinson 1953), consumption of top-competitors (Summerhayes 1943), differing resource ratios in heterogeneous environments (Tilman 1981), intraspecific competition (Lotka 1920; Volterra 1926), or some combination of the above (*e.g.* see Barabás et al. 2018 review on the integrated models of Chesson and colleagues).

However, human activity has degraded these means of maintaining diversity. Intermediate Disturbance Hypothesis shows moderate disturbance regimes, such as not-to-severe wildfire and flooding, can make habitat available for weaker competitors through removal of dominant species, but extreme disturbance reduces the abundance of all species (Hutchinson 1953). Today, however, even the most reliable disturbance regimes, have been fundamentally altered. Bungled, industry-focused forest management and climate change-exacerbated droughts fuel the extreme disturbances of megafires in California and other parts of the world (Williams 2013). Dams and concrete flood channels constructed for reservoirs, flood mitigation, and hydroelectricity convey a mundane consistency of utterly destroyed riparian habitat and minimized disturbances, unless a dam is breached or levees give way to the physics of river water, resulting in massive flooding (Poff et al. 2007). Fear-mongering by ranching interests prevents reintroduction of keystone predators on land, such as grey wolves (*Canis lupus*) (Niemiec at al. 2020). Where the wolves have been successfully reintroduced, such as Yellowstone National Park, the value of their top-down regulation is evident to the entire ecosystem as the trophic cascade led to the return of riparian vegetation that reduces sediment loading and allowed the Yellowstone River to deepen its channel (Bestcha & Ripple 2006). Besides the livestock industry, culinary traditions are driving extinctions of aquatic apex predators, such as sharks and blue-fin tuna (Brierly 2007). The loss of predator-mediated coexistence allows top-competitors in lower trophic levels to displace weaker competitors (Shurin et al. 2002) and the now more abundant top-competitors among primary consumers to decimate populations of foundational primary producers (Schmitz et al. 2000). The homogenization of ecosystems by global trade-driven introduction of non-native species (Keller et al. 2010), timber interests (Löf et al. 2016), and reduction of remaining habitat to tiny isolated patches by rampant urban sprawl (Felipe-Lucia et al. 2020) affords inadequate space for heterogeneity of resources and undermines the effects of resource ratios and niche differentiation on maintaining diversity. The increasing absence of factors regulating competitive populations thus renders classical coexistence theory of decreasing utility to the modern ecologist.

At the turn of the 21st century, Chesson and colleagues began integrating coexistence theory into models of transitive as well as relatively stable coexistence in what has been dubbed "modern coexistence theory". For example, by integrating resource partitioning and density-dependent invasion biology, his models show how species A that competes more with itself than with species B may still invade species B's territory until species A reaches a density threshold at which point it starts competing more with conspecifics in a type of allee effect (Chesson 2000). Or his Storage Effects model explains if a drought-deciduous shrub is more competitive in the rainy season, an evergreen shrub may be more competitive for uncommon rains and dew in the dry season. Over time, mean annual competitiveness of species A and B may become equal and competitive exclusion avoided. While he folded in a variation on neutral theory, his models are still fundamentally based in the assumption that the negative effects of top-competitors must be dampened in some way for species to achieve coexistence (Barabás et al. 2018).

This emphasis on suppressing competition ignores a lesser but significant body of work demonstrating positive plant-plant interactions, known as plant facilitation, can also promote coexistence and increased diversity (Hacker & Gaines 1997). Perhaps as a reflection of Anglo-American culture, it was the naturalist and Russian prince turned exiled anarchist philosopher Kropotkin who offered the first modern proposition arguing mutual aid between and among species is as important a force in evolution and species interaction as competition (1861). However, he pre-occupied his qualitative studies concerning cooperating populations in the harsh environments of the Siberian wilderness with philosophical arguments against early industrialists who exploited Darwin’s theories to justify subjugation of craftsmen for factory work and racist colonial hierarchies. Not long after Kropotkin, the American ecologist Clements (1916) proposed a theory on facilitated succession, in which facilitatory species improve growing conditions in harsh environments for successional communities, but his ideas were not well-supported with rigorous statistical analysis and were more or less abandoned following criticism of his notions of the global “super organism.” Decades later, Clements’ work on facilitated succession found some vindication in research on ecosystem engineers in successional communities. For example, pioneer species, such as N-fixing lupine-rhyzobia mutualists and lichens, colonize and improve growing conditions in areas left void of topsoil in the wake of massive disturbances, such as volcanic eruptions (Moral & Wood 1993) and glacial retreat (Chapin et al. 1994). But as Clements (1936) previously hypothesized, facilitators are displaced by the very species they facilitate, as subsequent successional communities tend to be more competitive by necessity within the context of enhanced habitat stability. Coexistence between the facilitator and facilitated is once again maintained by abiotic disturbance, such as a landslide, which allows facilitators still present in the seedbank to reestablish in the habitat mosaic (Mouw et al. 2012). In the end, facilitated succession's primary concerns are species interactions in dynamic ecosystems undergoing fundamental changes to biological communities.

In non-successional habitat, positive plant-plant interactions, generally known as plant facilitation and codified by the stress gradient hypothesis (SGH) (Bertness & Callaway 1994), can also promote coexistence and increased diversity (Brooker et al. 2008). Facilitator species improve growing conditions for neighboring heterospecifics through amelioration of biotic and abiotic stressors (Bertness & Callaway 1994). The SGH predicts that as stress gradients increase in severity, facilitated plants increase spatial associations with their benefactor, but interactions are not always positive, or even neutral. As the severity of a stress gradient decreases, either over space or as factors that once threatened vulnerable seedlings are no longer stressors for established adults, the SGH predicts interactions will shift toward competition and negative spatial associations. The SGH is still being refined but is well-supported in the literature (Callaway 2007).

Facilitation research has primarily concerned itself with nurse plant systems. Similar to other facilitative interactions, nurse plant facilitation is the indirect result of physiological activities that facilitators otherwise endeavor to alleviate stress for themselves, but in arid and semi-arid environments, nurse plants commonly facilitate other plant species and increase diversity through shading (e.g. Forseth et al. 2001), hydraulic lift (e.g. Caldwell et al. 1998), N-fixation (Pugnaire et al. 1996), or mobilization of nutrients (Carillo-Gracia et al. 1999). In limited cases, beneficiaries reciprocate through nutrient and moisture retention (Sortibrán et al.

2014; Navarro-Cano et al. 2015). Highlighting the fleeting stability nurse plant systems provide, nurse plants are further defined by a lack of traits for selecting which species are facilitated and commonly recruit harmful invasives (Badano et al. 2007; Von Holle 2013; Badano et al. 2015) and native competitors (e.g. Valiente-Banuet et al. 1991; Flores-Martinez et al. 1994). Despite nurse plant facilitation's non-selective nature, Valiente-Banuet et al. (1991) show roughly half of facilitated species maintain positive interactions throughout their life cycles with nurse plants. However, my analysis of life-histories of their target species, as well as target species in >100 other studies on nurse plant facilitation indicates that if a beneficiary is long-lived (>2-3yr), it eventually displaces the nurse from their once-shared microhabitat. Maestre et al. (2009) attribute this temporal dynamic from commensalism to competitive exclusion to the tendency of nurse plants to possess adaptations focused on stress-tolerance and long-lived beneficiaries to employ competition-focused evolutionary strategies.

While studies on facilitated primary succession and nurse plant systems provide valuable insights into mechanisms of community assembly, the tendency of focusing on these well-established categories of facilitation risks discovery of novel forms of facilitation between adults or seedlings. In their foundational paper on the SGH, Bertness & Callaway (1994) argue there is no reason why facilitation cannot occur between adult plants and presented such an interaction in marsh grasses alleviating salinity stress. More recent examples of facilitation between adults include pollinator attraction (*e.g* Ogilivie & Thomson 2016) and herbivore defense (e.g. Heil & Karban 2010), but they remain relatively rare in the literaure (Brooker et al. 2007). Further, few – if any – studies have considered pre-adult growth forms to be capable of alleviating stressors for neighbors. The focus on adults may partially be due to the fact that stress amelioration is commonly achieved through shading, hydraulic lift, pollinator attraction, or other means that require the well-developed canopies, deep roots, and flowers only found in adult growth forms. But as Bertness and Callaway (1994) argue that the bias on competition in community ecology may largely be the result of a bias toward ecosystems in which competition is obvious, the bias toward facilitation studies with adult facilitators and seedling beneficiaries may be due to a bias toward ecosystems in which facilitation is obvious. These biases are disconcerting because identifying and understanding novel mechanisms driving positive spatial associations and stable coexistence are vital to conservation efforts as human activity continues to accelerate its synergistic degradation of what remains of the world's biomes (Brooker et al. 2008). Positive species interactions conveying coexistence to native top-competitors that can compete with invasives will be even more valuable as a means of mitigating non-native plants that fundamentally alter the vegetative landscape and degrade habitat and associated ecosystem services.

STUDY SYSTEM

California coastal sage scrub (CSS) is a threatened, limiting Mediterranean-type habitat characterized by co-dominant shrubs, invasive annuals, seasonal droughts, fire regimes, and layered nutrient stressors, making this threatened ecosystem a good (and potentially valuable) candidate to investigate mechanisms of coexistence between competitive, long-lived plant species. A vast majority of unburned CSS sites are co-dominated by a habitat-level vegetative alliance between the long-lived (>25yr) woody shrubs California buckwheat (*Eriogonum fasciculatum*) and California sagebrush (*Artemisia californica*) (Westman 1981). *E. fasciculatum* and *A. californica* are also the most common woody plants to first establish after wildfires (Kirkpatrick & Hutchinson 1977), which means they must begin to coexist at germination, at least at a habitat level. Regardless of their well-documented co-dominance, research to date has yet to provide mechanisms for coexistence between these long-lived, top-competitors. Indeed, Westman (1981) identified substrate preferences driving niche differentiation for the ten most common co-dominant CSS shrubs, except *A. californica.* Kirkpatrick & Hutchinson (1977) discussed – but did not test or observe – that because of its broad realized niche and competitiveness, *A. californica* would eventually displace *E. fasciculatum,* unless wildfire disturbance burned shoot tissues down to smaller growth stages.

Here, however, adult *E. fasciculatum* and *A. californica* with canopies >1m d were observed growing in immediate proximity and at near exclusion of all other plant life in the UC Irvine's CSS Ecological Preserve, which the preserve manager said has not burned since at least as far back as the founding of the university over half-a-century ago in 1965. Subsequent statistical analysis testing patterns of spatial association across five CSS sites confirmed that adult *E. fasciculatum* and *A. californica* share an overall positive spatial association starting at scales of $1m^2$ (belt transects here) and 1m (independent data source).

Still, no coexistence theory on its own offered plausible mechanisms for the positive spatial associations between adult *E. fasciculatum* and *A. californica.* The exclusion of other plants as well as co-dominance in relatively stable habitat patches (Kirkpatrick & Hutchinson 1977) indicate both shrubs are competitive. Shade-intolerance and similarities in canopy morphology (Sawyer et al. 2003) suggest niche overlap, and yet the shrubs exhibit no obvious variance in aboveground growth forms whether together, solitary, or clustered with conspecifics. Patches of adult conspecifics are common (personal observations), suggesting neither shrub competes more with conspecifics than interspecifically. Before unmitigated real estate development reduced CSS to <10-15% of its historic range, regular disturbances by wildfires approximately every 25yr improved diversity by temporarily removing co-dominant shrub canopies (Westman 1981a), but the habitat in which the shrubs were initially observed coexisting has not burned in over half-a-century. The tendency for exclusively shared microhabitats discouraged hypotheses based in resource ratios. Chesson's invasion-based coexistence models could explain associations between younger plants but were not promising mechanisms for the frequent close spatial associations between adults. His Storage Effects model potentially explains neutral interactions and avoidance of exclusion, but not positive association at microhabitat scales. Nurse plant facilitation results in positive spatial associations, but once long-lived, competitive beneficiaries establish as adults, they destabilize these systems (Maestre et al. 2009). In productive environments, in which space is more limiting than nutrients and water, classical coexistence theory based in suppression of competition can sometimes explain positive spatial associations, but in drylands, where space is not as limiting as nutrients or water, positive spatial associations indicate positive facilitative interactions between plants (Arroyo et al. 20XX). However, facilitation between adult plants is very rare, especially between top-competitors, such as *E. fasciculatum* and *A. californica.* And so, the question remains as to how two long-lived, competitive plants share a positive and stable association at fine spatial scales in their severely limiting habitat. However, no research to date, of which I am aware, considers nurse plant facilitation in combination with other mechanisms of coexistence.

PHYLOGENIES AND NATURAL HISTORIES LEADING TO GENERAL HYPOTHESES

E. fasciculatum and *A. californica* assembled with their historic CSS plant communities as glaciers retreated from Southern California in the waning millenia of the Plastocene (Axelrod 1977) with several niches pre-differentiated. *A. californica* is a drought-deciduous, wind-pollinated shrub while *E. fasciculatum* maintains its canopies and inflorescence throughout all but the driest of years. *E. fasciculatum* is opportunistically deep-rooted (Kummerow et al. 1977; Hellmers et al. 1995) and *A. californica* has only shallow, fibrous roots (Westman 1981b). Similar variances in root morphology and drought-behavior between species permits groundwater partitioning in other systems, but *A. californica* roots remain metabolically active in CSS's long, dry season to quickly uptake dew or uncommon offseason rains (Westman 1981b). With sufficient moisture, *A. californica* grows a secondary leaf morphology in the summer, keeping it competitive whenever water is available (Gray 1982). *E. fasciculatum's* lignified roots can penetrate compacted soils up to 2.5m deep, but with its lateral roots that spread >3m d, the shrub prefers shallow soils with a thin humus layer (Kummerow et al. 1977; Hellmers et al. 1995).

Even if *E. fasciculatum* and *A. californica* can minimize water competition through some degree of groundwater partitioning, CSS's severely limited availability of nutrients would likely drive intense competition between co-occurring long-lived plants. When it rains, CaCO3 from ancient seashells in CSS's uplifted marine sediments leach into the groundwater, generating layered nutrient stressors associated with alkaline, calcareous soils (Rundel 2007). Rising mean annual temperatures are exacerbating seasonal and interannual droughts, in which nutrients are immobilized in undecomposed litter (Allison et al. 2013). However, if additional nutrients were made available – for example by a nurse plant acidifying CSS's alkaline soils - the costs of competition for nutrients would hypothetically be reduced and otherwise competitive species could coexist in microhabitat-scale positive spatial associations.

However, the bulk of research on CSS co-dominant shrub alliances predates foundational work on nurse plant facilitation and SGH. Consequently, the literature has never considered CSS's multiple potential stressors along their gradients, and how these abiotic gradients relate to species interactions between competitive shrubs at fine spatial scales. It is unsurprisingly then that facilitation has never been documented for *E. fasciculatum* or *A. californica,* but multiple studies demonstrate that the buckwheat family (Polygaceae) cover crop *Fagopyrum esculentum* acidifies soils to mobilize P otherwise bound to undecomposed litter and unavailable to plants (*e.g.* Possinger et al. 2013). CSS's alkaline, calcareous soils also immobilize nutrients, but acidification of similar soil profiles in other systems improves growing conditions for several otherwise stressed species (Pan et al. 2016). Considered together, these factors led to a series of experiments testing a general, Facilitation hypothesis: *E. fasciculatum* ameliorates layered nutrient stressors associated with CSS's alkaline, calcareous soils through soil acidification and thus facilitates any plant species that can tolerate its competitive effects. Indeed, nurse plant facilitation drives positive spatial associations in other moderately stressful Mediterranean climates (*e.g.* South Africa; Australia), but long-lived, competitive beneficiaries (>2-3yr) tend to eventually displace their benefactor (Maestre et al. 2009), and *A. californica* is a top-competitor. In addition to staying competitive for fleetingly available water in the dry season, *A. californica* inhibits recruitment of other plant species with allelopathic leaf leachates (Muller 1966) and sheltering mammalian seed predators (Bartholomew 1970). However, some native species are immune to the allelopathy (Halligan 1973; Karban 2007) while sheltered seed predators preferentially forage for the larger seeds of annuals (Halligan 1975). *E. fasciculatum* seeds, while larger than those of *A. californica,* are smaller than the seeds of most grasses and forbs. These factors led to a second general, Tolerance Hypothesis: Unlike other nurse plants, which cannot select beneficiaries, mutual tolerance of each other's competitive effects conveys exclusive facilitation of *A. californica* by *E. fasciculatum* and stability to the shrubs' positive spatial association and coexistence throughout the entirety of their ontongenies.

Chapter 1 explores mechanisms of *E. fasciculatum* nurse plant facilitation. Specifically, this study asks 1) Does *E. fasciculatum* acidify alkaline soils and facilitate *A. californica*? 2) Do association rates of *A. californica* and *E. fasciculatum* increase along a gradient of increasing soil pH in a manner consistent with SGH? Of course, if *E. fasciculatum* facilitates *A. californica* through alleviation of high soil pH, alkaline soils must be shown to be a stressor on *A.*

californica. So a final question of chapter 1 is 3) Does increasing soil pH reduce the abundance of *A. californica*?

Chapter 2 explores the interactions between *E. fasciculatum* and *A. californica* in their current ecological context of intensely invaded habitat. Invasive annuals have now displaced nearly all native grasses and forbs (Bowler 2000). One of the most prominent non-native plants is the allelopathic forb black mustard (*Brassica nigra*). Like other mustard species, *B. nigra* does not associate with mycorrhizae. *B. nigra* is thus able to facilitate its own invasiveness with a suite of chemicals, including isothiocyanate, in its root exudates that not only inhibit germination of other species but also indirectly harm their growth by killing mycorrhizae (Tawaha & Turk 20011). Still, co-dominant, native shrubs remain competitive as adults against invasives, but shrub seedlings compete poorly with fast-growing non-native annuals, and thus restrict further dispersal of otherwise competitive shrubs (Eliason & Allen 1997). Specifically, this study uses a greenhouse competition-facilitation experiment, complimented with *B. nigra* recruitment inhibition field experiments to answer the following questions: 1) Does *E. fasciculatum* facilitate *B. nigra* germination and growth? 2) Does *A. californica* inhibit the growth, survival, and reproductive potential of *B. nigra?* 3) If *A. californica* harms *B. nigra* and *B. nigra* harms *E. fasciculatum* in pairwise interactions, does *E. fasciculatum* tolerance of *A. californica* convey mutual defense against *B. nigra* when the shrubs grow in immediate proximity? Or does *E. fasciculatum* facilitation and *A. californica* competitive effects work antagonistically? To explore the greater significance of the ongoing competition between remaining native shrubs and invasive annuals in the field, multi-year transect data on the spread of *B. nigra* is analyzed. In particular, year-to-year association patterns between *B. nigra, E. fasciculatum, A. californica,* or other co-dominant native shrubs are analyzed for patterns consistent with facilitation, competition, or neutral interactions. Patterns of association for other invasive annuals and native shrubs with *E. fasciculatum* and *A. californica* growing within 1m of each other are compared to when *E. fasciculatum* and *A. californica* grow apart. Finally, a *B. nigra* legacy effects field experiment tests whether *E. fasciculatum* seedlings can facilitate germination and establishment of *A. californica* seedlings in soils from the previous year's *B. nigra* stands compared to soils taken from microhabitats of coexisting *E. fasciculatum* and *A. californica.*

Chapter 1:

Facilitation among seedlings and stable coexistence in late growth stages of two long-lived, co-dominant shrubs.

Abstract

While many models of species coexistence focus on how competition defines biological communities, over recent decades, a number of studies show positive plant-plant species interactions (facilitation) can also promote stable coexistence. The long-lived, co-dominant shrubs California buckwheat (*E. fasciculatum*) and California sagebrush (*A. californica*) share a well-documented positive association at the habitat level in their native California coastal sage scrub ecosystem, but mechanisms underlying their interactions remain unclear at finer spatial scales. Here, a hypothesis that *E. fasciculatum* acidifies CSS's alkaline soils and facilitates *A. californica* through amelioration of nutrient stress is tested in a greenhouse experiment and association tests in the field. Greenhouse results demonstrate facilitation at early growth stages and field observations show positive associations between *A. californica* and *E. fasciculatum* that have a positive linear relationship to increasing soil pH. In later life stages, water competition is known to determine the shrubs' interactions with each other. These results highlight the importance of understanding lifecycle-long interactions among species in evaluating facilitation's impacts on community structure.

Introduction

Models of species coexistence continue to focus primarily on negative species interactions (i.e. competition) as the primary drivers shaping plant communities despite the recognized ecological importance of positive interactions (Brooker 2006). While Neutral Theory has its proponents (Hubbell 1971), a preponderance of research implies coexistence is possible due to deterministic processes that reduce the abundance of competitive species. Factors mitigating competition include disturbance (Hutchinson 1953), herbivory (Summerhayes 1941), differing resource ratios (Tilman 1981), intraspecific competition (Lotka 1920; Volterra 1926), or some combination of the above (*e.g.* see Barabás et al. 2018 review on the integrated models of Chesson and colleagues). Alternatively, resource partitioning allows species to avoid interspecific competition all together in neutral interactions but may lead to greater intraspecific competition as individuals within a species' population vie for fewer resources (Chesson 2000). In recent decades, a growing but still relatively small body of literature has been investigating positive plant-plant interactions, known as plant facilitation (Michalet & Pugnaire 2016), however the importance of such interactions for coexistence is relatively unknown.

Facilitative plant species improve growing conditions for other species through the amelioration of stressors (Bertness & Callaway 1994) but past studies have focused almost exclusively upon facilitation by late growth stage plants, and little is known of such effects emanating from seedlings (Brooker et al. 2007). The ameliorated stressors include both biotic and abiotic actors, such as herbivory or salinity stress, respectively (Bertness & Callaway 1994). The most commonly studied plant facilitation systems are nurse plants, where plants at late growth stages ameliorate stressors for heterospecific seedlings beneath their canopies through shading or other means (Smit et al. 2007). This focus on facilitators in late growth stages may be due to the fact that observed stress amelioration is often achieved through shading (e.g. Forseth et al. 2001), hydraulic lift (e.g. Caldwell et al. 1998), pollinator attraction (e.g. Ogilivie & Thomson 2016), or other means that require well-developed canopies, deep roots, or flowers not typically present during early growth stages. Consequently, few – if any – studies have considered whether perennial facilitators are capable of facilitation beginning in their early growth stages.

Unlike alleviation of aboveground stressors or hydraulic lift, alleviation of other belowground stressors (e.g. nutrient stress) can potentially begin soon after germination and initiation of root growth. For example, lupines (*Lupinus spp.*) begin soil nitrogen-enrichment in early growth stages whether species are annuals (Arnone & Gordon 1990) or perennials

(Rudgers & Maron 2003), improving soil nutrient conditions for successional communities. While these examples do not show plants improving growing conditions for cooccurring heterospecifics, they demonstrate the potential for physiological alteration of soil conditions beginning soon after germination.

Adding further nuance to the study of plant facilitation systems is the fact that facilitated community interactions do not always remain positive, as plant facilitation's defining Stress Gradient Hypothesis (SGH) explains. The SGH suggests that facilitation and competition can occur simultaneously (Bertness & Callaway 1994). The SGH predicts positive associations increase as the severity of ameliorated stressors increase. When stressors decrease in severity over heterogenous spatial gradients or as facilitated species establish over time, competition begins to define species interactions (Malkinson & Tielbörger 2010). For example, in the semi-arid valley of Zapotitlán, Mexico, adult growth forms of the facilitative nurse shrub *Mimosa luisana* shade seedlings of the cactus *Neobuxbaumia tetetzo* (among other heterospecifics), which are vulnerable to soil water evaporation and desiccation (Valiente-Banuet et al. 1991; Flores-Martinez et al. 1994). Once facilitated *N. tetetzo* establish and are less vulnerable to water-stress, the cactus can shade the shade-intolerant *M. luisana* and kill the plant that once facilitated it. Thus, the shift to competitive interactions drives negative spatial associations and unstable coexistence over time.

This study tests a hypothesis for a mechanism by which the long-lived (25+ yr) co-dominant shrubs California buckwheat (*Eriogonum fasciculatum*) and California sagebrush (*Artemisia californica*) coexist from seedling to late growth stages in California coastal sage scrub (CSS) habitat. CSS is defined in part by gradients of alkaline, calcareous soils, which inhibit nutrient uptake (e.g. Fuglsang et al. 2007). Plants from the buckwheat family (Polygonaceae) are known to improve growing conditions with acidic root exudates that mobilize nutrients (Possinger et al. 2013). Based upon this potential mechanism of facilitation and *E. fasciculatum* and *A. californica* co-occurring dominance in a majority of CSS habitat (Kirkpatrick & Hutchinson 1977), this study sought to answer two questions: 1.) Do *E. fasciculatum* seedlings acidify alkaline soils and facilitate the growth of *A. californica* seedlings? 2.) Are spatial patterns of co-occurrence among late growth stage plants consistent with facilitation and the SGH along a gradient of increasing soil pH? The study of interactions between seedlings is becoming more important to our understanding of ecology as fire regimes increase in frequency in Southern California, as well as many other parts of the world, preventing biological communities from reaching late successional stages.

METHODS

Target Species

Eriogonum fasciculatum is a long-lived (> 50 yr) (Sawyer et al. 2009), drought-tolerant, facultatively evergreen shrub (Westman 1981b) that, along with *A. californica,* co-dominates a majority of CSS habitat undisturbed by wildfire for >25 yr (Kirkpatrick & Hutchinson 1977). Roots can penetrate compacted soils up to 2.5 m deep but commonly spread >3 m laterally (Kummerow et al. 1977; Hellmers et al. 1995). *E. fasciculatum* tolerates shallow soils with thin humus layers, in which it outcompetes most other plants (Kirkpatrick et al. 1977). Canopies generally grow 0.6-0.8 m tall and >1 m wide (USDA NRCS).

Artemisia californica is a long-lived (25+ yr; Sawyer et al. 2009), wind-pollinated, drought-deciduous shrub that co-dominates 96 % of CSS patches with other perennials (Westman 1981a). Roots remain fibrous and shallow throughout the shrub's ontogeny (Gordon & White 1994). Canopies generally grow 0.6 – 1.5 m tall and >1 m wide (USDA NRCS). *Artemisia californica* senesces its winter rainy season leaves in the summer, but keeps its shallow, fibrous roots metabolically active in the dry season to rapidly uptake water from morning dew and offseason rain events to regrow a secondary leaf morphology (Westman 1981b).

Greenhouse Experiments

A primary goal of this study was to test if *E. fasciculatum* seedlings facilitate *A. californica* seedlings under alkaline soil conditions. *E. fasciculatum* and *A. californica* seedlings were grown in mono- and polycultures for 120 days in the University of California Irvine greenhouse. *Eriogonum fasciculatum* seeds were gathered from CSS at UC Irvine's Ecological Preserve in mid-to-late July, 2015. Due to low seed viability of genetically local *A. californica* seeds, *A. californica* seeds were purchased from irrigated shrubs from Larner Seeds (Bolinas, California). *A. californica* and *E. fasciculatum* were geminated in monoculture sand trays in the UC Irvine greenhouse for 12-15 days in July 2015 until the first true leaf emerged. Seedlings were then transplanted to homogenized 7:2:1 autoclaved potting soil:sand:gravel medium in 7 L pots. Soil microbes and mycorrhizae have been shown to mediate positive plant-plant interactions (*e.g.* Johnson et al. 1997, Sortibrán et al. 2014, Montesinos-Navarro et al. 2016), so pots were inoculated with 300 *g* of live field soil taken from stands of coexisting *E. fasciculatum* and *A. californica*. Eight monoculture controls for each species contained two seedlings, and eight polycultures contained one seedling of each species. The study system includes direct interactions between roots, which were observed to be entangled. It was not possible, then to separate entangled roots without substantial tissue loss and root DW and root:shoot ratios were not included in analysis. To minimize light competition, pots were rotated 90° to the next clock-wise cardinal direction 3x weekly and relative positions of pots were re-randomized weekly within the greenhouse. Pots were fertilized with water soluble fertilizer thrice weekly delivered in “hard” alkaline (pH=7.9-8.4), Ca^{2+}-rich (28-90 $mg \cdot L^{-1}$) tap water (2018 IRWD Report) to mimic extreme calcareous, alkaline soil field conditions. Greenhouse tap water pH recordings with a HANA field meter (HANA Instruments, Smithfield, RI) showed mean water pH of 7.8 – 8.7. After 120 days, shoot tissue was harvested and dried in open envelopes in the greenhouse for five days. Shoot Dry Mass (DW) was recorded for five replicates of each treatment due to potential root damage from pH readings in each treatment’s remaining three replicates (see below). To compare the effects of intra- and interspecific interactions on shoot growth performance for each shrub species, a single-factor, fixed-effects ANOVA with Tukey HSD post hoc test (Rstudio v 0.98.1062) analyzed variance in mean shoot DW in each treatment as the dependent variable and each target species in its monoculture and the polyculture were independent variables. Shapiro Wilk’s tests confirmed normality in the data. A decrease in mean shoot DW was interpreted as negative competitive interactions. No difference in mean shoot DW was interpreted as neutral interactions or ‘tolerance.’ An increase in shoot DW in the polyculture treatment was interpreted as facilitation.

To record the effect of *E. fasciculatum* on soil acidification in the greenhouse, a subset of three pots from each treatment were used for pH readings directly from the rhizosphere. A HANA Field pH meter (HANA Instrument, RI) with fresh electrode was plunged directly into the rhizosphere (10-12 cm deep) to take mean pH readings from three plunges in each pot at 0 h, 1 h, and 24 h following flooding mesocosms with “hard” tap water. A repeated measures ANOVA (Rstudio v 0.98.1062) was used to analyze variance in pH over the 24 hr period, with mean pH at each time interval as the dependent variable vs. *E. fasciculatum* monocultures, *A. californica* monocultures, or polycultures as the independent variable and time intervals as a fixed effect.

Field Experiments

Positive spatial associations among plant species can be driven by similar habitat requirements in more mesic and productive ecosystems, but in drylands positive spatial associations are commonly interpreted as indicators of facilitative interactions that would otherwise compete for restricted water and nutrients (Saiz & Alados 2012; Soliveres et al. 2014). In order to test if positive species interactions occur between *E. fasciculatum* and *A. californica* during late growth stages, an analysis of spatial associations in the field was conducted.

The presence of *E. fasciculatum* and *A. californica* in late growth forms (canopies > 1 m diameter) as well as all other plant species grouped together were recorded in 560 1m^2 quadrats along 56 1 x 10 m belt transects. Sampling sites were located in five CSS preserves and nature parks (see Table S1) along a N-S coastal gradient from San Onofre State Beach (33.376068, -117.567275) to Zuma Canyon State Park in Malibu (34.044757, -118.809499). At each site, the 10 m^2 quadrats were haphazardly chosen to filter microsites that supported growth of both target species. Within each 10m^2 quadrat, three parallel belt transects were randomly sampled, except in Zuma Canyon and San Onofre Beach. In these two latter sites, 1x10 m belt transects were sampled in the only areas in which *E. fasciculatum* and *A. californica* were observed growing: along washes with thin soils in Zuma Canyon and along the edges of vernal pools atop seaside bluffs in San Onofre Beach. Associations were analyzed at 1 m^2, because it was assumed *E. fasciculatum, A. californica* and other species would interact at a species-to-species level for limited water, nutrients, and light.

The 56 belt transects were aggregated with species data in five 50 m point-intercept transects from five additional CSS sites that were independently surveyed the same year by UC Irvine's Center for Environmental Biology (CEB) along a coast-to-inland gradient (see Table S1 for locations). The presence of *E. fasciculatum, A. californica* and all other plant species grouped together in CEB transects was counted in adjacent 1m blocks to conform surveying methods as much as possible with belt transects. For example, if *E. fasciculatum* was present at the 4 m point-intercept and *A. californica* was present at the 5 m point-intercept, then they were counted as co-occurring at a scale of 1 m. Since both shrub species typically have canopies > 1m in late growth stages, these counting methods mean some shrubs were likely counted twice, but these methods better reflect the relatively large area of vegetative cover and effects on the distribution of other species that *E. fasciculatum* and *A. californica* in adult growth forms exhibit.

Spatial associations were analyzed with chi-squared contingency tests (Rstudio v 0.98.1062), as indicators of negative, positive, or neutral species interactions. To test a prediction that *E. fasciculatum* and *A. californica* in adult growth forms need to partition space to accommodate their > 1 m diameter, shade-intolerant canopies, chi-square analysis was also run on associations at point-intercepts (0 m) in CEB transects. Additionally, we were interested in whether *E. fasciculatum* and *A. californica* did not share a positive spatial association with other plant species. The exclusiveness of the spatial associations between the competitive shrubs were analyzed by comparing the number of *E. fasciculatum* and *A. californica* that occurred within 1 m^2 of any other plant species when each shrub species grew within 1 m^2 of the other shrub species and when each shrub species grew > 1 m^2 apart from the other shrub species using a series of 2x2 chi-squared contingency tests.

To test whether increasing soil alkalinity negatively affects absolute *A. californica* abundance and if *A. californica* association rates with *E. fasciculatum* differ significantly among sites with a gradient in soil pH, a multifactor logistic regression (Rstudio v 0.98.1062) was used to analyze interactive effects of *E. fasciculatum* abundance and mean soil pH on *A. californica* abundance from belt-transects at the five sites sampled here. The SGH predicts that association rates of a beneficiary species with a facilitative species will increase as the alleviated stressor increases in intensity, even as the absolute abundance of the beneficiary decreases with increasing stress (citation). Likewise, demonstrating that the hypothesized stressor is indeed a stressor on a proposed beneficiary and negatively affects its abundance is important to demonstrate when testing the SGH. In other words, increasing soil alkalinity must be shown to interact negatively with the abundance of *A. californica*.

Because the logistic regression analysis above showed association rates differing significantly among sites and their respective soil pH ($p = 0.0140$, Table 1), a linear regression analysis was used to test for a relationship between the percent deviation from expected values for the rate at which *A. californica* associates with *E. fasciculatum* versus mean pH at each site. Percent deviations from expected values were calculated for the ratio of *A. californica* co-occurring with *E. fasciculatum* at 1 m^2 (belt-transects) and 1 m (point-intercept transects). Percent deviations were derived from chi-square contingency tests for association, which are a conservative method of analyzing spatial associations and inherently control for the relative abundance of both target species. Percent deviations for *A. californica* cooccurrence ratios with *E. fasciculatum* from ten field sites were then linearly regressed (Rstudio v 0.98.1062) against a gradient of increasing mean soil pH for each site. Mean soil pH was recorded with chemical indicators of randomly sampled soil cores in the field and confirmed with pH electrode 1:1 slurry assays in the lab by the USDA Soil Conservation Service for the USDA Web Soil Survey (USDA Web Soil) online database. Soil pH sampling methods were acquired through email correspondence with USDA soil ecologists.

RESULTS

Facilitation, tolerance, and soil acidification in greenhouse

In greenhouse treatments, *E. fasciculatum* both tolerated and facilitated *A. californica.*

Shoot DW (g) differed significantly between greenhouse treatments (n=15, $F_{3,16} = 13.13$, $p < 0.0005$; Fig. 1). In polycultures, *E. fasciculatum* significantly increased *A. californica* mean shoot DW by 80.2% (Tukey HSD post hoc; $p = 0.012$) compared to *A. californica* monocultures. *A. californica* did not significantly affect *E. fasciculatum* mean shoot DW (Tukey HSD post hoc; $p = 0.997$) compared to *E. fasciculatum* monocultures.

In order to test how each species treatment affected soil pH, three pots from each greenhouse treatment was flooded with alkaline tap water. The pH changed significantly in monocultures and across time, but with no interactive effect (n = 9, culture: $F_{2,18} = 5.465$, $p = 0.014$; time: $F_{2,18} = 20.638$, $p < 0.0001$; culture·time: $F_{4,18} = 1.672$, $p = 0.20$; Fig. 2). Overall, Tukey HSD post hoc analysis shows *E. fasciculatum* monocultures maintained a significantly 4.2 % lower mean pH over the 24 hr period compared to *A. californica* monocultures ($p = 0.01164$). In *E. fasciculatum* monocultures, mean soil pH dropped 3.5 % from 1-24 hr after a 6.4 % increase from 0-1 hr, but pH was statistically equal among sampling times. *A. californica* monocultures did not lower pH at 24 hr after a significant 11.4 % increase from 0-1 h ($p = 0.0005$). Final mean soil pH after 24 hr was 6.2 % higher in *A. californica* monocultures compared to *E. fasciculatum* monocultures. Polyculture mean pH was intermediate between *E. fasciculatum* ($p = 0.1256$) and *A. californica* ($p = 0.7701$) monocultures over the 24 hr period.

Associations in Field Surveys

After haphazardly choosing 10 m^2 quadrats filtered for suitable habitat for both *E. fasciculatum* and *A. californica,* species interactions were analyzed at a scale of 1 m^2. Associations in the field were first analyzed with the 1x10 m belt transects excluding data from the five 50 m CEB point-intercept transects. *Eriogonum fasciculatum* and *A. californica* exhibited a positive overall significant pattern of spatial association at 1 m^2 across five sites sampled with belt transects ($X^2 = 15.72$, $P < 0.0001$; Table 1). A site-by-site analysis of percent deviations and standardized residuals derived from chi-square contingency tests on belt transects (*see supplementary materials*), shows the association was primarily driven by -56.9 % deviation from what would be expected by chance alone for the number of times *A. californica* occurred without *E. fasciculatum* at the UCI site ($X^2 = 25.9$, $p < 0.0001$; Table 1) which had the highest recorded mean soil pH (8.2). The positive spatial association between *E. fasciculatum* and *A. californica* was also seen at 1x2 m scales ($X^2 = 5.12$, $p = 0.0237$; Table 1). Analysis of CEB point-intercept transects shows the shrubs do not associate at point-intercept (0m) ($X^2 = 0.1$, $p = 0.7518$), but begin a significantly positive overall association at 1m ($X^2 = 7.07$, $p < 0.0078$). Associations between *E. fasciculatum* and *A. californica* were not significantly negative at any of the ten sites.

The positive spatial association between the shrubs is nearly exclusive. *Artemisia californica* did not associate with all other plant species regardless of whether *A. californica* grew within 1 m^2 of *E. fasciculatum* ($X^2 = 7.3137$, df = 1, $p = 0.0078$) or > 1 m^2 from *E. fasciculatum* ($X^2 = 12.8023$, df = 1, p = 0.0.0038). *Eriogonum fasciculatum,* likewise, exhibited a negative association with other plant species whether within 1 m^2 of *A. californica* ($X^2 = 20.37$, df = 1, $p < 0.0001$) or when growing > 1 m^2 from *A. californica* ($X^2 = 49.02$, df = 1, $p < 0.0001$).

SGH along pH gradient in the field and alkaline soil stress on A. californica

In order to test if variance in spatial associations correlated with increasing soil pH, *A. californica* association rates with *E. fasciculatum* were linearly regressed versus increasing soil pH in a test of the SGH. Alkaline soils co-vary with the positive association of *A. californica* with *E. fasciculatum.* Percent deviations of observed *A. californica* co-occurrence rates with *E. fasciculatum* at 1 m^2 (belt transects) and 1 m scales (point-intercept transects) from what would be expected by chance alone showed a significantly positive linear relationship with increasing mean soil pH across the 10 field sites ($R^2 = 0.4991$, $p = 0.0224$, Fig. 3). Multivariate Logistic Regression analysis of belt transects shows when the presence of *E. fasciculatum* is held at zero, increasing mean soil pH negatively interacts with *A. californica* occurrence (ESE = -1.3941, $p < 0.0001$). When mean soil pH is held at 0, *E. fasciculatum* negatively correlates with *A. californica* (ESE = -4.6762, $p = 0.0397$), but as mean soil pH and *E. fasciculatum* increase together, the two variables have a positive interactive effect on *A. californica* (ESE = 0.760, $p = 0.0140$) across the five field sites surveyed here.

DISCUSSION

This study's findings support the hypothesis that by acidifying soils, *E. fasciculatum* facilitates *A. californica* with implications at both seedling and late growth stages. At the seedling stage, *E. fasciculatum* lowers soil pH and facilitates *A. californica* shoot growth, while *A. californica* did not affect *E. fasciculatum* growth or lower soil pH. Field surveys in turn showed positive spatial associations between late growth stage shrubs that were contingent upon soil alkalinity, with positive associations increasing with soil alkalinity. This pattern in the field might be due to a legacy of facilitation between seedlings, facilitatory interactions among the shrubs in late growth stages, or both. Nevertheless, unlike most known plant facilitation systems, which require facilitators in late growth stages and shift to competition as beneficiaries mature, results here indicate facilitation can begin in the seedling stage and result in patterns of positive associations between adults. This positive association between *E. fasciculatum* and *A. californica* in late growth stages is unusual, especially between two competitive, dominant species.

The greenhouse experiment shows strong evidence that *E. fasciculatum* seedlings facilitate *A. californica* seedlings (Fig. 1). Both *E. fasciculatum* and *A. californica* are known to be competitive plants as evidenced by their dominance of a majority of CSS habitat patches (e.g. Westman 1981a) as well as by results presented here of both shrub species' negative spatial associations with all other plants combined (Table 1). However, if either *E. fasciculatum* or *A. californica* were more competitive than the other at early growth stages, then one would have harmed the shoot growth of the other in polycultures, which was not observed after 120 days of growth. For two species to achieve coexistence through the Lotka-Volterra Model of Coexistence, both species must exhibit intraspecific competition that is greater than their interspecific competition. If this were occurring here, then both *E. fasciculatum* and *A. californica* would have exhibited increased shoot growth in polycultures and decreased shoot growth in their respective monocultures. Resource partitioning may result in neutral interactions as well, but this would likely render equal shoot growth for both shrubs in polycultures compared to their respective monocultures. In polycultures, however, only *A. californica* increased shoot growth while *E. fasciculatum* did not experience any loss or gain to shoot growth. Furthermore, the potential for light competition was minimized and root partitioning in regularly watered 7 L pots would be negligible. Therefore, facilitation by *E. fasciculatum* best explains the 80.2 % increase in *A. californica* shoot DW.

Results from the greenhouse and the field are consistent with the hypothesis that *E. fasciculatum* acidifies soils to ameliorate soil alkalinity stress for *A. californica.* Logistic regression analysis indicates that increasing soil pH interacts negatively with the occurrence of *A. californica* when the occurrence of *E. fasciculatum* is held constant (Table 1). But when both pH and *E. fasciculatum* increase together, the interaction on the occurrence of *A. californica* becomes positive. In other calcareous soil systems, several species of trees and shrubs are known to release acidic root exudates to reduce pH in their rhizosphere, mobilizing N and P otherwise bound to Ca^{2+}, and improving nutrient uptake for themselves (Pan et al. 2016). Here, *E.*

fasciculatum reduced soil pH in the greenhouse while pH remained elevated for 24 hr in *A. californica* monocultures (Fig. 2). Intermediate pH in polycultures from the greenhouse pH experiment also suggests the possibility of density effects, in which two *E. fasciculatum* in monocultures may acidify soils more than one *E. fasciculatum* in polycultures. Antagonistic manipulation of soil pH between the shrubs is not likely, because all treatments saw an initial increase in soil pH 1 hr after flooding with alkaline tap water, indicating the tap water raised pH and not *A. californica*. If *E. fasciculatum* facilitates *A. californica* through its modification of soil conditions, which alleviates its own nutrient stress, selection would likely favor *E. fasciculatum* to begin doing so at the seedling stage when plants are most vulnerable to nutrient stress.

The positive relationship of association rates of *A. californica* with *E. fasciculatum* in the field as soil pH increases further supports the hypotheses that facilitation is the means of coexistence and acidification of alkaline soils is the means of facilitation. Consistent with what the SGH predicts, percent deviations of association rates of the beneficiary *A. californica* with its facilitator *E. fasciculatum* increase significantly along a gradient of increasing soil pH (Fig. 3). Further, site-by-site analysis shows the positive association between adults is primarily driven by co-occurrence in CSS habitat patches at the upper limit of recorded soil pH (Table 1 and closed circles in Fig. 3). Alkaline soils can be stressors for many plant species and affect ecosystem-level processes (e.g. decomposition, soil respiration, nutrient cycling), Further, the predictably varying patterns of fine-scale association with *E. fasciculatum* might have implications for other CSS plant species, as well as ecosystem-level processes (e.g. decomposition, soil respiration, nutrient cycling) affected by alkaline soils.

This study did not specifically test for facilitation between *E. fasciculatum* and *A. californica* during late growth stages. The patterns of positive associations observed in the field between the two species in late growth stages could be determined as a function of the shrubs' positive species interactions and establishment niche dynamics as seedlings in alkaline soils. For positive spatial associations to be maintained in the absence of facilitation though, another mechanism would need to be at work to prevent competitive exclusion. For example, facilitation between seedlings could overcome the effects of competition in early growth stages while both species' roots overlap. As the shrubs establish and become competitive, variance in root depth could allow for partitioning of soil water and maintain positive spatial associations in late growth stages. Indeed, some mechanism of coexistence is likely at work in the sites sampled here and in CEB transects because the spatial associations between these two competitive shrub species never become significantly negative even at the lower end of soil pH readings (Table 1). Therefore, it may be useful in future studies to not only investigate facilitation by facilitators in early growth stages, particularly when the means of facilitation is physiological alteration of soil chemistry, but to also consider facilitation in combination with other means of coexistence.

Table 1.1 Statistical results from individual chi-square contingency tests and logistic regression P-values Bonnferoni-adjusted for multiple comparisons within transect sets.
Chi-square contingency tests for association between E. fasciculatum and A. californica

	pH	n	df	X^2	P
All belt transects 1m^2		560	1	23.02	< 0.0001***
UCI	8.2	210	1	10	< 0.0001***
Crystal Cove	7	120	1	1.23	0.2674
Newport	6.9	150	1	0.01	0.9203
San Onofre	6.3	50	1	0.64	0.4237
Zuma	7.2	30	1	Fishers	0.1216
All belt transect 1x2m		280	1	5.12	0.0237*
CEB pt-intercept					
All transects at 0m		250	1	0.1	0.7518
All transects at 1m		250	1	7.07	0.0078*
Cattle Crest	7	50	1	Fishers	0.09796
Gypsum	6.2	50	1	Fishers	0.43568
Laguna Laurel	7.1	50	1	Fishers	0.007317
Shoestring	8.2	50	1	Fishers	0.00629
Weir Creek	7.3	50	1	Fishers	0.2673
Chi-squared contingency tests for association between E. fasciculatum and Other species					
EF & Other		391	1	64.28	<0.0001***
AC+		236	1	20.37	<0.0001***
AC-		155	1	49.02	<0.0001***
Chi-squared contingency test for association between A. californica and Other species					
AC & Other		328	1	18.26	<0.0001***
EF+		240	1	7.3137	0.0068*
EF-		88	1	12.802	0.0003**

Logistic regression interactions with A. californica

	n	df	ESE	P
E. fasciculatum	560	2	-4.68	0.0397*
soil pH	560	2	-1.39	<0.0001***
E. fasc. x pH	560	2	0.76	0.014*

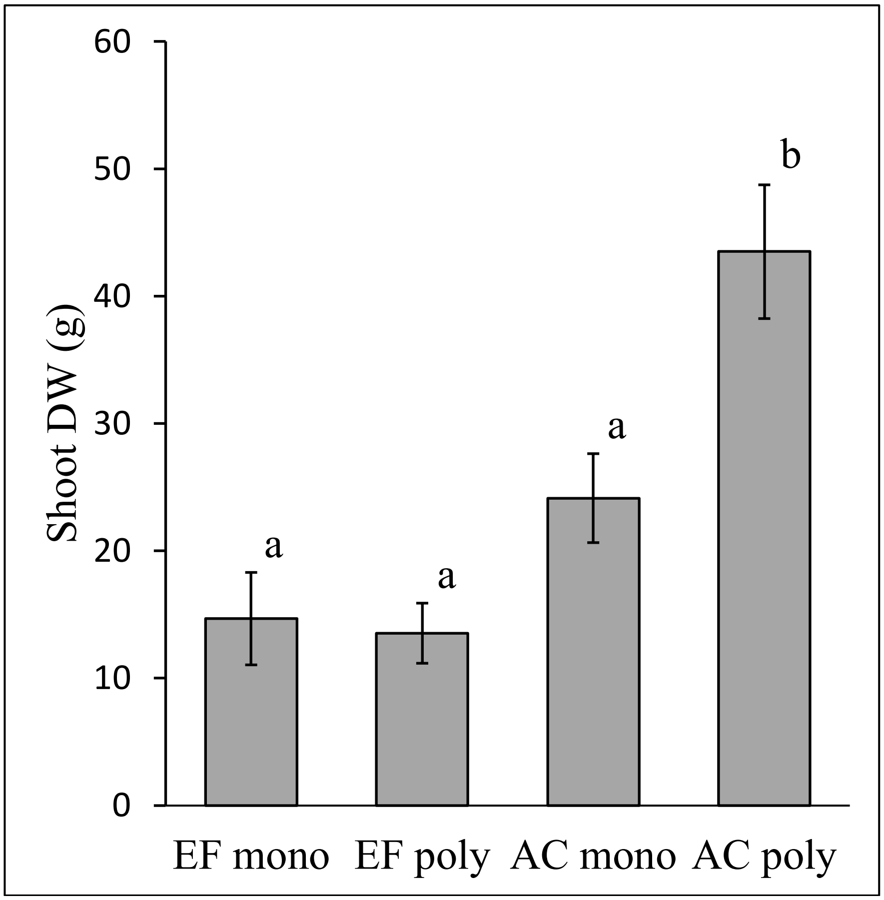

Figure 1.1 Mean shoot DW (g) (± 1 S.E.) of *E. fasciculatum* and *A. californica* seedlings in mono- and polycultures after 120 days in greenhouse mesocosms (ANOVA n=15, $F_{3,16} = 13.13$, $p < 0.0005$). Letters indicate significant differences between pairwise means (Tukey HSD post hoc, $\alpha = 0.05$).

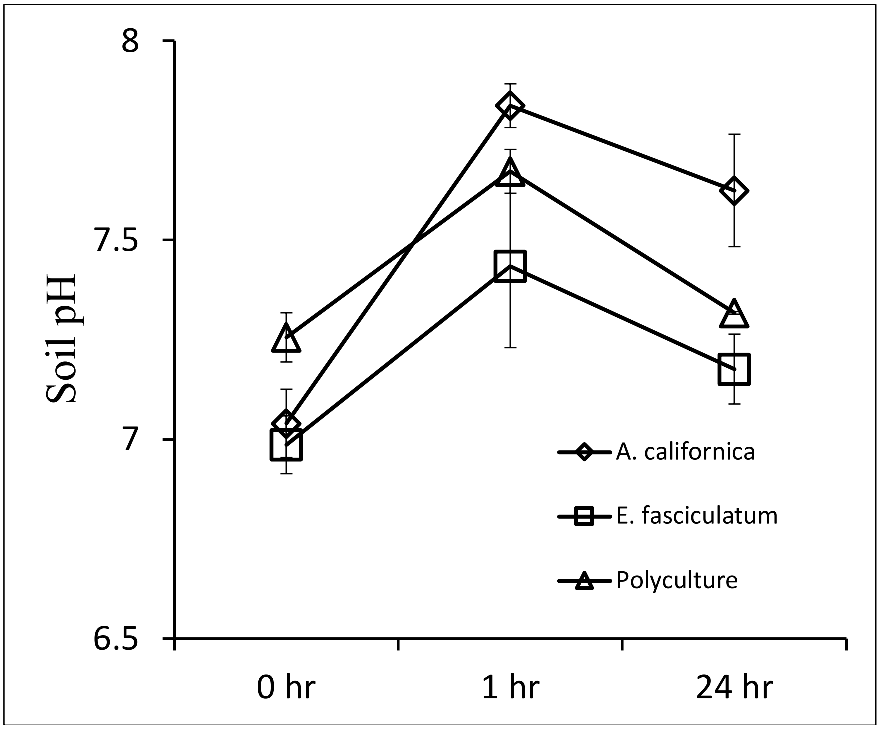

Figure 1.2 Moving averages (+/- 1 S.E.) of soil pH of *E. fasciculatum* and *A. californica* mono- and polycultures at 0 hr, 1 hr, and 24 hr after flooding greenhouse mesocosms with alkaline (pH = 7.8) greenhouse tap water. Soil pH changed significantly in monocultures and across time, but with no interactive effect (Repeated measures ANOVA n = 9, culture: $F_{2,18} = 5.465$, p = 0.014; time: $F_{2,18} = 20.638$, $p < 0.0001$; culture·time: $F_{4,18} = 1.672$, p = 0.20). Tukey HSD post hoc analysis (n = 9) showed significant differences between monocultures (p = 0.0116) and *A. californica* from 0 – 1 hr (p = 0.0005) and 0 – 24 hr (p = 0.0123).

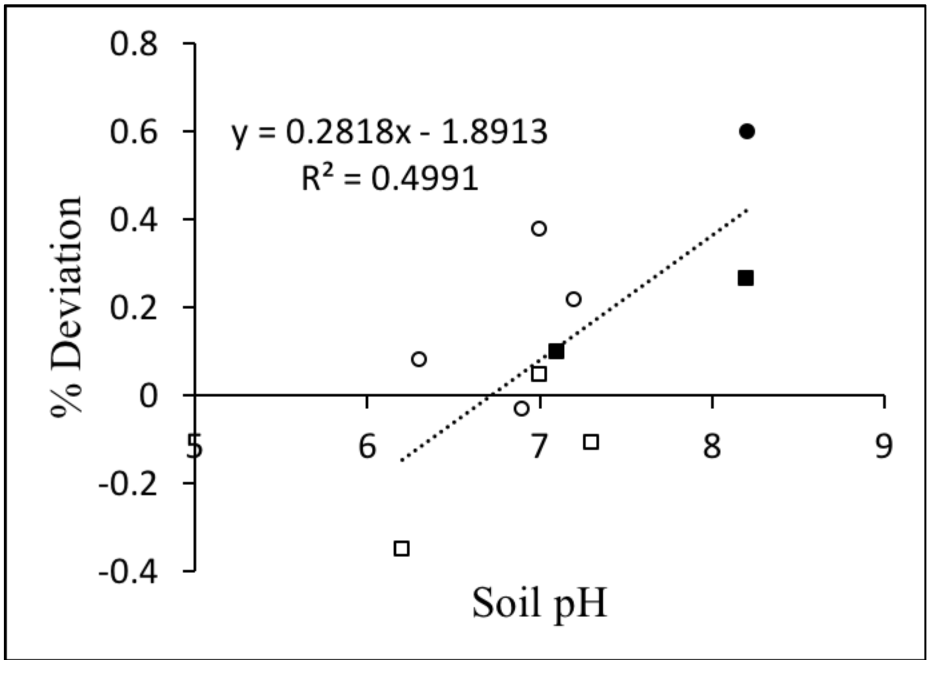

Figure. 1.3 Percent deviation of observed from expected association rates of *A. californica* with the facilitative shrub *E. fasciculatum* versus mean soil pH (Linear regression p = 0.0224). Expected rate of association derived from chi-square contingency tests for association or Fishers Exact Tests. Closed markers indicate a significant positive association. Open markers indicate no significant association. Squares are derived from CEB transects. Circles are derived from belt transects.

Chapter 2:

An invasive annual changes species interactions between dominant native shrubs.

ABSTRACT

Context-dependent species interactions require considering the effects of tertiary species, on pairwise species interactions. While the general importance of tertiary species is well recognized, few – if any – studies have investigated the effects of invasive plants on interactions between a facilitator and the native plant community. A separate study indicated invasive black mustard (*B. nigra*) disrupts otherwise positive interactions between the native facilitator *E. fasciculatum* and a competitive beneficiary *A. californica*. Here, a greenhouse experiment tests how *B. nigra* affects interactions between *E. fasciculatum* and *A. californica* during early growth stages. An additive field experiment tests if *A. californica* can protect *E. fasciculatum* from facilitating germination and growth of *B. nigra* during late growth stages. As seedlings, both *E. fasciculatum* and *A. californica* increased *B. nigra* shoot growth in pairwise interactions. However, in three-way species interactions, *E. fasciculatum* and *A. californica* seedlings together reduced *B. nigra* SLA, height, and inflorescence count while not increasing *B. nigra* shoot growth. *B. nigra* diminished *E. fasciculatum* facilitation of *A. californica.* However, light competition with *B. nigra* resulted in an increase in height for *A. californica,* which reduced *A. californica* shading of shade-intolerant *E. fasciculatum.* During late growth stages, *A. californica* prevented *E. fasciculatum* from facilitating recruitment of *B. nigra*. This indirect positive effect on *E. fasciculatum* is not likely limited to the presence of *B. nigra.* Rather, any herbaceous plant with vertical-dominant growth may effect an increase in *A. californica* seedling height and reduce light competition with *E. fasciculatum.* This study demonstrates the importance of studying interactions between competitive, native perennials in the current ecological context of intensely invaded ecosystems.

INTRODUCTION

Context-dependent species interactions demonstrate the importance of considering the effects of tertiary species on pairwise interactions (*e.g.* Wooten 1994; Chamberlain et al. 2014). This phenom is well-known in even the most obligatory of mutualisms, such as context-dependent parasitism in pollinator syndromes (see Palmer et al., 2003 for further explanation). Plant facilitation is another potentially positive but highly context-dependent species interaction, in which species interactions often shift to competition as ameliorated stressors lessen in severity (Bertness & Callaway 1994). Despite the context-dependent nature of plant facilitation, research has yet to adequately address how tertiary species affect pairwise species interactions in these systems. Furthermore, facilitators can also recruit harmful invasives (*e.g.* Rodríguez & Miriti 2009), yet it remains even less understood how facilitated harmful invasives affect pairwise interactions between native species.

Invasive plants degrade biotic and abiotic ecosystem processes by fundamentally altering the vegetative landscape (D'Antonio & Vitousek 1992; Stylinski & Allen 1999), either directly through competitive exclusion of native flora or indirectly through changing soil conditions and disturbance regimes. For example, in Southern California's threatened, semi-arid coastal sage scrub (CSS) ecosystem, fast-growing invasive annuals have competitively displaced nearly all native grasses and forbs (Bowler 2000). The loss of native flora has severely degraded habitat quality for several sensitive animal species (Bowler 2000). At the ecosystem level, these fundamental changes to vegetative cover often negatively impact native plant productivity and diversity (Goldstein & Suding 2014). The loss in productivity in turn degrades the ability of native plants within watersheds to improve air and water quality. Fast-growing invasive annuals increase the frequency and intensity of wildfires in CSS by senescing shoot tissues early in the growing season, adding easily ignitable material to the fuel load and extending the fire season (Minnich & Dezzani 1998). The resulting increase in fire intervals further reduces habitat heterogeneity by inhibiting the ability of longer-lived native perennials to recover shoot tissues and realize post-fire successional stages. At the same time, long-lived plants are less likely to reach late growth stages and their interactions with other species during seedling stages may become more determinant to the distribution of perennials. It is therefore a priority of ecology to identify, understand, and cultivate positive interactions between native flora, such as plant facilitation - especially between competitive natives - that potentially improve resistance and resilience to invasion (Brooker 2006). It is equally important to study how invasive plants affect interactions between native plants, especially while natives are in early growth stages.

A previous study indicated the CSS native facilitatory shrub California buckwheat (*Eriogonum fasciculatum*) potentially benefits from complimentary competitive effects of its primary beneficiary, the dominant native shrub California sagebrush (*Artemisia californica*) (Chapter 1). In the previous study, experiments in the field and greenhouse suggested the mechanism of facilitation is acidification of CSS's calcareous soils and alleviation of alkalinity stress. The positive interactions between the shrubs began at the seedling-to-juvenile growth stage, and positive spatial associations persist into adult growth forms. These spatial associations were nearly exclusive of all other plant species. The question then arose as to whether *E. fasciculatum's* positive spatial association with *A. californica* allows *E. fasciculatum* to benefit from complimentary recruitment inhibition of other plant species. While *E. fasciculatum* and *A. californica* still co-dominate a majority of remaining CSS habitat patches, dispersal of their offspring is inhibited because shrub seedlings compete poorly for sunlight and seasonally limited water with fast-growing non-native annuals (*e.g.* Eliason & Allen 1997). For example, Cione et al. (2002) showed *A. californica* established most successfully with a labor-intensive combination of manual removal of invasive grasses, application of grass-specific herbicides, and immobilization of nitrogen, as well as an accidental low severity wildfire. Further, a pilot study indicated the potential for the highly invasive allelopathic annual forb black mustard (*Brassica nigra*) to disrupt the otherwise positive interactions between *E. fasciculatum* and *A. californica* in the seedling-to-juvenile growth stages. While co-occurring *E. fasciculatum* and *A. californica* in late growth stages (canopies > 1 m diameter) are expected to resist facilitating recruitment of *B. nigra,* the outcome of how the two native shrub species will interact with *B. nigra* in the seedling-to-juvenile growth stages remains unclear and is the primary focus of the study presented here.

The study here asked the following questions: 1.) How does *B. nigra* affect interactions between *E. fasciculatum* and *A. californica* during seedling-to-juvenile growth stages, when the shrub species are most vulnerable*?* 2.) During late growth stages, does *A. californica* protect *E. fasciculatum* from facilitating recruitment of *B. nigra?* 3.) At either growth stage, do the two shrub species synergistically harm *B. nigra*? 4.) *Finally,* to further test the proposed mechanism of facilitation*,* does artificially lowering the soil pH in *A. californica* monocultures mimic the facilitative effects of *E. fasciculatum* on *A. californica*? Disrupting positive interactions between native flora and inhibiting dispersal and establishment of native, competitive shrubs likely generates a positive feedback loop for further enhancing dispersal and establishment of invasive annuals and acceleration of habitat degradation (Greer et al. 2014). Improving our understanding of facilitative plant systems requires consideration of these systems within their greater ecological context. For most ecosystems, this means studying facilitation within the context of intensely invaded plant communities. The dynamics of invaded facilitative systems, or invasions of any positive interactions between native species that improve biodiversity and habitat quality, demand further study if ecologists are to maximize biodiversity's adaptive potential in ecosystems undergoing fundamental changes to precipitation, temperature, wildfire regimes, eutrophication, and geographic range.

Methods:

STUDY SYSTEM

California coastal sage scrub (CSS) is a threatened, semi-arid, Mediterranean-type ecosystem occupying < 10-15 % of its historic range (Westman 1981). Poorly managed urban sprawl and N-loading from automobile emissions continue to exacerbate edge effects and facilitate the establishment of non-native plants.

Excessive $CaCO_3$ deposits (2600 $\mu g\ Ca^{2+} \cdot g^{-1}$ soil; Westman 1981b) from ancient seashells in CSS's uplifted marine sediments generate layered nutrient stressors for plants. When CSS's mean 250-450 mm of annual precipitation falls in the short winter rainy season (Rundel 2007), CSS's calcareous soils "harden" soil water and raise pH up to 8.2 (2018 IRWD Report). As $CaCO_3$ disassociates, Ca^{2+} ions bond to and immobilize PO_4^{3-} and NO_3^- (Strom et al. 2005). CO_3^{2-} ions degrade proton gradients roots establish for nutrient uptake by pulling H_3O^+ out of the rhizosphere in a reaction that forms HCO_3^-, thus raising soil pH, but soils lack bicarbonate buffering effects seen in other systems (e.g. Fuglsang et al. 2007). Thus, decreasing water-stress increases nutrient stress from changes in soil alkalinity.

TARGET SPECIES

Eriogonum fasciculatum facilitates *A. californica* via acidification of alkaline soils (presumably to mobilize nutrients; Schlau *manuscript in preparation*), but competitively excludes most other plants through water competition with its shallow, woody roots that spread > 3 m laterally (Kirkpatrick et al. 1977) and upto 2.5 m deep (Kummerow et al. 1977; Hellmers et al. 1995).

Co-dominant, long-lived *A. californica* likewise competitively excludes most other species. Allelopathic terpene leachates inhibit germination and growth of other plants. Fibrous, shallow roots remain metabolically active during the dry season to uptake dew and offseason rain events to grow a secondary leaf morphology that allows the hardy shrub to outcompete most other plants for water. Both of the shrubs have lateral dominant growth forms that spread > 1 m. The wide canopies shelter mammalian herbivores and seed predators that remove heterspecifics and compete with other plants for sunlight.

Brassica nigra was chosen as the tertiary invasive species because of its high abundance and fecundity in CSS, and the potential of its fungicidal allelopathy to disrupt potentially mycorrhizal-mediated, positive facilitatory interactions between *E. fasciculatum* and *A. californica. Brassica nigra* does not associate with mycorrhizae, and in fact, kills soil fungi with root exudates (Schreiner & Koide 1993). Isothiocynate is part of a suite of secondary metabolites produced in the leaves and seeds that render the tissues unpalatable to most invertebrate (Van Dam & Raaijmakers 2006) and mammalian (Orrock et al. 2008) herbivores. Upon germination or leaching, isothiocynate root exudates inhibit germination and growth of other plants (Turk et al. 2003, Tawaha & Turk 2003).

Greenhouse Competition-Facilitation Experiment

In order to test how *B. nigra* affects interactions between *E. fasciculatum* and *A. californica* during early growth stages, seedlings of all three species were put into direct competition in a full factorial design in the UC Irvine greenhouse (n=15 ea. treatment; see supplemental figure). Additionally, a general hypothesis that *E. fasciculatum* facilitates *A. californica* with acidic exudates, which alleviate alkaline soil stress, was further tested. In order to mimic the effects of reduced soil pH by *E. fasciculatum*, 36 g of elemental sulfur were added to 15 additional *A. californica* monoculture replicates. As a control for plant-soil feedback effects on soil pH, 12 more pots were filled with soil mix and watered, but contained no plants.

Local *E. fasciculatum* and *A. californica* seeds were provided by the Irvine Ranch Conservancy and sourced from Irvine Ranch Natural Landmarks in Irvine, CA. *Brassica nigra* seeds were gathered from over a dozen plants scattered throughout the UC Irvine CSS Ecological Preserve. All seeds germinated for the greenhouse experiment were collected after the 2018-2019 winter rainy season, which experienced above average precipitation. Target species were germinated in trays on a misting bench and transplanted to 7 L pots after emergence of true leaves.

The 7 L pots contained UCI native (CSS) soil mix (1.5 pumice:1 sand:1 redwood mulch:1 peat moss). Soil pH levels were recorded for all pots using 1:1 slurry method with a HANA field pH meter prior to transplanting seedlings from germination trays, but after conditioning the soil with local “hard” tap water (28-90 mg $Ca^{2+} \cdot L^{-1}$; ph 7.8-8.2; IRWD 2018) for 8 days in order to raise the pH to the higher end of field conditions. Soil pH was remeasured prior to destructive harvest.

In order to compare the effects of pairwise and three-way species interactions on overall shoot growth performance after 89 days in the greenhouse, log response ratios (mean effect size) of mean shoot DW (g) with pooled standard deviation were used. Log response ratios in pairwise interactions were calculated by taking the natural log of mean shoot DW for the target species in pairwise interaction treatments divided by the mean shoot DW of the target species in monoculture controls. Pooled standard deviations were used to calculate 95% confidence intervals. First, variance of each log response ratio was calculated with equation 1:

$$\square^{2}_{LRR} = (SD_{e})^{2}/(N_{e} \bullet \overline{X}_{e}^{2}) + (SD_{c})^{2}/(N_{c} \bullet \overline{X}_{c}^{2}) \qquad \text{eq 1}$$

where $\square^{2}_{LRR}$ = variance of log response ratio, SD = standard deviation, N = sample size, $\overline{X}$ = mean, subscript "e"= pairwise effect on mean shoot DW of target species, subscript "c" = monoculture control. That is, the variance is the standard deviation squared divided by the product of the sample size times mean squared for both exclusion and control added together.

Second, the standard deviation of the log response ratio (σ_{LRR}) was calculated by taking the square root of the variance, as described in equation 2:

$\sigma_{LRR} = (\sigma^2_{LRR})^{0.5}$ eq 2

Third, to get the 95% confidence interval the standard error (SE_{LRR}), which is the standard deviation divided by the square root of the sample size, must be known. The sample size in this case is a pooled sample size, calculated with equation 3:

$N_{pooled}=N_e \bullet N_c/(N_e+N_c)$ eq 3

Fourth, dividing the standard deviation by the square of the pooled sample size renders the standard error, as calculated in equation 4:

$SE_{LRR}= \sigma_{LRR}/(N_{pooled})^{0.5}$ eq 4

Firth, the 95% confidence interval is then SE_{LRR} times the critical value of the t-distribution with a two-tailed $\alpha = 0.05$. The mean effect size +/- the 95% confidence interval is thus calculated with equation 5:

σ^2_{LRR} +/- (critical value of the t-distribution $\bullet$ SE_{LRR}) eq 5

As indicated from a previous study (*unpublished*), *E. fasciculatum* was expected to facilitate the other target species, increasing their overall shoot growth. *Brassica nigra* was expected to compete with both shrub species seedlings and decrease shoot growth. *Artemisia* *californica* was expected to not affect *E. fasciculatum* shoot growth and decrease *B. nigra* shoot growth.

A primary goal of this study was to test how a tertiary species affects pairwise interactions. To test if a tertiary species changed how the two other target species interacted, observed three-way interactions were compared to expected additive three-way interactions. Additive three-way interaction effects were predicted from the means of the respective pairwise interaction effect log response ratios with 95% confidence intervals calculated by the pooled standard deviations method outlined above. Standard deviations were calculated from the means of the pairwise effects. Deviations of observed from predicted three-way interactions were considered as significant, and thus as non-additive interactions, if the predicted effect's 95% confidence interval lied outside the observed effect's 95% confidence interval. Synergistic three-way interactions were indicated by significantly stronger observed three-way interaction effects than that of the predicted. Antagonistic three-way interactions were indicated by a significantly weaker effect than predicted.

Log response ratios (see results and Fig. 2.3) indicated a significant three-way interaction that antagonistically weakened negative effects on *E. fasciculatum* observed with *E. fasciculatum* in pairwise interactions with both *B. nigra* and *A. californica*. Therefore, linear regression analysis was used to compare *E. fasciculatum* shoot DW to the shoot DWs of *A. californica* and *B. nigra* in pairwise interactions, as well as *E. fasciculatum* shoot DW versus the sums of *A. californica* and *B. nigra* shoot DWs in three-way interactions. Negative relationships in pairwise interactions were interpreted as competition and positive relationships in three-way interactions were interpreted as indicative of an interaction between *A. californica* and *B. nigra* that was somehow alleviating the negative effects on *E. fasciculatum* seen in pairwise interactions.

In order to further tease apart the processes behind observed patterns in species interactions, shoot DW (covariable) was further analyzed with ANCOVAs for relationships to specific leaf area (SLA), soil pH, and shoot height (dependent variables) in each species interaction treatment (independent variables). When ANCOVAs indicated a significant difference among slopes, linear regressions were used to test if any particular treatment effected a significant relationship between the growth metric and total shoot DW for each target species. For example, if pairwise interactions with *B. nigra* effected a significant positive relationship in linear regression analysis between shoot height and shoot DW for *A. californica,* the result was interpreted as *A. californica* growing taller due to interactions with *B. nigra.*

Another goal of this study was to test for complimentary competitive effects between *E. fasciculatum* and *A. californica.* Log response ratios showed a significant three-way interaction, in which *E. fasciculatum* and *A. californica* together resulted in a *B. nigra* shoot DW significantly lower than the expected interactive effect. Plants can harm other species in ways that may not be detected from comparing measurements of total shoot DW alone. Therefore, inflorescence count and leaf mass (dependent variables) for *B. nigra* were also compared between species treatments (independent variable) with seperate ANOVAs and interpreted in conjunction with ANOCVA and subsequent linear regressions for indication of competition-driven tradeoffs among SLA, shoot height, and reproductive potential a la Westoby's Leaf-Height-Seed Hypothesis (Westoby 1980).

Root DW and root:shoot ratios were not included in analysis due to the experimental design relying on direct interactions between rhizospheres and resulting entanglement of belowground tissues. These interactions resulted in roots that were impossible to separate from the roots of other plants without extensive tissue loss.

In a previous experiment with two shrubs per treatment, *E. fasciculatum* facilitated *A. californica* at no cost to its own shoot growth. However, at four shrubs per pot in the present study, *A. californica* grew so much faster than *E. fasciculatum,* and *A. californica* lateral-dominant growth form appeared to quickly shade *E. fasciculatum* seedlings. Both shrubs seedlings shoots emerged on the same day, so germination priority effects were likely not a confounding variable. This led to a hypothesis that *A. californica* may harm its facilitator *E. fasciculatum* through light competition at this density. To test this hypothesis, *E. fasciculatum* shoot biomass was linearly regressed versus *A. californica* distal canopy width with the prediction that wider *A. californica* canopies would have a negative relationship to *E. fasciculatum* shoot biomass.

Field Recruitment Facilitation-Inhibition Experiments

In order to test whether *A. californica* protects *E. fasciculatum* from facilitating germination and growth of *B. nigra* during late growth stages, an additive field experiment was carried out in the UC Irvine Coastal Sage Scrub Ecological Preserve. Long-lived, facilitated perennials tend to eventually outcompete their facilitator after the beneficiary has established and becomes less vulnerable to previously alleviated stressors. However, in a previous study, we showed a significant, positive spatial association between *E. fasciculatum* and *A. californica* persisted into adult growth stages with canopies > 1 m (see Schlau above). The persistence of tolerance instead of competitive exclusion between the two shrubs suggests that positive or, at a minimum, neutral species interactions continue into adult growth forms. These observations led to a hypothesis that *A. californica* protects *E. fasciculatum* from recruiting harmful invasives and *E. fasciculatum* facilitation of *A. californica* enhances *A. californica* recruitment inhibition. However, a pilot study suggested *E. fasciculatum* exploits its root plasticity to partition soil water with *A. californica,* leaving open the possibility of exclusion of other flora via complimentary water competition.

In order to separate the effects of water competition from allelopathic facilitation, 30 seeds of randomly collected, genetically local *B. nigra* were scattered in 12x12x10cm buried pots filled with field soil and adjacent 12 cm^2 plots of tilled bare ground. The top 8-10cm of topsoil were removed to minimize the presence of pre-existing *B. nigra* in the seedbank. Ten replicates of each paired treatment were placed directly beneath the southern-most facing canopy edge of *E. fasciculatum,* an adjacent *A. californica,* where shrub species' canopies crossed, and under shaded controls isolated by 0.5-1.0m from the nearest shrub (n=80; n=10 replicates per treatment). All shrub canopies were > 1 m diameter. The experiment was established in late December 2017 before a belated rainy season began. The number of germinations were recorded 3 times a week. February and March of 2018 experienced several weeks of record low rainfall, so all subtreatments were watered by hand with 200mL of hard tap water three-way a week until April 24th, which was two weeks after the last observed *B. nigra* germination. All *B. nigra* died within two weeks after germination, presumably due to water-stress. Treatments were left in the field and reseeded to 30 *B. nigra* seeds before the 2018-2019 winter rainy season. In the second growing season, several *B. nigra* had established in some treatments and were destructively harvested after the first flowers began to close, indicative of pollination. *B. nigra* inflorescence count was used as a proxy for reproductive potential. Shoot and root tissue were separated and dried in ovens at 65° C for 72 hr. Shoot DW was analyzed as a metric of growth performance and productivity. Root:shoot ratios were calculated and analyzed as an indication of nutrient stress or alleviation of that stress. Variance in germinations (dependent variable) were compared with one-way and two-way fixed effect ANOVAs. In the one-way ANOVA, canopy treatment was the independent variable. In the two-way ANOVA, canopy treatment and buried pots or tilled ground were the independent variables. Variance in growth during the second year in the three dependent variables (shoot DW, root DW, and root:shoot ratios) were analyzed with one-way fixed-effects ANOVAs with canopy treatment as the independent variable. Residuals were not normally distributed for germinations or growth, presumably because of intense facilitation in buried pots under *E. fasciculatum* canopies, so type 3 SS were used in analysis.

All statistical analysis was performed with R(v0.98.1062).

RESULTS

ANCOVAs compared relationships between growth metrics versus shoot DW to tease apart treatment effects on growth behavior. An ANCOVA of the relationships between Log-transformed height to Log-transformed shoot DW for *A. californica* showed a significant relationship between monocultures and pairwise treatments with *B. nigra* (Est. Std. -0.54828, p = 0.0391, Table S1).

Subsequent linear regression analysis of the effects of each treatment on the *A. californica's* relationship between Log-transformed height and shoot DW (Fig. S2.2, Table S1) showed that the presence of other plant species corresponded with *A. californica* to grow taller. In pairwise interactions, *E. fasciculatum* effected a moderately, significantly positive relationship ($R^2 = 0.22$, p = 0.0765). *B. nigra* effected a strongly significant, positive relationship ($R^2 = 0.65$, p = 0.0008). There was a significant three-way interaction on *A. californica,* which was strongly significant, positive linear relationship ($R^2 = 0.48$, p = 0.0063).

Furthermore, in order to test if *A. californica* was outcompeting *E. fasciculatum* for sunlight, *E. fasciculatum* shoot DW was linearly regressed against *A. californica* distal canopy width. *Artemisia californica* distal canopy width had a strongly significant, negative relationship with *Eriogonum fasciculatum* shoot DW ($R^2 = 0.59$, p = 0.00586; Fig. S2.1).

Thus, in order to test if competition between *A. californica* and *B. nigra* dampened negative effects on *E. fasciculatum, E. fasciculatum* shoot DW was linearly regressed against the shoot DW of *A. californica, B. nigra,* and the sum of *A. californica* and *B. nigra* shoot DW in three-way interactions. *Artemisia californica* shoot DW had a moderately significant, negative relationship with *E. fasciculatum* shoot DW in pairwise interactions ($R^2 = 0.33$, $p = 0.0521$; Fig. 2.2A). *Brassica nigra* shoot DW had no significant effect on *E. fasciculatum* shoot DW ($R^2 = 0.06$, $p = 0.384$, Fig. 2.2B). However, *E. fasciculatum* shoot DW had a significant, positive relationship to the summed shoot DW of *A. californica* and *B. nigra* in three-way interaction treatments ($R^2 = 0.41$, $p = 0.026$, Fig. 2.2C).

In order to test if interactions between seedlings were positive, negative, or neutral, and if observed three-way interactions were significantly synergistic or antagonistic to expected three-way interaction effects, log response ratios (effect sizes) were analyzed for each target species' mean shoot DW. Log response ratio analysis of *A. californica* shoot growth performance indicates a significant additive three-way interaction (Fig. 2.3). In pairwise interactions, *E. fasciculatum* enhanced *A. californica* mean shoot growth performance by 101.9 % compared to monoculture controls (Fig. 2.3). In three-way species interaction treatments, there was an additive effect on *A. californica. B. nigra* reduced *E. fasciculatum* facilitation to a still significant 85.3 % increase in *A. californica* shoot growth, the 95% confidence interval of which was within the 95% confidence interval of expected additive effects (Fig. 2.3). In pairwise interactions, *B. nigra* did not affect a significant change in *A. californica* shoot DW (Fig. 2.3).

Log response ratio analysis of *E. fasciculatum* shoot growth performance indicates both *A. californica* and *B. nigra* significantly reduce *E. fasciculatum* shoot DW in pairwise interactions (Fig. 2.3). However, there was a significantly antagonistic three-way interaction effect on *E. fasciculatum. Artemisia californica* and *B. nigra* together still significantly reduced

E. fasciculatum shoot DW, but the effect size was significantly lower than the expected additive effect.

Log response ratios for *B. nigra* show both *E. fasciculatum* and *A. californica* significantly increased *B. nigra* shoot growth in pairwise interactions compared to monoculture controls (Fig. 2.3). However, there was a significant three-way interaction on *B. nigra*. Three-way interactions produced an antagonistic effect, which was less than the expected additive effect, and did not increase *B. nigra* shoot growth compared to *B. nigra* monocultures (Fig. 2.3).

In order to further test a hypothesis that *E. fasciculatum* acidifies soils and facilitates *A. californica* through amelioration of soil alkalinity stress, the response ratios of lowered soil pH on *A. californica* shoot growth was compared to respective monoculture treatments. Reducing soil pH with sulfur soil amendments increased *A. californica* shoot biomass by 40.0 % (Fig. 2.3).

In order to test how *E. fasciculatum* and *A. californica* did not facilitate shoot growth of *B. nigra* in three-way interaction treatments, statistical analyses were run on a series of growth metrics. An ANCOVA of Log-transformed data for *B. nigra* SLA versus shoot DW showed a moderately significant, negative effect in Monocultures (Est. Std. = -1.229685, p = 0.0575) and three-way interaction treatments (Est. Std. = -0.768761, p = 0.0783; Table S3). Linear regression analysis (Fig. S2.4, Table S3) showed significantly negative relationships in pairwise treatments with *A. californica* (R^2 = 0.39, p = 0.013) and *E. fasciculatum* (R^2 = 0.28, p = 0.0308). In three-way interaction treatments, the antagonistic relationship was moderately significant and negative (R^2 = 0.27, p = 0.0858).

In order to further test how species interaction treatments effect *B. nigra* growth, an ANCOVA was conducted analyzing the relationship between *B. nigra* log-transformed height and log-transformed shoot growth. The ANOCVA showed three-way interactions effected the relationship (Est. Std. = 0.29053, p = 0.00394; Fig S2.5, Table S2.4). Subsequent linear regression analysis showed a significantly positive relationship in pairwise interactions with *A. californica* ($R^2 = 0.50$, p = 0.00656) and in three-way interactions ($R^2 = 0.34$, p = 0.0343).

Additionally, ANOVAs of three-way species interactions showed total *B. nigra* leaf mass did not differ significantly among treatments ($F_{3,50} = 1.366, p = 0.265; data\ not\ shown$), but inflorescence count ($F_{3,50} = 2.895, p = 0.0452; data\ not\ shown$) and shoot height ($F_{3,50} = 6.203, p = 0.0015; data\ not\ shown$) did. Tukey HSD post hoc analysis revealed that *E. fasciculatum* and *A. californica* did not affect *B. nigra* shoot height or inflorescence count in pairwise interactions. However, the shrubs together in three-way interaction treatments decreased *B. nigra* shoot height 33.1 % (p = 0.0009, *data not shown*) and reduced inflorescence count 66.8 % (p = 0.0452, *data not shown*) compared to monoculture controls. While three-way interactions reduced *B. nigra* shoot height, three-way interactions increased the ratio of height to total shoot DW (Fig. S2.5, Table S2.4).

Additive Field Experiment

ANOVAs of the additive field inhibition-facilitation experiment showed germination differed significantly among canopy treatments ($F_{3,72} = 8.381, p \ll 0.0001$; Fig. 2.4A) and pot-bare ground subtreatments ($F_{1,72} = 17.473, p \ll 0.0001$; Fig. 2.4B), but with no interactive effect ($F_{3,72} = 2.053, p = 0.114$; Fig. 2.4B). Shoot biomass ($F_{3,24} = 9.972, p = 0.0034$), root biomass ($F_{3,24} = 10.03, p = 0.0002$), and root:shoot ratios ($F_{3,24} = 4.775, p = 0.007$) also differed significantly among canopy treatments (Fig. 2.5A-C, respectively). Tukey HSD post hoc analysis showed that germination, shoot growth, and root growth under *A. californica,* mixed canopies, and shaded bare ground treatments were statistically equal. However, under *E. fasciculatum* canopies *B. nigra* germination increased 103.2 % (p = 0.0043; Fig. 2.4A), shoot biomass increased 433.3 % (p = 0.0012; Fig. 2.5A), root biomass increased 160.7 % (p = 0.0112;

Fig. 2.5B), and root:shoot ratios decreased by 44.5 % (p = 0.0149; Fig. 2.5C) compared to shaded controls. Facilitation of *B. nigra* establishment was driven by germination in buried pots (Fig. 2.4B) and no *B. nigra* survived in bare ground subtreatments beneath *E. fasciculatum* canopies.

Discussion

This study sought to test 1) how an invasive tertiary species affects facilitative interactions between two co-occurring, dominant, long-lived, native shrub species during early growth stages, and 2) if a competitive beneficiary protects its facilitator from recruiting a potentially harmful invasive species during late growth stages. During early growth stages, *B. nigra* reduces *E. fasciculatum* facilitation of *A. californica,* but also mitigates *A. californica* harmful competitive shading effects on *E. fasciculatum.* In three-way interaction treatments, *E. fasciculatum* and *A. californica* seedlings decrease *B. nigra* SLA, height, and reproductive potential. In late growth stages, *A. californica* protects *E. fasciculatum* from facilitating germination and growth of *B. nigra.* In further support of a hypothesis that *E. fasciculatum* facilitates *A. californica* by reducing soil pH and alleviating alkalinity stress, reducing soil pH in the greenhouse with elemental sulfur increased *A. californica* shoot growth. The presence of *B. nigra* appears to inhibit *E. fasciculatum* from reducing soil pH. Taken together, results presented here demonstrate not only significant effects of a tertiary invasive species on interactions between two ecologically important native plants, but also significant negative effects of the co-occurring native shrub species on the invasive.

As nutrients become more available, competition can shift from a battle over N and P to a fight for available light as faster-growing individuals acquire more biomass (Bobbink et al. 2010). *Eriogonum fasciculatum* more than doubled *A. californica* shoot biomass (Fig. 2.3), but facilitation and competition occur simultaneously (Bertness & Callaway 1994). If *E. fasciculatum* soil acidification makes additional nutrients available, this would explain why faster-growing *A. californica* shaded *E. fasciculatum* (Fig. S2.1) and reduced *E. fasciculatum* shoot biomass by 74.2% (Fig. 2.3) at densities here of four shrubs per 7 L pot. At lower densities of two shrubs per 7 L pot - tested in a previous experiment - *E. fasciculatum* facilitates *A. californica* and *A. californica* does not reduce *E. fasciculatum* shoot growth (Schlau *manuscript in preparation*). Results presented here also coincide with our previous field study, showing the two shrubs share a positive spatial association in adult growth stages at $1m^2$ and 1m scales, but not at 0 m. In order for positive interactions to be maintained between the shade-intolerant shrubs, *E. fasciculatum* and *A. californica,* the shrubs likely must first partition space and thus access to sunlight.

Surprisingly though, in three-way species interaction treatments, negative effects on *E. fasciculatum* shoot growth were greatly diminished compared to *E. fasciculatum* pairwise interactions with *A. californica* or pairwise interactions with *B. nigra* (Fig. 2.3). *B. nigra* appears to elicit a plasticity growth response in *A. californica* to grow taller compared to *A. californica* controls (Fig. S2.2). In further support for the possibility that competition between *A. californica* and *B. nigra* mitigates both species competitive effects on *E. fasciculatum, E. fasciculatum* shoot DW had a significantly positive linear relationship with the sum of *A. californica* and *B. nigra* shoot DW (Fig. 2.2). In other words, when *B. nigra* (or possibly any vertical growth-dominant species) elicits a growth plasticity response in *A. californica* to grow taller, the harmful competitive shading effects of *A. californica* on *E. fasciculatum* may be reduced as more light reaches the facilitator's leaves.

Greenhouse results also suggest that in three-way species interaction treatments, *B. nigra* interferes with *E. fasciculatum* facilitation of *A. californica.* Since *B. nigra* does not harm *A.*

californica shoot growth in pairwise interactions, the three-way interaction effect is not likely the result of *B. nigra* directly harming *A. californica* while *E. fasciculatum* facilitates *A. californica*. Instead, two possibilities would explain how *B. nigra* works antagonistically against *E. fasciculatum* facilitation: First, *B. nigra* allelopathically disrupts *E. fasciculatum* facilitation. This would be likely if mycorrhizae mediate or control facilitation, as *B. nigra* kills soil fungi (Schreiner & Koide 1993). Coinciding with this first idea, soil pH was significantly lower in all treatments with *E. fasciculatum,* except when *B. nigra* was also part of the treatment (Fig. S2.6). Bozzolo and Lipson (2012) show the loss of mycorrhizae does not significantly reduce *A. californica* shoot growth in monocultures, but soil sterilization reduced *E. fasciculatum* shoot growth by 70%. Second, *B. nigra* harmful effects on *E. fasciculatum* growth may diminish *E. fasciculatum's* ability to facilitate *A. californica* as *E. fasciculatum* is forced to reallocate limited resources toward competition and away from facilitation. However, this study presents no evidence of *E. fasciculatum* shunting resources from facilitation to competition in analysis of the relationship of *E. fasciculatum* SLA, leaf count, or height versus shoot DW (*data not shown*). Density effects are also not likely the cause of reduced facilitation in three-way interactions. In a previous greenhouse experiment, in which treatments had one of each shrub, *E. fasciculatum* increased *A. californica* shoot DW by 80.2%. Here, *E. fasciculatum* increased *A. californica* shoot DW by 101.9%. Additive effects would likely result in a more proportional increase in facilitated growth.

If *B. nigra* competed more with conspecifics than with *E. fasciculatum* and *A. californica,* this would explain *B. nigra's* increase in shoot DW in pairwise interactions. However, in three-way interactions, there was no increase in *B. nigra* shoot growth. *Brassica nigra* shoot growth did not decrease in three-way species interactions compared to monocultures (Fig. 2.3), but *B. nigra* decreased SLA as its shoot growth increased (Fig. S2.4, Table S3). Total leaf mass remained equal across all treatments (*data not shown*). If SLA decreases while total leaf mass remains relatively constant, then *B. nigra* is growing its leaves thicker and smaller. Such a plasticity growth response is typical of plants under low-N-stress and/or water-stress, which may result from belowground competition for nutrients or water (Violle et al. 2009). Field results suggest *E. fasciculatum* is highly competitive for soil water (see below). In three-way species interaction treatments, *B. nigra* height (*data not shown*) and inflorescence (*data not shown*) also decreased compared to the invasive's growth performance in monocultures. While *B. nigra* overall shoot growth did not change in three-way interactions and height decreased, the three-way interactions did significantly increase the ratio of height to shoot DW (Fig. S2.5, Table S4). Pairwise interactions between *B. nigra* and either *A. californica* and *E. fasciculatum* were intermediate for SLA v. shoot biomass, height, and inflorescence count. According to Westoby's Leaf-Height-Seed strategy (Westoby 1998), plants have finite resources to allocate to different traits associated with either leaf growth, shoot growth, or reproduction. In this model, the allocation of limited resources to increase the invasive's height per unit shoot mass would require redirecting resources from leaf growth (reduced SLA per unit shoot mass) and reproduction (reduced inflorescence count). Scaled up to field conditions, such an interaction could potentially reduce dispersal of *B. nigra*.

While it remains unclear whether *E. fasciculatum* continues facilitation of *A. californica* during late growth stages in the field, *E. fasciculatum* appears to benefit from its spatial association to *A. californica* and its beneficiary's competitive effects. *Artemisia californica* prevented *E. fasciculatum* from facilitating germination and growth of the harmful invasive *B. nigra* when water competition was reduced with buried pots under mixed canopy treatments of shrubs (Fig. 2.4B). Even without immediate proximity to *A. californica, E. fasciculatum* inhibited germination (Fig. 2.4B) and growth of *B. nigra* on tilled bare ground treatments. *E.*

fasciculatum shallow roots may have allowed for *E. fasciculatum* to inhibit germination of *B. nigra* via water competition in CSS's well-drained soils and short rainy seasons. Even under conditions with sufficient precipitation for *B. nigra* to establish, *E. fasciculatum* facultatively deep tap root (up to 2.5 m soil depth) may outcompete *B. nigra* for limited soil water at greater soil depth, especially after CSS's short winter rainy season has ended. These results suggest for heterospecifics to benefit from *E. fasciculatum* facilitation, as *A. californica* does- at least as seedlings - heterospecifics must first tolerate competition with *E. fasciculatum* for seasonally available water or establish themselves prior to *E. fasciculatum*. In short, when water is limited – even with above average rainfall in well-drained soils - *E. fasciculatum* in adult growth stages does not facilitate recruitment of *B. nigra.* When water competition is minimized (i.e. in buried pots or a year with a longer rainy season and above average rainfall), *A. californica* may offer some protection to *E. fasciculatum* from facilitating recruitment and growth of *B. nigra,* and perhaps other invasive species.

This study also sought to further test a hypothesis that *E. fasciculatum* acidifies soils and facilitates *A. californica* through amelioration of soil alkalinity stress. CSS's calcareous soils generate layered nutrient stressors by immobilizing P and N (Strom et al. 2005) and degrading proton gradients roots establish for nutrient uptake (Fuglslang et al. 2007). In other systems, several species have been shown to acidify calcareous soils and alleviate layered nutrient stressors for themselves. In pairwise interactions here, *E. fasciculatum* reduced soil pH and effected a positive growth response in *A. californica* compared to controls (Fig. S2.3, Table S2). Both the y-intercept and slope differed from controls, indicating that the relationship between soil pH and *A. californica* shoot biomass was not a plasticity response due to competition with *E. fasciculatum.* Reducing soil pH with sulfur soil amendments likewise increased *A. californica* shoot growth albeit to a lesser extent (Fig. 2.3). Reducing soil pH did not have a significant linear relationship to shoot biomass either (*data not shown*). However, in three-way species interactions and pairwise *E. fasciculatum* treatments the slopes for soil pH and *A. californica* shoot biomass seem to be converging at pH 6.2 (Fig. S2.3). A polynomial regression of *A. californica* shoot biomass versus soil pH in low pH treatments shows a significant relationship with an optimal pH at approximately 6.2 (*data not shown*). Mean soil pH in pairwise interactions between *E. fasciculatum* and *A. californica* was also 6.2 (Fig. S2.5). Further testing with a controlled gradient of soil pH is needed to adequately address this question, but data here suggests that *E. fasciculatum* may reduce soil pH to an optimal range for *A. californica* and presumably increase nutrient availability. Alternatively, it is quite possible that *E. fasciculatum* facilitates *A. californica* while freeing its co-dominant native shrub from some degree of intraspecific competition.

CONCLUSIONS

This study reinforces the importance of considering pairwise species interactions within the context of their greater biological community. After CSS plant communities assembled in the wake of early glacial retreat from mid-latitudes in the Pleistocene (Axelrod 1978), the co-dominant vegetative alliance between *E. fasciculatum* and *A. californica* suppressed overall native plant diversity (see collective works of Whittaker and colleagues). In the current ecological context of the Anthropocene, however, this means studying the native shrubs' pairwise interactions within the context of intensely invaded habitats. Surprisingly here, however, the tertiary species – an abundant, fast-growing, allelopathic, invasive annual – appears capable of mitigating a native heterospecific beneficiary's negative competitive effects upon its facilitator during early growth stages. It is likely that any tertiary plant species with a vertical-dominant growth form may elicit a plasticity growth response in *A. californica* to grow taller and mitigate the negative effects of *A. californica* light competition on *E. fasciculatum.* Once established, the beneficiary protects its facilitator from recruiting a harmful invasive. Several studies show that competitive native species can improve a biological community's resistance to invasion (e.g. Maron & Marler 2007). If *E. fasciculatum* and *A. californica* do indeed increase the native plant community's resistance and resilience to invasion, as results here suggest as a possibility, the shrubs' niche has shifted with their environment from suppressor to protector of native plant diversity. Regardless, when *E. fasciculatum* or *A. californica* disperse seeds into re-occurring patches of *B. nigra* (and probably other invasive annuals)*,* it may be advantageous for the two shrubs' seeds to disperse in close proximity.

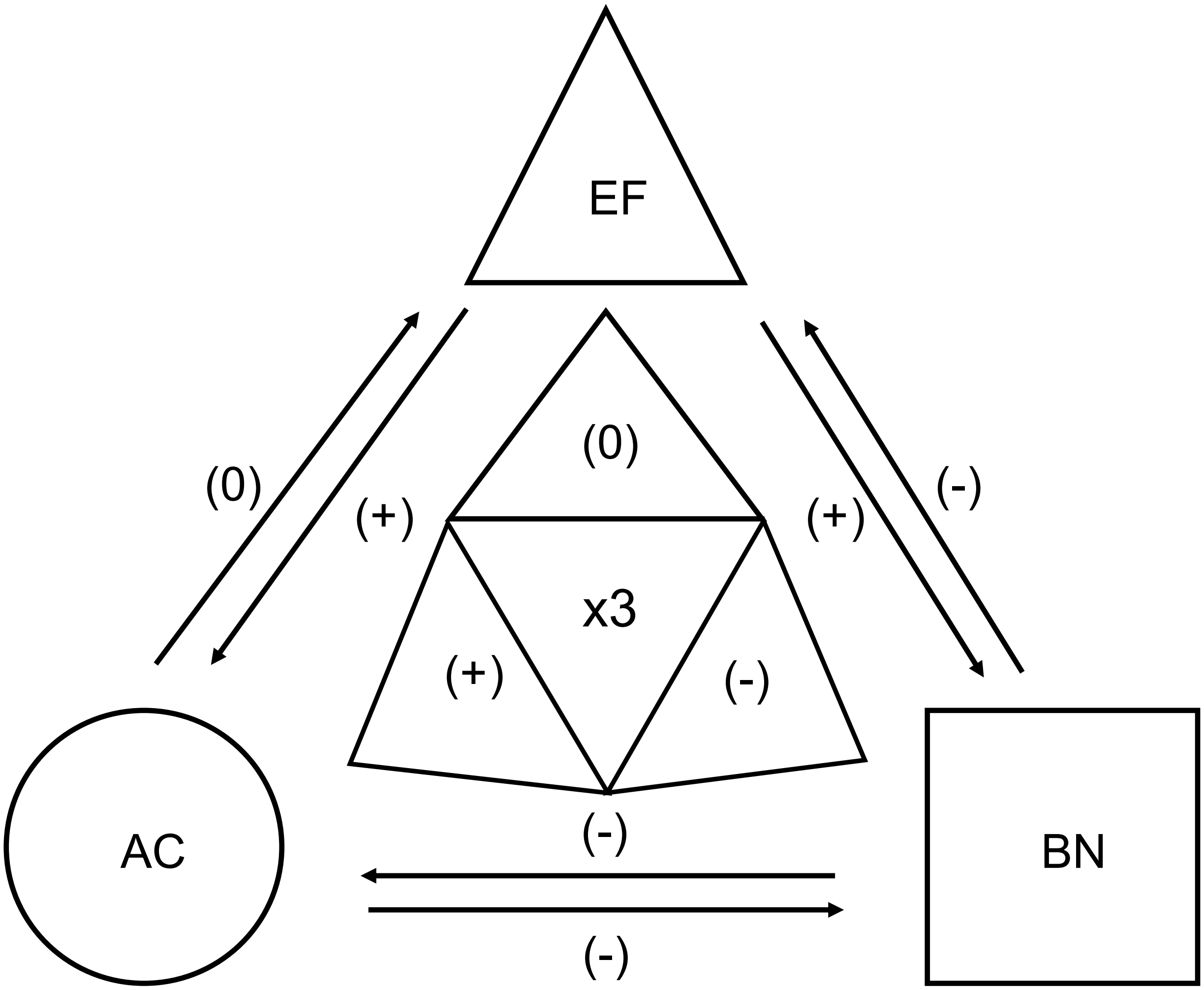

Figure 2.1 Conceptual model of hypothesized system of pairwise and three-way species interactions between *E. fasciculatum* (EF), *A. californica* (AC), and *B. nigra* (BC). Outer arrows represent pairwise species interactions. Internal radiating triangles represent effect of two species on the third. Interaction signs represent (+) positive; facilitation, (-) negative; competition, and (0) neutral species interactions.

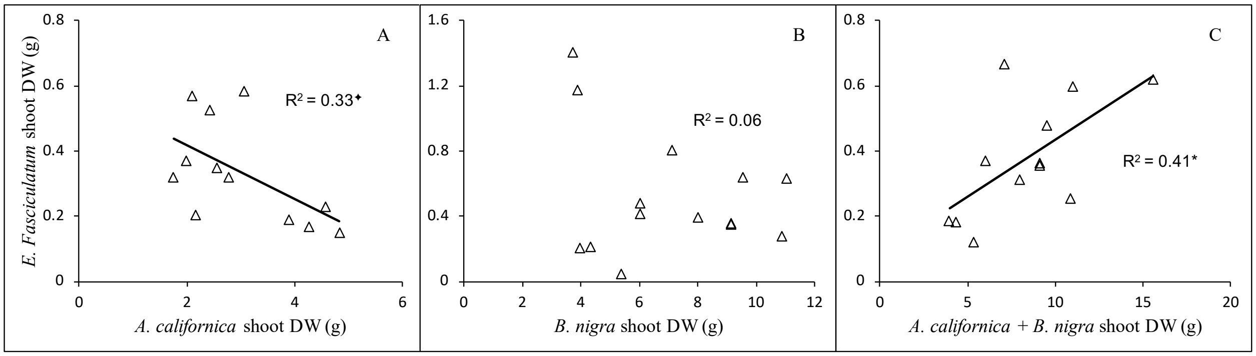

Figure 2.2 *E. fasciculatum* shoot DW (g) versus A.) *A. californica* shoot DW (g), B.) *B. nigra* shoot DW (g), and C.) *A. californica* + *B. nigra* shoot DW (g). Linear regression analysis showed *A. californica* shoot DW had a moderately significant, inverse relationship to *E. fasciculatum* shoot DW (n = 12, p = 0.0521✦), *B. nigra* shoot DW had no affect (n = 14, p = 0.384), and *A. californica* + *B. nigra* shoot DW had a significant, positive relationship to *E. fasciculatum* shoot DW (n = 12, p = 0.026*).

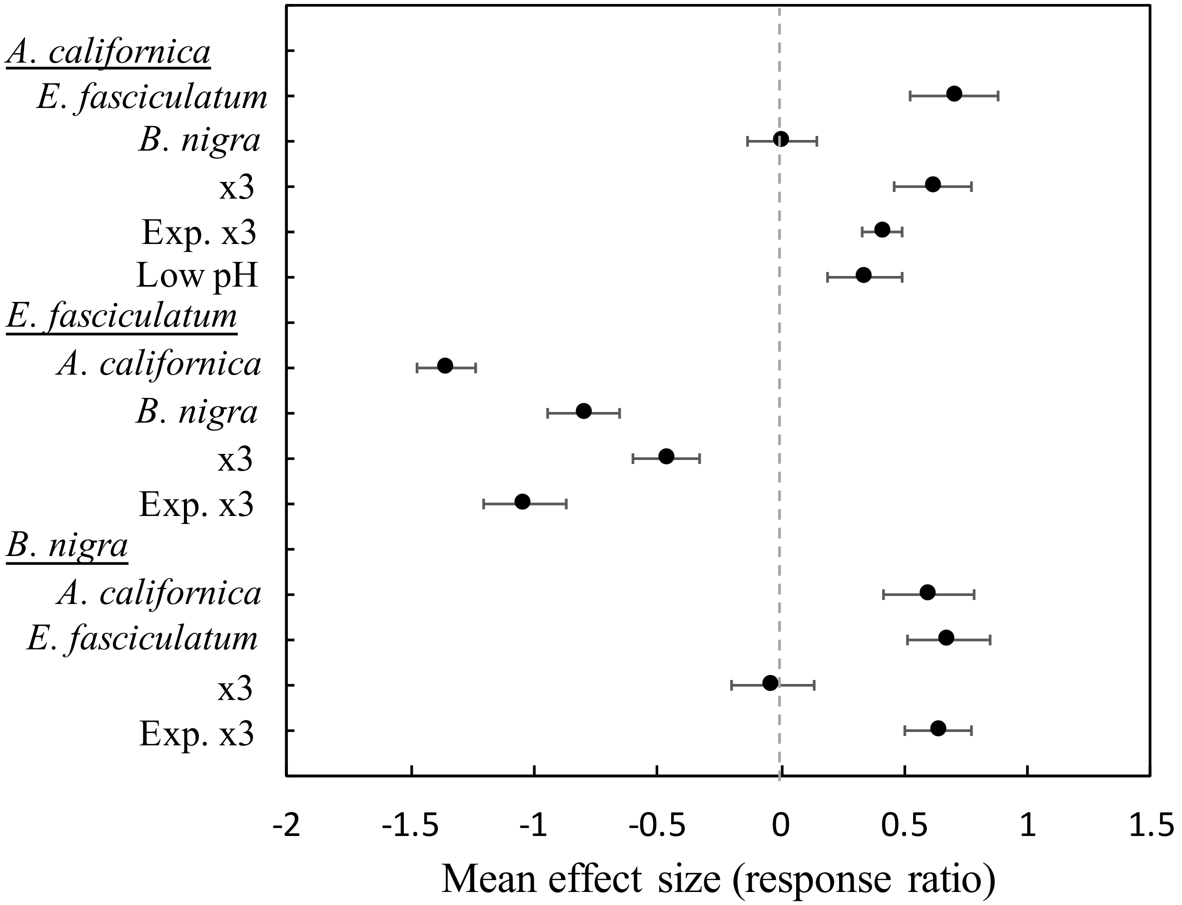

Figure 2.3 Mean effect size (response ratio) for targest species (underlined species name) in pair-wise interactions (species name), three-way species interactions (x3), expected three-way species interactions (Exp. X3), and lowered pH for *A. californica* (Low pH). Bars around the means denote 95% confidence intervals. Mean effect size is significantly different from zero if the 95% con fidence interval does not include zero.

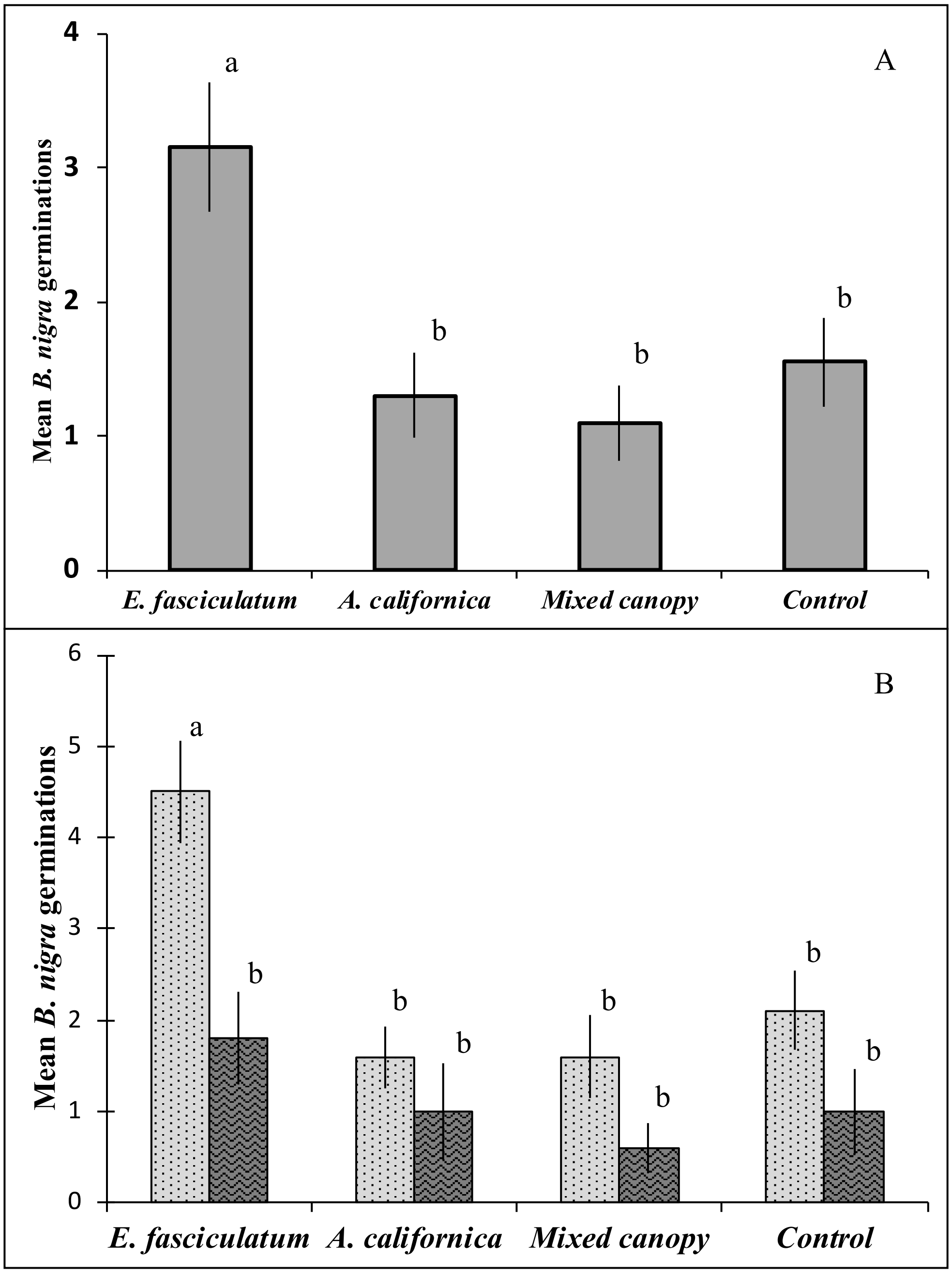

Figure 2.4 A.) Mean *B. nigra* germinations under *E. fasciculatum, A. californica,* mixed canopies, and shaded controls. B.) Mean *B. nigra* germinations in buried pots (light gray with dots) and tilled bare ground (dark grey with waves) under *E. fasciculatum, A. californica,* mixed canopies, and shaded controls. Letters indicate significant differences from Tukey HSD post hoc analysis of permutation ANOVAs.

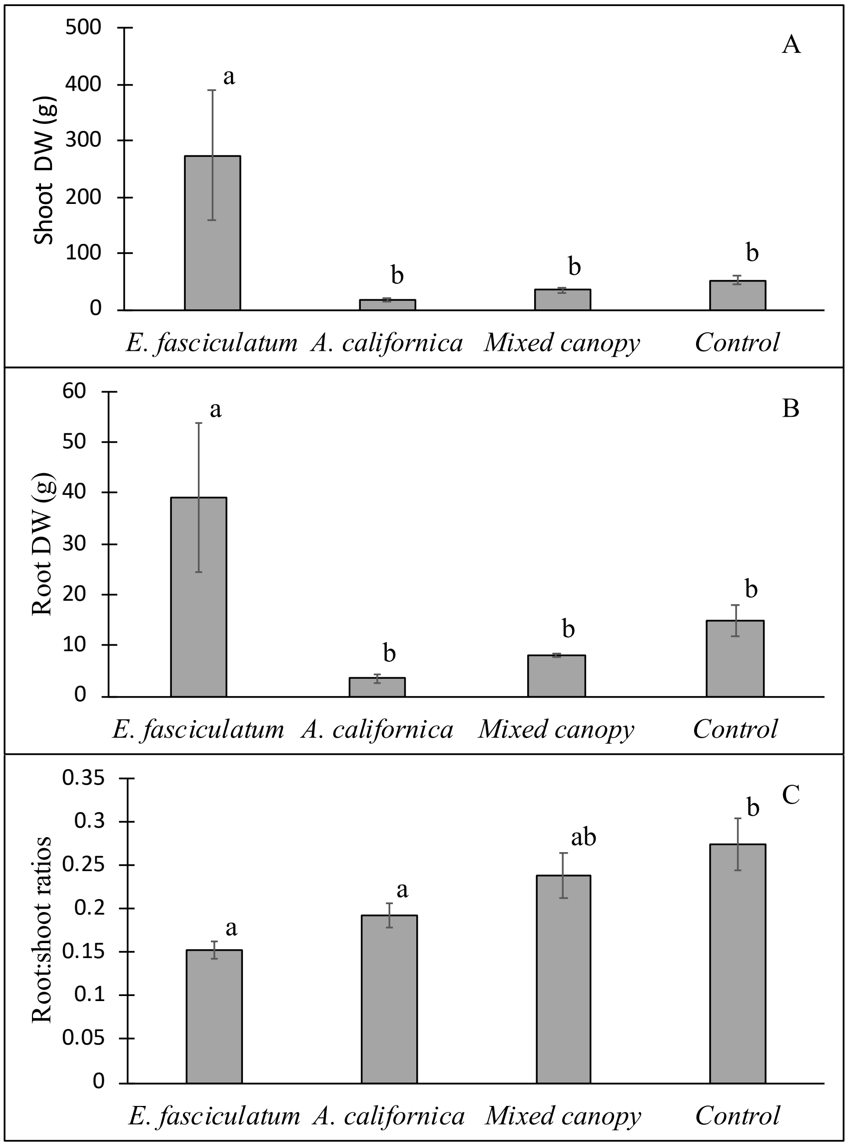

Figure 2.5 A.) *B. nigra* mean shoot DW (g) ($F_{3,24} = 9.972$, $p=0.0034$; B.) *B. nigra* mean root DW (g) ($F_{3,24} = 10.03$, $p=0.0002$; and C.) *B. nigra* mean root:shoot ratios ($F_{3,24} = 4.775$, $p=0.007$) under *E. fasciculatum, A. californica,* mixed canopies, and shaded controls. Letters indicate significant difference between means with Tukey HSD post hoc test on Permutation ANOVAs.

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Appendix A: Supplemental Figures & Tables for Chapter 2

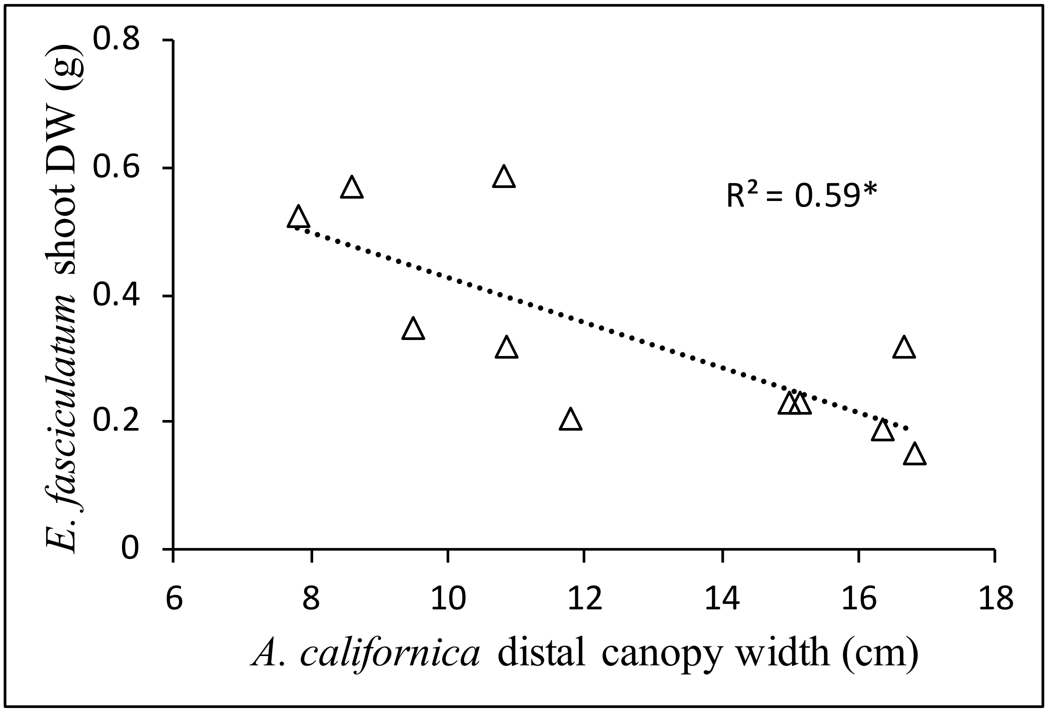

Figure S2.1 *E. fasciculatum* shoot DW (g) versus *A. californica* distal canopy width (cm). *A. californica* distal canopy width had a strongly significant, negative relationship to *E. fasciculaum* shoot DW (n = 11, p = 0.00586).

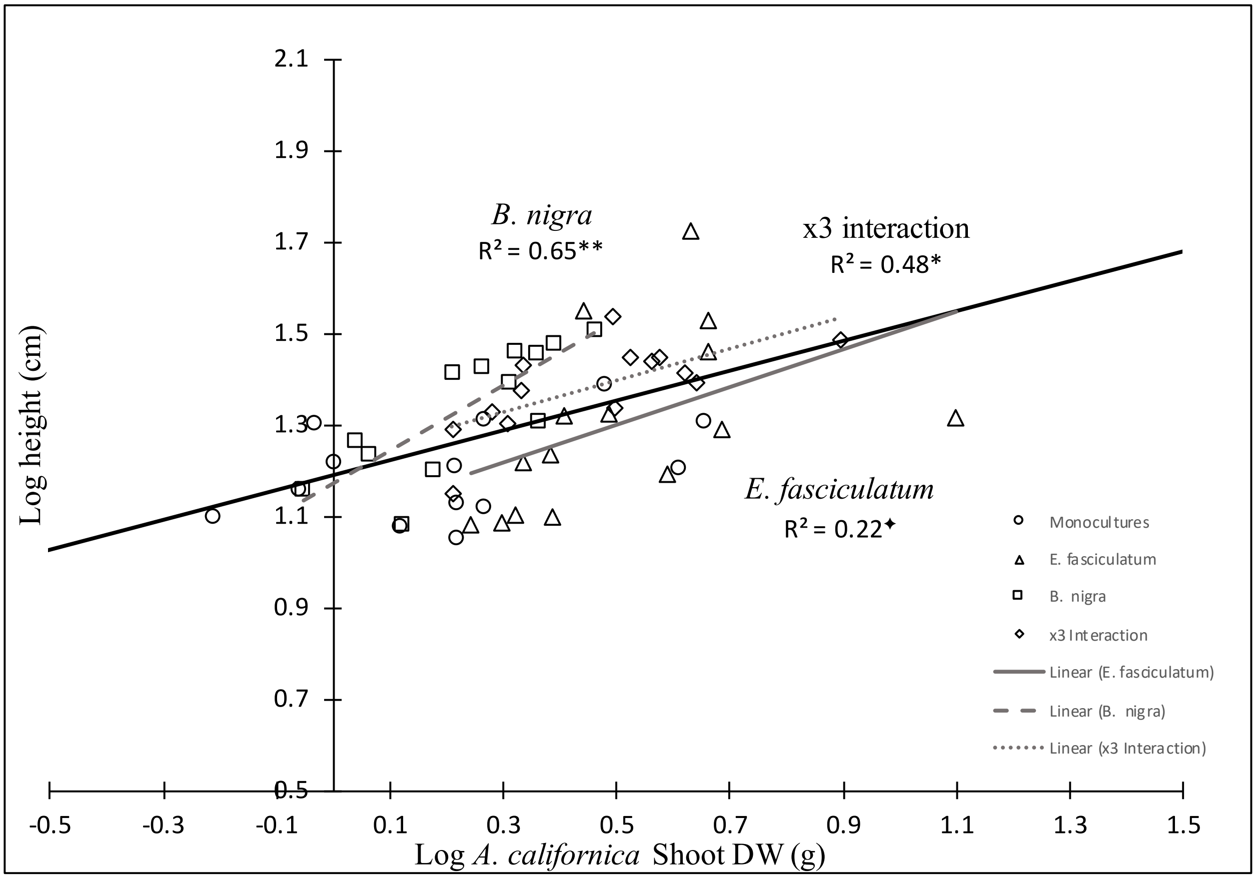

Figure S2.2 Log-transformed height (cm) versus log-transformed shoot DW (g) for *A. californica* in monocultures (circles), pairwise with *E. fasciculatum* (triangles with solid grey line), pairwise with *B. nigra* (squares with dashed line), and three-way interactions (diamonds with dotted line). R^2 values with ✦ $p < 0.08$, * means $p < 0.05$, and **$p < 0.005$.

Table S1. ANCOVA summary table with linear regression analysis for *A. californica* log-transformed height (cm) versus log-transformed shoot DW (g).

	ANCOVA		Linear Regressions			
	Est. Std.	p-value		R2	p-value	Line Eq.
Intercept	1.172471	2.00E-16				
Monoculture	0.719736	0.002**		0.11	0.14404	y = 1.516x+ 13.381
E. fasciculatum	-0.078483	0.435		0.22	0.0765✦	y = 0.8639x+ 19.023
B. nigra	-0.009032	0.904		0.65	0.0008**	y = 9.2658x+ 6.1196
x3 interaction	0.052214	0.617		0.48	0.0063*	y = 1.8798x+ 18.852
Mono vs E. fasc.	-0.306705	0.250				
Mono vs B. nigra	-0.545828	0.0391*	All treatments	0.40	0.00004176***	
Mono vs x3	-0.370878	0.188				

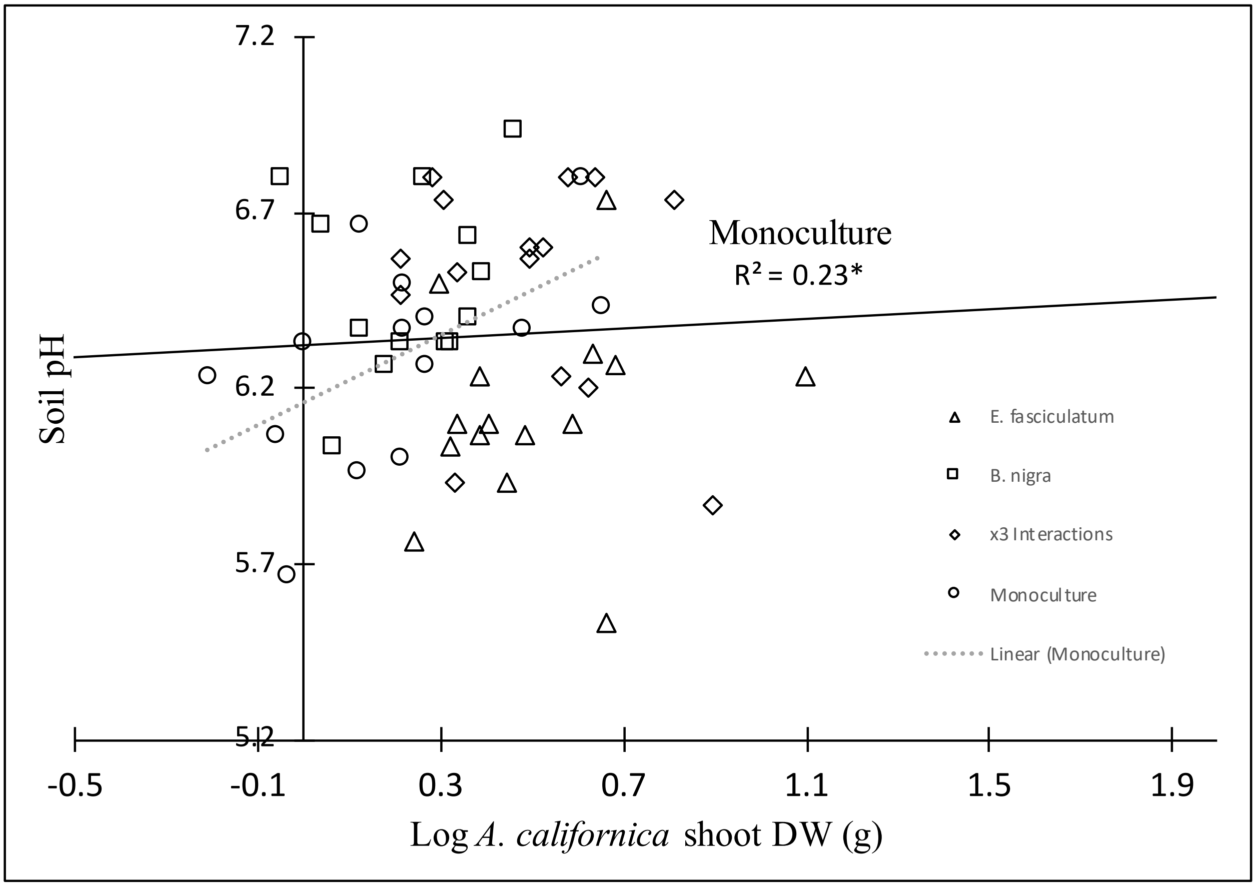

Figure S2.3 Soil pH versus log-transformed shoot DW (g) for *A. californica* in monocultures (circles with dotted grey line), pairwise with *E. fasciculatum* (triangles), pairwise with *B. nigra* (squares), and three-way interactions (diamonds). R^2 values with * means $p < 0.05$.

Table S2. ANOCVA and linear regression analysis for soil pH versus Log-transformed shoot DW (g) for *A. californica*.

	ANCOVA			Linear regressions		
	Est. Std.	p-value		R2	p-value	Line Eq.
Intercept	0.82326	<2e-16				
Monoculture	-0.02286	0.365		0.23	0.0459*	y = 0.1337x + 6.0399
E. fasciculatum	-0.04434	0.0204*		-0.04334	0.529	y = 0.0166x + 6.0693
B. nigra	-0.01554	0.351		-0.08	0.566	y = 0.116x + 6.2857
x3 interaction	-0.03412	0.0255*		-0.02	0.413	y = -0.0493x + 6.6643
Mono vs E. fasc.	0.03899	0.263				
Mono vs B. nigra	0.04242	0.34	All treats	0.22	0.00630**	
Mono vs x3	0.06746	0.0470*				

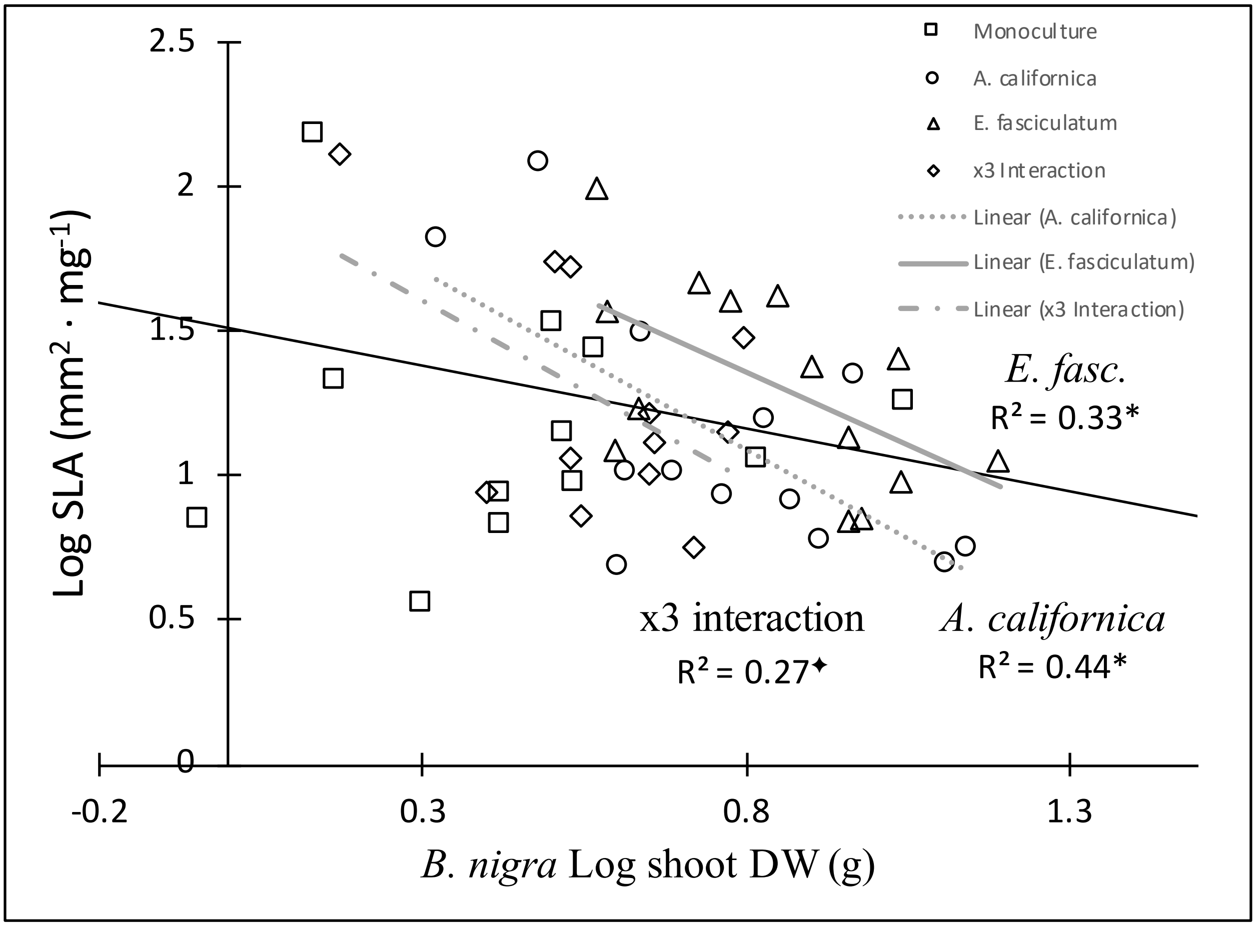

Figure S2.4 Log-transformed SLA ($mm^2 \cdot mg^{-1}$) versus log-transformed shoot DW (g) for *B. nigra* in monocultures (squares), pairwise with *E. fasciculatum* (triangles with solid grey line), pairwise with *A. californica* (circles with dotted line), and three-way interactions (diamonds with dotted- dashed line). R^2 values with ✦ $p < 0.08$ and * means $p < 0.05$.

Table S3. ANCOVA and linear regression analysis for Log-tranformed SLA (mm2 · mg-1) versus Log-transformed shoot DW (g) for *B. nigra*.

	ANCOVA			Linear Regressions		
	Est. Std.	p-value		R2	p-value	Line equation
Intercept	1.969	5.30E-06				
Monocultue	-1.229685	0.0575✦		-0.1	0.896	Y = -0.0601 + 1.2003
A. californica	0.098966	0.849		0.39	0.013*	y = -1.2249x + 2.0681
E. fasciculatum	0.193168	0.741		0.28	0.0308*	y = -1.0078x + 2.1623
x3 interaction	-0.768761	0.0783✦		0.27	0.0858✦	y = -1.2297x + 1.9691
Mono vs A. cali.	0.004755	0.995				
Mono vs E. fasc.	0.22186	0.785	All treatments	0.18	0.0291	
Mono vs x3	1.169626	0.116				

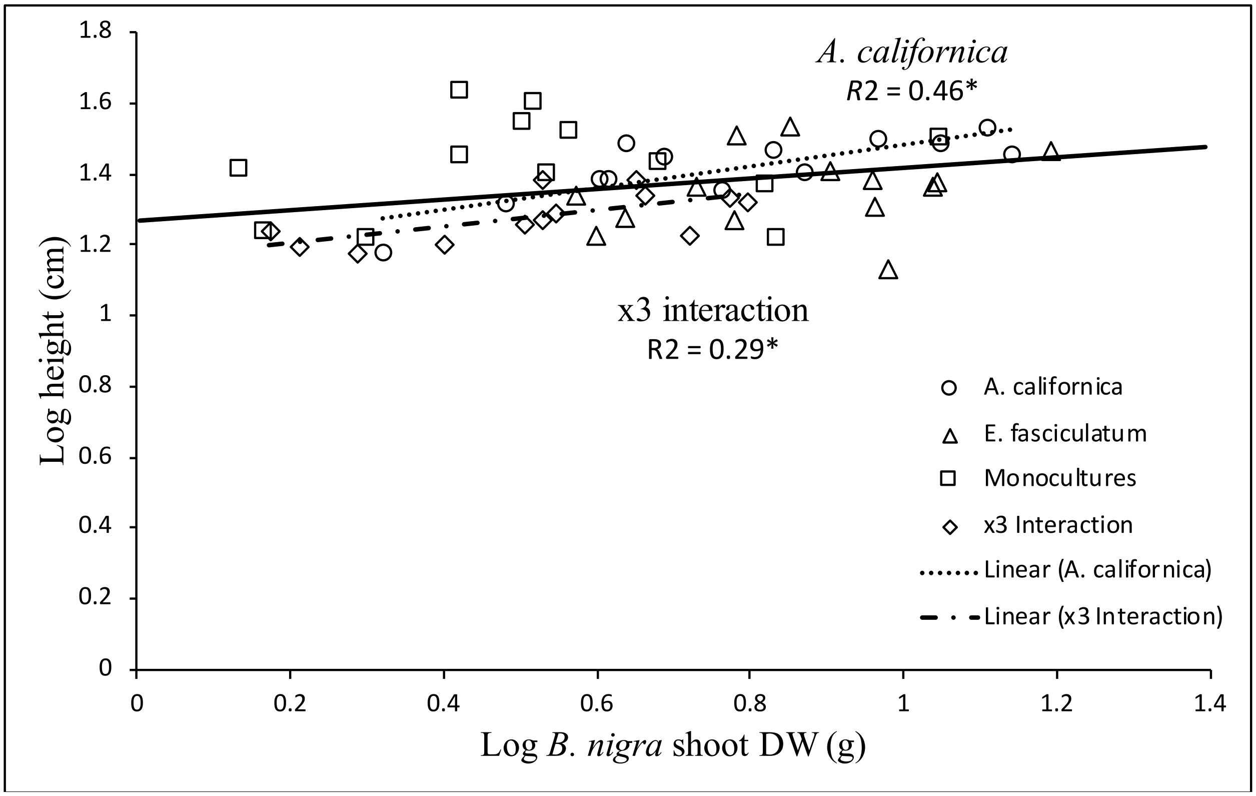

Figure S2.5 Log-transformed hieght (cm) versus log-transformed shoot DW (g) for *B. nigra* in monocultures (squares), pairwise with *E. fasciculatum* (triangles with solid grey line), pairwise with *A. californica* (circles with dotted line), and three-way interactions (diamonds with dotted-dashed line). R^2 values with * means $p < 0.05$.

Table S4. ANCOVA and linear regression analysis for Log-transformed height (cm) versus Log-transformed shoot DW (g) for *B. nigra*.

	ANCOVA			Linear Regressions		
	Est. Std.	p-value		R2	p-value	Line equation
Intercept	17.3578	<0.0001				
Monocultue	0.22798	0.115		0.0078	0.763	y = 0.0633x + 1.3953
A. californica	0.01819	0.884		0.50	0.00656*	y = 0.3038x + 1.1785
E. fasciculatum	0.07331	0.638		0.05	0.420*	y = 0.1415x + 1.2336
x3 interaction	0.29053	0.00394✦		0.34	0.0343✦	y = 0.228x + 1.1603
Mono vs A. cali.	0.07581	0.682				
Mono vs E. fasc.	-0.08643	0.680	All treatments	0.32	<0.001	
Mono vs x3	-0.25054	0.1488				

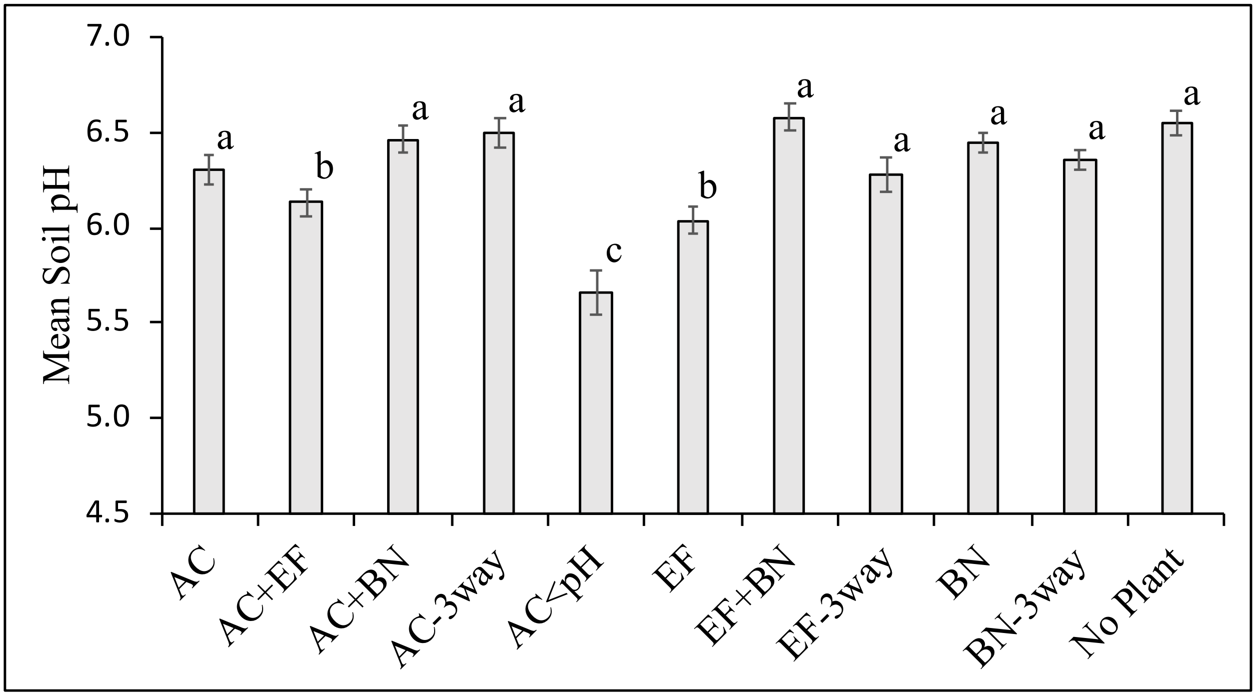

Figure S2.6 Final mean (+/- 1 SD) soil pH after 89 days in the greenhouse. Includes data for 15 pots with ‘no plants’ as control. Letters indicate significant difference between treatments with Tukey HSD post hoc analysis following an ANOVA.