



Three-way species interactions reverse the positive pairwise effects of two natives on an exotic invader

Benjamin M. Schlau¹ · Travis E. Huxman² · Kailen A. Mooney³ · Jessica D. Pratt⁴

Received: 2 June 2022 / Accepted: 10 February 2023 / Published online: 2 March 2023
© The Author(s) 2023

Abstract

The disruptive effects of tertiary species on otherwise positive pairwise species interactions (e.g. context-dependent parasitism in pollinator syndromes) is well-known. However, few—if any—studies have investigated how invasive plants affect interactions between facilitative plants and their native plant communities. Further, if tertiary invasive species can change interactions among native species from positive to negative, then a tertiary native should be capable of the same phenom for pairwise interactions between natives and invasives. Our previous research indicates invasive black mustard (*Brassica nigra*) changes interaction signs for otherwise positive species interactions between the dominant, native facilitator California buckwheat (*Eriogonum fasciculatum*) and its co-dominant beneficiary California sagebrush (*Artemisia californica*) in semi-arid California coastal sage scrub habitat. Here, *E. fasciculatum* and *A. californica* seedlings increased *B. nigra* shoot growth in pairwise species interactions in the greenhouse. However, in three-way species interactions, *E. fasciculatum* and *A. californica* together reduced *B. nigra* SLA, height, and reproductive potential while not increasing shoot DW. In three-way species interactions, *B. nigra* did not significantly reduce *E. fasciculatum* facilitation of *A. californica*. Also surprisingly, light competition with *B. nigra* resulted in an increase in *A. californica* height, which reduced the negative effects of *A. californica* light competition on shade-intolerant *E. fasciculatum*. In an additive field experiment, *A. californica* protected *E. fasciculatum* from facilitating germination and growth of *B. nigra* when water competition was minimized. Taken together, this study demonstrates the importance of studying species interactions between competitive, native perennials in the current ecological context of invaded ecosystems.

Keywords Plant facilitation · Coexistence · Invasive species · Context-dependent species interactions · Competition

Introduction

Tertiary species can alter pairwise species interactions (e.g. Wootton 1994; Chamberlain et al. 2014), even in obligatory mutualisms, such as context-dependent parasitism observed in pollinator syndromes (see Palmer et al. 2003 for further explanation). Another positive but highly context-dependent species interaction is plant facilitation, in which interactions shift to competition as alleviated stressors weaken along spatial and temporal gradients (Bertness and Callaway 1994). However, the literature has yet to illuminate how non-native tertiary species affect pairwise species interactions in plant facilitation systems, despite the well-documented occurrences of facilitative plants recruiting harmful invasives (e.g. Rodríguez-Buritica and Miriti 2009). Conversely, if a tertiary species can alter interactions between native species (Chamberlain et al. 2014), a tertiary native could theoretically

Communicated by Ling Zhang.

✉ Benjamin M. Schlau
bschlau@scrippscollege.edu

- ¹ W. M. Keck Science Department, Claremont McKenna, Pitzer, and Scripps Colleges, 925 N Mills Ave., Claremont, CA 91711, USA
- ² Department of Ecology and Evolutionary Biology, University of California, Irvine, 449 Steinhaus Hall, Irvine, CA 92697, USA
- ³ Department of Ecology and Evolutionary Biology, University of California, Irvine, 3112 Biological Sciences 3, Irvine, CA 92697, USA
- ⁴ Department of Ecology and Evolutionary Biology, University of California, Irvine, 361 Steinhaus Hall, Irvine, CA 92697, USA

change pairwise interaction signs between native and invasive plants, but to our knowledge, this has never been demonstrated.

Invasive plants fundamentally alter ecological processes, both directly through exclusion of native flora (D’Antonio and Vitousek 1992) and indirectly through changes to soil properties and disturbance regimes (Stylinski and Allen 1999). For example, in semi-arid California coastal sage scrub (CSS), fast-growing invasive annuals have competitively excluded nearly all native annual and perennial grasses and forbs (Bowler 2000). At the community level, the loss of native plant diversity degrades habitat quality for animals (Bowler 2000). At the ecosystem level, invasive annuals reduce overall plant productivity (Goldstein and Suding 2014) and increase wildfire frequency by senescing easily ignitable shoot tissues early in the growing season (Minnich and Dezzani 1998). The decrease in fire intervals degrades habitat heterogeneity by inhibiting native perennials from reestablishing and reaching late post-fire successional stages (Minnich and Dezzani 1998). As fires occur more frequently, long-lived shrubs and trees may never reach late growth stages. Therefore, species interactions among long-lived perennials and other shorter-lived species during early growth stages may become more determinant to the distribution of all plant species.

Our previous work indicates the CSS dominant native facilitatory perennial California buckwheat (*Eriogonum fasciculatum*) benefits from the competitive effects of its nearly exclusive beneficiary, the codominant native perennial California sagebrush (*Artemisia californica*) (Schlau et al. 2021). Greenhouse and field experiments suggest *E. fasciculatum* acidifies CSS’s calcareous soils and alleviates alkalinity stress for *A. californica*. Positive interactions begin as early as the seedling stage and result in nearly exclusive positive spatial associations maintained into late growth forms when canopies are > 1 m in diameter. These previous findings led to a question as to whether *E. fasciculatum*’s positive spatial association with *A. californica* conveys the benefits of complimentary recruitment inhibition of other plant species, especially harmful invasives. *Eriogonum fasciculatum* and *A. californica* co-dominate a majority of remaining CSS habitat patches (e.g. Eliason and Allen 1997). However, slower-growing, long-lived perennial seedlings compete poorly for light and water with faster-growing invasive annuals (e.g. Eliason and Allen 1997; Cione et al. 2002). Indeed, an exploratory study suggested the highly invasive, allelopathic annual forb black mustard (*Brassica nigra*) disrupts otherwise positive species interactions in a plant facilitation system with *E. fasciculatum* and *A. californica* at early growth stages. Co-occurring *E. fasciculatum* and *A. californica* in late growth stages (canopies > 1 m diameter) are expected to not facilitate—and even inhibit recruitment

of *B. nigra*, but recruitment outcomes at the seedling-to-juvenile growth stages remains far more unclear and is the primary focus of the study presented here.

This study asked the following questions: (1) Does *B. nigra* affect interactions between *E. fasciculatum* and *A. californica* at the seedling-to-juvenile growth stages? (2) At late growth stages, does *A. californica* prevent *E. fasciculatum* from recruiting *B. nigra*? (3) At any growth stage, do *E. fasciculatum* and *A. californica* synergistically harm *B. nigra*? (4) Finally, in an investigation into mechanisms of facilitation, does reducing soil pH in *A. californica* monocultures mimic facilitative effects of *E. fasciculatum* on *A. californica*? The dynamics of invaded facilitative systems, or invasions of any positive interactions between native species that enhance primary productivity, biodiversity, resistance to invasion, and habitat quality, demand extensive investigation if biologists are to enhance biological communities’ potential to adapt to changes in water availability, temperature, wildfire severity, eutrophication, invasive species, and geographic range (Brooker 2006). Furthermore, as increasing temperatures and invasives increase the frequency of wildfires in various ecosystems, studying species interactions between newly establishing seedlings of competitive, native perennials can further inform long-term land management decisions. In other words, improving our understanding of facilitative plant systems in the Anthropocene requires studying facilitation within the greater ecological context of intensely invaded plant communities.

Methods

Study system

California coastal sage scrub (CSS) is a Mediterranean-type ecosystem that occupies < 10–15% of its pre-colonial range (Westman 1981). White flight, the resulting poorly managed urban sprawl, and soil N-loading (soil eutrophication), primarily from heavy diesel truck emissions and heavy construction equipment, continue to facilitate the establishment of non-native plants into increasingly isolated habitat patches (Talluto and Suding 2008). CaCO_3 soil deposits ($2600 \mu\text{g Ca}^{2+} \text{ g}^{-1}$ soil; Westman 1981) from ancient seashells uplifted with marine sediment produce multifaceted nutrient stress for local plant communities. As a mean 250–450 mm of annual precipitation falls during short winter rainy seasons, calcareous soils “harden” soil water up to pH 8.2 (Irvine Ranch Water District Comprehensive Financial Annual Report 2018). As CaCO_3 disassociates, Ca^{2+} ions bond to PO_4^{3-} and NO_3^- , making them unavailable to plants (Strom et al. 2005). By pulling H_3O^+ out of the rhizosphere, CO_3^{2-} ions degrade proton gradients that roots establish to uptake nutrients (e.g. Fuglsang et al. 2007).

Target species

Eriogonum fasciculatum is a long-lived (> 25 year) perennial, facultatively evergreen and shade-intolerant shrub. *Eriogonum fasciculatum* facilitates *A. californica* via acidification of alkaline soils (presumably via mobilize of nutrients; Schlau et al. 2021), but outcompetes most other plant species for water with woody roots that spread > 3 m laterally (Kirkpatrick et al. 1977) and up to 2.5 m deep (Kummerow et al. 1977; Hellmers et al. 1995).

Co-dominant *A. californica* similarly competitively excludes most other species (Kirkpatrick et al. 1977). Terpene leachates inhibit germination and growth of several species (Halligan 1973, 1975; Karban 2007). Fibrous, shallow roots quickly absorb soil water from morning dew and offseason rain events to outcompete most other plants for water (Gray 1982). Both *A. californica* and *E. fasciculatum* canopies exhibit lateral dominant growth forms > 1 m wide to outcompete heterospecific seedlings for light.

Brassica nigra was chosen as the tertiary invasive due to its dominance of disturbed habitat and high fecundity in CSS, as well as its potential to allelopathically alter facilitatory interactions between *E. fasciculatum* and *A. californica*. Unlike most Angiosperms, *B. nigra* does not form mutualistic interactions with mycorrhizae, and instead, releases fungicidal root exudates, which indirectly harms other plant species by preventing associations with mycorrhizae (Schreiner and Koide 1993). When *B. nigra* germinates or leaches isothiocyanate into soils, the forb directly inhibits germination and growth of at least some other plant species (Turk and Tawha 2003; Tawaha and Turk 2003; Oduor et al. 2020).

Greenhouse competition-facilitation experiment

To test how *B. nigra* seedlings affect pairwise species interactions between *E. fasciculatum* and *A. californica* seedlings, the three species were grown in 7 L pots in the UC Irvine greenhouse utilizing a full factorial design ($n = 15$ ea. treatment). An additional 15 replicates of *A. californica* monocultures with 36 g elemental sulfur soil amendments were used to mimic plant-soil feedback of *E. fasciculatum* and test a hypothesis that *E. fasciculatum* alleviates alkalinity stress for *A. californica* with acidic root exudates. As a control for plant-soil feedback effects on soil pH, 12 more replicates were filled with soil and watered, but contained no plants.

Genetically local *E. fasciculatum* and *A. californica* seeds were furnished by the Irvine Ranch Conservancy's Irvine Ranch Natural Landmarks in Irvine, CA. *Brassica nigra* seeds were harvested from the UC Irvine CSS Ecological Preserve. All seeds germinated for greenhouse experiment were collected after the above average 2018–2019 winter

rainy season. Plants were started in germination trays under misting benches, then replanted in 7 L pots following growth of first true leaves.

Each 7 L pot contained four plants. Pairwise species interactions were tested with two of each target species. Three-way species interactions were tested with two of the treated species and one each of the other two species. For example, in order to test the effects of *B. nigra* and *A. californica* on *E. fasciculatum* growth, two *E. fasciculatum* grew with one *B. nigra* and one *A. californica*.

The 7 L pots contained UCI native CSS soil mix (1.5 pumice:1 sand:1 redwood mulch:1 peat moss by volume). Previous research indicated *E. fasciculatum* facilitation of *A. californica* was conditional on alkaline soil (Schlau et al. 2021). Soil pH levels were recorded for all pots using 1:1 slurry method with a HANA field pH meter prior to planting but after conditioning the soil with local “hard” tap water (28–90 mg $\text{Ca}^{2+} \cdot \text{L}^{-1}$; pH 7.8–8.2; IRWD 2018) for 8 days to raise the pH to a closer approximation of field conditions (mean pH 7.8) observed in previous experiments. All plants were continuously watered with “hard” tap water. Soil pH was remeasured again prior to destructive harvest for analysis of plant-soil feedback effects. All pot positions were rerandomized weekly.

To test how pairwise and three-way species interactions affected overall shoot growth after 89 days in the greenhouse, log response ratios (mean effect size) of mean shoot DW (g) with pooled standard deviation were analyzed. Log response ratios in pairwise species interactions were derived from the natural log of mean shoot DW for target species in pairwise species 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 (see Online Appendix for full description of calculations).

As indicated from a previous study (B. Schlau *unpublished*), *E. fasciculatum* was expected to facilitate shoot growth of the other target species. *Brassica nigra* was expected to decrease shoot DW of both *E. fasciculatum* and *A. californica*. *Artemisia californica* was expected to not affect *E. fasciculatum* shoot DW and decrease *B. nigra* shoot DW (see Fig. 1 for graph of proposed species interactions).

A primary objective of this study was to test the effects of a tertiary, invasive species on positive pairwise species interactions between codominant, native species. To test if the tertiary species affected species interactions between the two native species, three-way species interaction effect sizes were analyzed with expected values for additive three-way species interactions. To predict additive three-way species interaction effects, means of the respective pairwise species interaction effect log response ratios with 95% confidence intervals derived from pooled standard deviations (again, see Online Appendix for full description of equations).

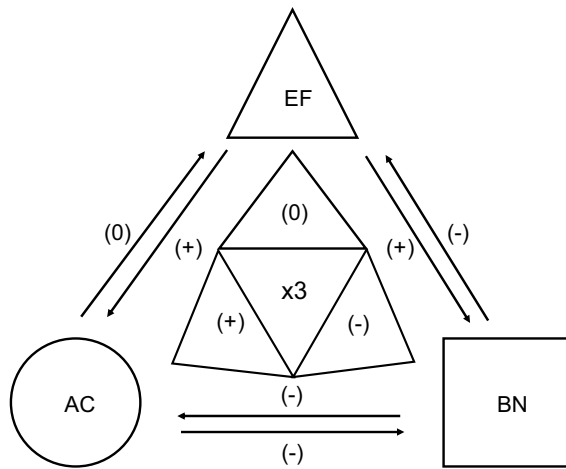


Fig. 1 Conceptual model of hypothesized system of pairwise and three-way species interactions between *E. fasciculatum* (EF), *A. californica* (AC), and *B. nigra* (BN). Outer arrows represent pairwise species interactions. Internal radiating triangles represent effect of two species on the third. Interaction signs represent (+) positive/facilitation, (–) negative; competition, and (0) neutral species interactions

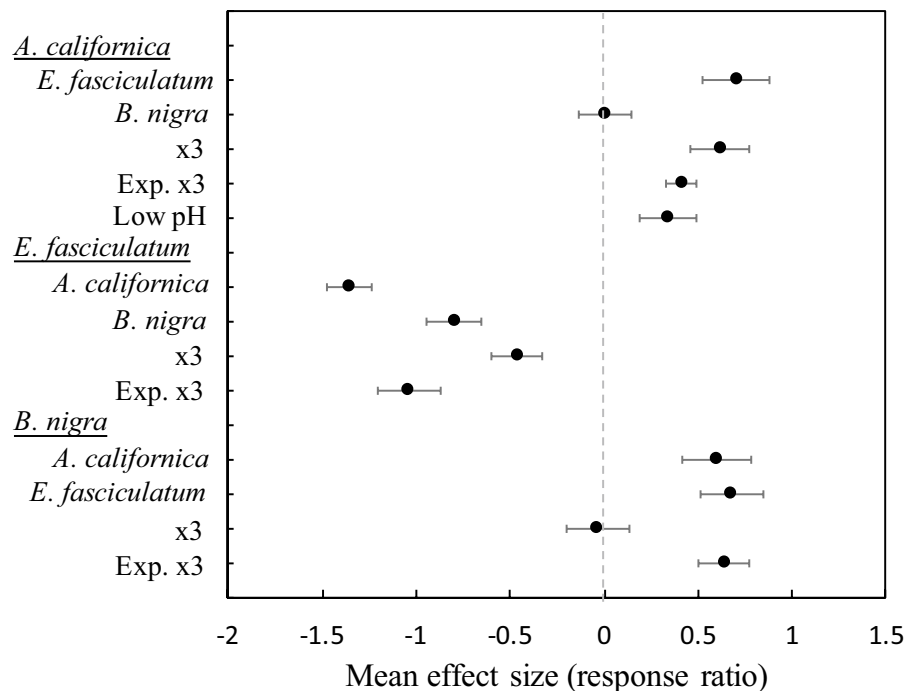
Standard deviations were determined with pairwise effect means. Deviation of observed from predicted three-way species interactions were considered significant, and therefore non-additive interactions, if the predicted effect's 95% confidence interval lied outside the observed effect's 95% confidence interval. Synergistic three-way species interactions were indicated by significantly stronger observed three-way species interaction effects than predicted. Antagonistic

three-way species interactions were indicated by significantly weaker effects than predicted.

Log response ratios showed a significantly antagonistic three-way species interaction that weakened negative effects on *E. fasciculatum* shoot DW observed with *E. fasciculatum* in pairwise species interactions with *B. nigra* and *A. californica* (see “Results” section, Fig. 2). Thus, relationships of *E. fasciculatum* shoot DW to the shoot DWs of *A. californica* and *B. nigra* in pairwise species interactions as well as *E. fasciculatum* shoot DW versus the sum of *A. californica* and *B. nigra* shoot DWs in three-way species interactions were analyzed with linear regressions. Negative relationships in pairwise species interactions were interpreted as competition and positive relationships in three-way species interactions were interpreted as an interaction between *A. californica* and *B. nigra* that alleviated negative effects of *B. nigra* and *A. californica* on *E. fasciculatum* observed in pairwise species interactions.

In order to investigate processes behind observed patterns in species interactions, shoot DW (covariable) was analyzed with ANCOVAs for relationships to specific leaf area (SLA), soil pH, and shoot height (dependent variables) in each species interaction treatment (independent variables). If an ANCOVA suggested a significant difference among slopes, linear regressions were used to test if any particular treatment effected a significant relationship between the dependent variable (e.g. SLA) and the covariable (shoot DW). For example, if pairwise species interactions with *B. nigra* resulted in an observed significant positive relationship with linear regression analysis between *A. californica*

Fig. 2 Mean effect size (response ratios) of target species (underlined species name) shoot DW in pair-wise species interactions (species name), three-way species interactions (x3), expected three-way species interactions (Exp. x3), and reduced pH for *A. californica* (Low pH).



shoot height and shoot DW, the result was interpreted as *A. californica* growing taller due to interactions, such as light competition, with *B. nigra*.

Another of this study's objectives was to test for interactions between *E. fasciculatum* and *A. californica* that increase resistance and resilience to invasion by *B. nigra* and potentially other invasives. Plants harm other plant species in ways that may not be detected from analyzing total shoot DW alone. Therefore, *B. nigra* leaf mass and inflorescence count (dependent variables) were compared among species treatments (independent variables) with ANOVAs and analyzed in conjunction with results from ANCOVA and linear regression analysis for indications of competition-driven tradeoffs among SLA, shoot height, and reproductive potential (i.e. inflorescence count). Aggregated results were interpreted with Westoby's Leaf-Height-Seed Hypothesis (Westoby 1998).

Root DW and root:shoot ratios were not included in analysis due to entanglement of belowground tissues. The entangled roots that were impossible to separate while avoiding extensive root tissue damage.

At a density of two shrubs per 7 L pot in a previous experiment, *E. fasciculatum* facilitated *A. californica* shoot growth with no loss in its own shoot DW (Schlau et al. 2021). However, at four shrubs per treatment in the greenhouse here, *A. californica* appeared to shade *E. fasciculatum* seedlings. The shading led to an impromptu hypothesis that *A. californica* may harm its facilitator *E. fasciculatum* with light competition at this experiment's planting density. As a test of this impromptu hypothesis, a linear regression was used to test for a relationship between *E. fasciculatum* shoot DW versus *A. californica* distal canopy width and using a prediction that *A. californica* distal canopy width would have a significantly inverse relationship to *E. fasciculatum* shoot DW.

Field recruitment facilitation-inhibition experiments

To test whether *A. californica* inhibits *E. fasciculatum* from recruiting *B. nigra* when the native shrubs are in late growth stages, an additive field experiment was conducted in the UC Irvine Coastal Sage Scrub Ecological Preserve. Long-lived, perennials that benefit from facilitation by a heterospecific tend to eventually outcompete their facilitator once the beneficiary establishes and is less vulnerable to previously alleviated stressors. Unlike other plant facilitation systems, a significant, positive spatial association between *E. fasciculatum* and *A. californica* persists into late growth stages when canopies are > 1 m diameter (Schlau et al. 2021). The persistence of mutual tolerance instead of competitive exclusion indicates positive or, at a minimum, neutral species interactions persist into later

growth stages. This previous study led to a hypothesis that *A. californica* can protect *E. fasciculatum* from facilitating recruitment of harmful invasive species and *E. fasciculatum* facilitation of *A. californica* enhances the competitive effects of *A. californica* recruitment inhibition.

To tease apart facilitation from water competition, 30 genetically local *B. nigra* seeds were sown in 12 × 12 × 10 cm buried pots of field soil and adjacent 12 cm² plots of tilled bare ground. Topsoil was removed to a depth of 8–10 cm to minimize the possibility of *B. nigra* seedbanks from previous years. Ten replicates of each paired treatment (i.e. buried pots and adjacent tilled ground) were positioned beneath south-facing canopy edges of *E. fasciculatum*, an adjacent *A. californica*, where the two species' canopies crossed, and under shaded controls isolated by 0.5–1.0 m from the nearest plant ($n = 80$; $n = 10$ replicates per treatment). All shrub canopies were > 1 m diameter to better ensure each shrub exhibited the full metabolism of a perennial in late growth stages. The experiment was initially setup in the second half of December 2017. The germinations were counted × 3 a week between March 1st and April 20th 2018. In February and March of 2018, the study area experienced record low rainfall. Starting March 14th, seeds were watered with 200 mL of tap water × 3 a week until April 24th, which was the 14th day following the last observed germination. All *B. nigra* died within 2 weeks after germination, presumably due to water-stress. Field treatments were left in place for the following growing season and reseeded to 30 *B. nigra* in October before the 2018–2019 winter rainy season began. After the second rainy season, several *B. nigra* had established and destructively harvested after the first flowers closed. *Brassica nigra* reproductive potential was estimated with inflorescence count as a proxy. Root and shoot tissue were dried in ovens at 65 °C for 72 h. Shoot DW was analyzed as a metric of growth performance and productivity. Root:shoot ratios were analyzed for indications of alleviation of nutrient stress. Mean germinations (dependent variable) were compared with one-way and two-way fixed effect ANOVAs. A one-way ANOVA was utilized to compare germinations with canopy treatment as the independent variable. The two-way ANOVA was utilized to test for interactive effects between canopy treatment and tilled bare ground or buried pots (independent variables). The three dependent variables (shoot DW, root DW, and root:shoot ratios) were compared with one-way fixed-effects ANOVAs with canopy treatment as the independent variable. Residuals were not normally distributed for any dependent variables, so type 3 SS were used in analysis.

Statistical analyses were performed with R(v0.98.1062). Graphs were designed with MS Office Suite 2016.

Results

Log response ratios (mean effect sizes) showed *E. fasciculatum* increased *A. californica* mean shoot DW 101.9% in pairwise species interactions and an intermediate 85.3% in three-way species interactions compared to *A. californica* monoculture controls (Fig. 2). *Brassica nigra* did not significantly affect *A. californica* shoot DW in pairwise species interactions (Fig. 2).

Log response ratios of *E. fasciculatum* shoot DW indicated both *A. californica* and *B. nigra* significantly reduced *E. fasciculatum* shoot DW in pairwise species interactions (Fig. 2). In three-way species interactions, *A. californica* and *B. nigra* significantly decreased *E. fasciculatum* shoot DW, but was significantly less than the expected additive effect (Fig. 2).

Both *E. fasciculatum* and *A. californica* significantly increased *B. nigra* shoot DW in pairwise species interactions compared to monoculture controls (Fig. 2). However, three-way species interactions of *A. californica* and *E. fasciculatum* on *B. nigra* produced an antagonistic effect that did not affect *B. nigra* shoot DW compared to *B. nigra* monocultures (Fig. 2).

Finally, reducing soil pH with elemental sulfur significantly increased *A. californica* shoot DW by 40.0% (Fig. 2).

Artemisia californica Log-transformed shoot height to Log-transformed shoot DW showed a significant relationship between pairwise treatments with *B. nigra* and monoculture controls (ANCOVA: Est. Std. = 0.54828, $p=0.0391$, Fig. S2, Table S1). *Artemisia californica* Log-transformed shoot height increased with Log-transformed shoot DW in pairwise species interactions with *E. fasciculatum* in a moderately significant relationship (Linear Regression: $R^2=0.22$, $p=0.0765$, Fig. S2, Table S1). *Artemisia californica* Log-transformed shoot height increased

significantly with Log-transformed shoot DW in pairwise species interactions with *B. nigra* ($R^2=0.65$, $p=0.0008$, Fig. S2, Table S1) and in three-way species interactions ($R^2=0.48$, $p=0.0063$, Fig. S2, Table S1).

Artemisia californica distal canopy width significantly decreased *E. fasciculatum* shoot DW in pairwise species interactions (Linear Regression: $R^2=0.59$, $p=0.00586$; Fig. S1). Subsequent linear regression analysis of *E. fasciculatum* shoot DW versus the shoot DW of *A. californica*, *B. nigra*, and the sum of *A. californica* and *B. nigra* suggest competitive effects between *A. californica* and *B. nigra* weakened both species competitive effects on *E. fasciculatum*. In pairwise species interactions, increasing *A. californica* shoot DW decreased *E. fasciculatum* shoot DW significantly ($R^2=0.33$, $p=0.0521$; Fig. 3A), and *B. nigra* shoot DW had no significant effect on *E. fasciculatum* shoot DW ($R^2=0.06$, $p=0.384$, Fig. 3B). However, as the sum of *A. californica* and *B. nigra* shoot DW increased, *E. fasciculatum* shoot DW significantly increased in three-way species interactions ($R^2=0.41$, $p=0.026$, Fig. 3C).

A series of ANCOVAs were used to tease apart the processes by which *A. californica* and *E. fasciculatum* did not increase *B. nigra* shoot DW in three-way species treatments. Log-transformed *B. nigra* SLA versus log-transformed *B. nigra* shoot DW had moderately significant, negative effects in Monocultures (Est. Std. = -1.229685, $p=0.0575$) and in three-way species interactions (Est. Std. = -0.768761, $p=0.0783$; Table S3). In pairwise species interactions with *E. fasciculatum* and *A. californica*, linear regression analysis (Fig. S4, Table S3) showed significant, inverse relationships (with *E. fasciculatum*: $R^2=0.28$, $p=0.0308$; and with *A. californica*: $R^2=0.39$, $p=0.013$). In three-way species interaction, the antagonistic, inverse relationship was moderately significant ($R^2=0.27$, $p=0.0858$). A second ANCOVA showed three-way species treatments affected the relationship of *B. nigra* Log-transformed shoot height versus

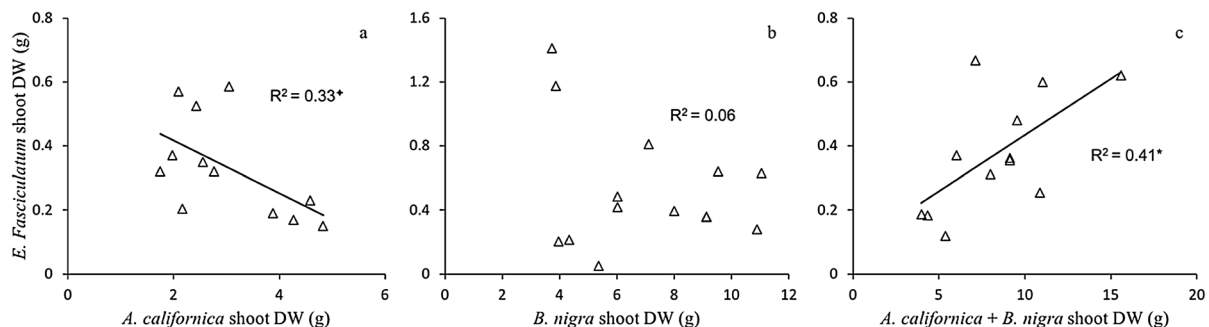


Fig. 3 *E. fasciculatum* shoot DW (g) versus **a** *A. californica* shoot DW (g), **b** *B. nigra* shoot DW (g), and **c** the sum of *A. californica* and *B. nigra* shoot DW (g). Linear regressions showed *A. californica* shoot DW had a moderately significant, inverse relationship to *E. fas-*

ciculatum shoot DW ($n=12$, $p=0.0521^*$), *B. nigra* shoot DW had no affect ($n=14$, $p=0.384$), and the sum of *A. californica* and *B. nigra* shoot DW had a significant, positive relationship to *E. fasciculatum* shoot DW ($n=12$, $p=0.026^*$)

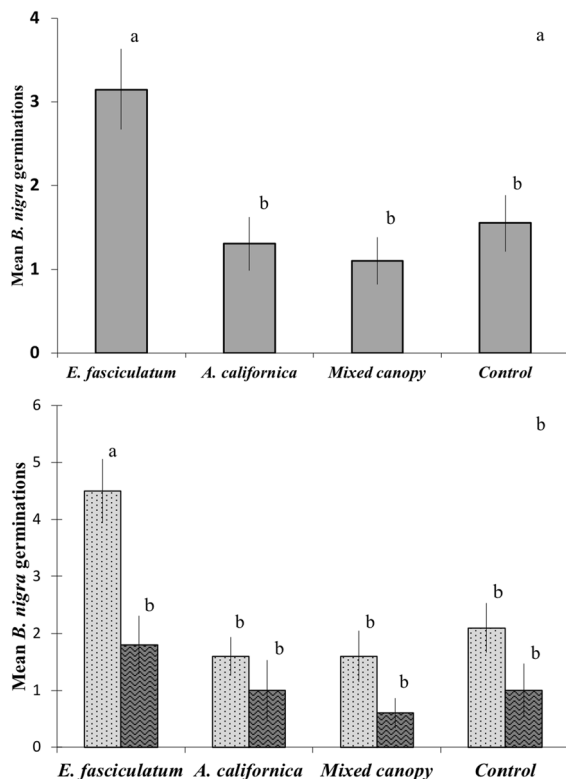


Fig. 4 **a** Mean *B. nigra* germinations under *E. fasciculatum*, *A. californica*, mixed canopies, and shaded controls. **b** Mean *B. nigra* germinations in buried pots (light gray with dots) and tilled bare ground (dark gray with waves) under *E. fasciculatum*, *A. californica*, mixed canopies, and shaded controls. Letters indicate significant differences from Tukey HSD post hoc analysis of permutation ANOVAs

shoot DW (Est. Std. = 0.29053, $p = 0.00394$; Fig. S5, Table S4). *B. nigra* shoot height increased with shoot DW in a significantly positive relationship in pairwise species interactions with *A. californica* (Linear Regression: $R^2 = 0.50$, $p = 0.00656$) or in three-way species interactions ($R^2 = 0.34$, $p = 0.0343$).

ANOVAs of three-way species interactions saw total *B. nigra* leaf mass was equal among treatments ($F_{3,50} = 1.366$, $p = 0.265$; data not shown). However, shoot height ($F_{3,50} = 6.203$, $p = 0.0015$; data not shown) and inflorescence count ($F_{3,50} = 2.895$, $p = 0.0452$; data not shown) varied significantly among species interaction treatments. Tukey HSD post hoc analysis of pairwise species interactions suggested *A. californica* and *E. fasciculatum* had no effect on *B. nigra* shoot height or inflorescence count. However, in three-way species interactions *A. californica* and *E. fasciculatum* together 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 *B. nigra* monocultures.

Additive field experiment

Among canopy treatments, *B. nigra* germination differed significantly in the field ($F_{3,72} = 8.381$, $p < 0.0001$; Fig. 4a) and between pots and tilled bare ground ($F_{1,72} = 17.473$, $p < 0.0001$; Fig. 4b), but with no interactive effect (TWO-WAY ANOVA: $F_{3,72} = 2.053$, $p = 0.114$; Fig. 4b). Shoot DW (ANOVA: $F_{3,24} = 9.972$, $p = 0.0034$), root DW (ANOVA: $F_{3,24} = 10.03$, $p = 0.0002$), and root:shoot ratios (ANOVA: $F_{3,24} = 4.775$, $p = 0.007$) differed significantly among canopies (Fig. 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 equal, but *E. fasciculatum* increased mean *B. nigra* germination by 103.2% ($p = 0.0043$; Fig. 4a), shoot DW 433.3% ($p = 0.0012$; Fig. 5a), root DW 160.7% ($p = 0.0112$; Fig. 5b), and decreased root:shoot ratios by 44.5% ($p = 0.0149$; Fig. 5c) compared to *B. nigra* under shaded controls. *Eriogonum fasciculatum* recruitment of *B. nigra* only occurred in buried pots (Fig. 4b) as no *B. nigra* survived on tilled bare ground under *E. fasciculatum*.

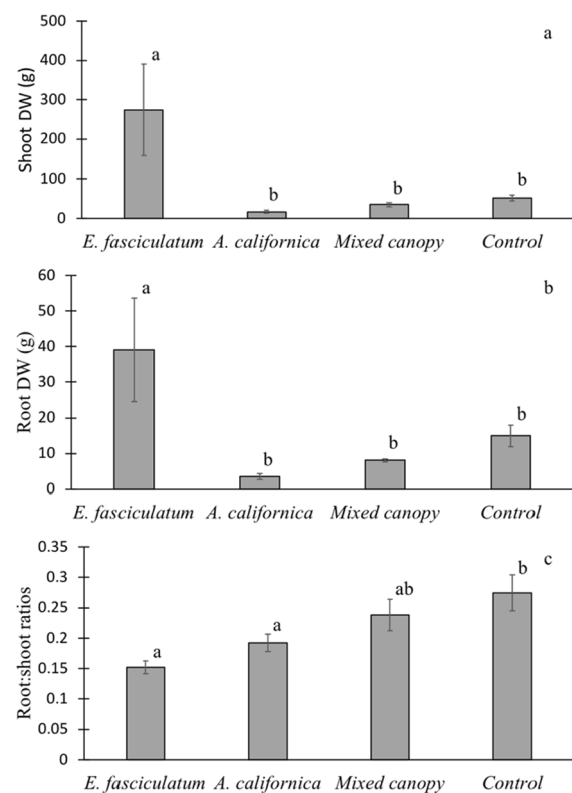


Fig. 5 **a** *B. nigra* mean shoot DW (g) ($F_{3,24} = 9.972$, $p = 0.0034$; **b** *B. nigra* mean root DW (g) ($F_{3,24} = 10.03$, $p = 0.0002$; and **c** *B. nigra* mean root:shoot ratios ($F_{3,24} = 4.775$, $p = 0.007$) under *E. fasciculatum*, *A. californica*, mixed canopies, and shaded controls. Letters indicate significant difference between means with Tukey HSD post hoc test on Permutation ANOVAs.

Discussion

This study tested (1) how a harmful invasive annual affects facilitative species interactions between two dominant, native perennials from seedlings to juveniles, and (2) can a competitive native perennial protect its facilitator from facilitating recruitment of a harmful invasive. While *E. fasciculatum* facilitation of *B. nigra* seedlings was expected in pairwise interactions, it was not predicted that *A. californica* would also facilitate *B. nigra* (Fig. 2). Once these observations are considered, the most surprising results were that in three-way species interactions among seedlings, the combined effects of *E. fasciculatum* and *A. californica* on *B. nigra* reversed the facilitation observed in pairwise interactions with *B. nigra* (Fig. 2). As seedlings, *B. nigra* does not significantly reduce *E. fasciculatum* facilitation of *A. californica*, and three-way species interactions lessen harmful competition with shade-intolerant *E. fasciculatum* for light. In three-way species interactions among seedlings, *E. fasciculatum* and *A. californica* also decrease *B. nigra* SLA, height, and inflorescence count. During later growth stages in the field, *A. californica* inhibits *E. fasciculatum* from recruiting *B. nigra*. Finally, results support the hypothesis that *E. fasciculatum* facilitates *A. californica* by acidification of alkaline soils. However, the presence of *B. nigra* inhibits *E. fasciculatum* soil acidification. Considered together, results presented here demonstrate significant effects of a tertiary invasive species on interactions between two ecologically important native plant species and demonstrate the potential for native perennials to increase resistance and resilience to biological invasion.

If pairwise interactions with *A. californica* and *E. fasciculatum* free *B. nigra* from intraspecific competition, *B. nigra* shoot DW could increase, but this scenario does not explain all the results. Three-way species interactions did not affect *B. nigra* shoot DW (Fig. 2), but SLA decreased as *B. nigra* shoot DW increased (Fig. S4, Table S3), and total leaf mass remained the same in all treatments (data not shown). When total leaf mass remains constant as SLA decreases, then a plant is growing its leaves thicker with less leaf area. Thicker leaves with reduced surface area is a common growth response of plants experiencing N-stress and/or water-stress (Violle et al. 2009). Field results below indicate *E. fasciculatum* is highly efficient at water competition. Further, *B. nigra* height (data not shown) and inflorescence (data not shown) also decreased in three-way species interactions compared to *B. nigra* monoculture controls. Three-way species interactions did not change *B. nigra* shoot DW. However, *E. fasciculatum* and *A. californica* together decreased *B. nigra* shoot height while significantly increasing the ratio of *B. nigra* shoot height to shoot DW (Fig. S5, Table S4). Between three-way species interactions and *B. nigra* monocultures, pairwise species interactions between *B. nigra* and

either *A. californica* and *E. fasciculatum* were intermediate for SLA v. shoot DW in an ANCOVA as well as ANOVAs of *B. nigra* height and inflorescence count. Westoby's Leaf-Height-Seed Strategy (1998) hypothesizes plants have finite resources to allocate to leaf growth, shoot growth, or reproduction. Resources allocated to shoot height per unit shoot DW would then be at the coast of leaf growth (reduced SLA per unit shoot DW) and reproduction (reduced inflorescence count). Scaled up to population level in the field, the effects of *E. fasciculatum* and *A. californica* together could reduce *B. nigra* fitness.

Indeed, field results suggest when *E. fasciculatum* cannot exclude *B. nigra* through water competition, *A. californica* can inhibit its codominant native facilitator from recruiting *B. nigra*. *Eriogonum fasciculatum* reduced germination (Fig. 4b) and growth of *B. nigra* on tilled bare ground treatments (Fig. 5a–c). The shallow lateral roots of *E. fasciculatum* could outcompete *B. nigra* seeds for limited water and prevent germination in CSS's well-drained soils during the short rainy season. When sufficient precipitation allows *B. nigra* to establish, *E. fasciculatum* tap roots (up to 2.5 m soil depth) could outcompete *B. nigra* for water at greater soil depths during CSS's long dry season. When buried pot treatments minimized water competition—or possibly when sufficient precipitation reduces the significance of *E. fasciculatum* water competition—field results suggest *A. californica* can prevent *E. fasciculatum* from facilitating germination and growth of *B. nigra* (Figs. 4b, 5a–c).

As nutrient availability increases, competition over N and P can be less deterministic than competition for light as fast-growing species produce more biomass than slower-growing heterospecifics (Bobbink et al. 2010). In pairwise interactions with *Eriogonum fasciculatum*, *A. californica* shoot DW more than doubled (Fig. 2), but competition and facilitation can occur concurrently (Bertness and Callaway 1994). The mobilization of nutrients otherwise unavailable in calcareous soils by *E. fasciculatum* would explain why *A. californica* shaded *E. fasciculatum* (Fig. S1) and reduced *E. fasciculatum* shoot DW 74.2% (Fig. 2) at densities 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* shoot growth and *A. californica* does not affect *E. fasciculatum* shoot DW (Schlau et al. 2021). Results here also align with a previous field study (Schlau et al. 2021), in which the two shrubs shared a positive spatial association in late growth stages at 1 m² and 1 m scales, but not at 0 m (point-intercepts). In order for *E. fasciculatum* to maintain positive spatial associations with *A. californica* into late growth stages, the two shade-intolerant perennial species likely may have to partition space to access sunlight.

Negative effects on *E. fasciculatum* shoot DW were significantly antagonistic in three-way species interactions compared to expected additive effects from pairwise

interactions (Fig. 2). *Brassica nigra* appears to trigger a plasticity growth response in *A. californica* to grow taller (Fig. S2). *Eriogonum fasciculatum* shoot DW's significantly positive linear relationship with the sum of *A. californica* and *B. nigra* shoot DW in three-way interactions provides further evidence that interactions between *A. californica* and *B. nigra* diminish both species competitive effects on *E. fasciculatum* (Fig. 3C). When *B. nigra* (or possibly any vertical growth-dominant species) effects taller growth in *A. californica*, the shading effects of *A. californica* on *E. fasciculatum* seen in pairwise interactions (Fig. S1) may weaken.

Despite *B. nigra* competitive effects on *E. fasciculatum* in pair-wise interactions, in three-way interactions, *B. nigra* did not significantly reduce facilitation of *A. californica* by *E. fasciculatum* (Fig. 2) and mean soil pH was not reduced in pots with *B. nigra*, even when *E. fasciculatum* was also present (Fig. S5). This suggests the possibility that facilitation is not driven by *E. fasciculatum* acidifying soils as hypothesized here and previously shown (Schlau et al. 2021). Data presented here does not offer an explanation of alternate means of facilitation. However, *B. nigra* may inhibit soil acidification by *E. fasciculatum* after a critical growth period. Indeed, Rangkadilok et al. (2002) found that secondary metabolite production in *B. nigra* changes with the plant's growth stages. The latter explanation could be answered with subsequent tests where soil pH is monitored at regular time intervals following planting. A third possibility is that soil acidification is the mechanism of facilitation in pairwise interactions between *A. californica* and *E. fasciculatum*, but not in three-way species interactions. Instead, if and when *B. nigra* triggers the growth plasticity response in *A. californica* to grow taller, *A. californica* may devote more resources to shoot tissues to be more competitive for light and increase its shoot DW (Fig. S2).

Another goal of this study was to test a hypothesis that *E. fasciculatum* acidifies CSS's calcareous soils and alleviates soil alkalinity stress for *A. californica*. In pairwise species interactions, *E. fasciculatum* reduced soil pH and increased *A. californica* shoot DW compared to *A. californica* monocultures (Fig. S3, Table S2). In pairwise interactions with *E. fasciculatum*, the y-intercept and slope differed significantly, suggesting the relationship between soil pH and *A. californica* shoot DW was not driven by competition with *E. fasciculatum*. Coinciding with these results, utilizing elemental sulfur to reduce soil pH resulted in a weaker but still significant increase in *A. californica* shoot DW (Fig. 2). While soil pH did not have a significant relationship to *A. californica* shoot DW (data not shown), the slopes for *A. californica* shoot DW versus soil pH converged at pH 6.2 in both pairwise interactions with *E. fasciculatum* and in three-way species interactions (Fig. S3). What's more, a subsequent polynomial regression of *A. californica* shoot DW versus soil pH in sulfur-treated pots has a significant

relationship with an optimal soil pH at approximately 6.2 (data not shown). Mean soil pH in treatments with *E. fasciculatum* and *A. californica* was also 6.2 (Fig. S5). Further testing with a controlled gradient of soil pH is needed to adequately address the question of optimal soil pH for *A. californica* shoot DW as well as if *E. fasciculatum* reduces soil pH to that optimal range, but data here suggests *E. fasciculatum* reduces soil pH to an optimal range for *A. californica* growth and is the means of facilitation.

Conclusions

The experiments presented here emphasize the significance of studying positive pairwise species interactions within their greater ecological context. In the Anthropocene, this means considering species interactions within intensely invaded habitats. Most surprisingly, the tertiary species here—a fast-growing, allelopathic invasive (*B. nigra*)—appears to reduce competitive effects of a heterospecific beneficiary (*A. californica*) upon its facilitator (*E. fasciculatum*) during seedling-to-juvenile growth stages. During late growth stages, the competitive beneficiary protects the facilitative plant from recruiting the harmful invasive. The codominance of *A. californica* and *E. fasciculatum* once reduced native biodiversity in CSS (see collective works of Whittaker et al.), but today, competitive native species can enhance resistance and resilience to invasion (e.g. Maron and Marler 2007). If this is true of *E. fasciculatum* and *A. californica*, as results presented here suggest, then the niche of the two native species may shift from suppressor to protector of native biodiversity. In more applicable terms, restoration of disturbed CSS habitat may benefit from cultivated co-dispersal of these native perennials, especially into re-occurring populations of *B. nigra*—and likely any invasive annual. Indeed, as fire intervals decrease and habitat becomes continually isolated, species interactions between seedlings of dominant perennials will likely become even more determinant to post-fire vegetative succession.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11258-023-01304-6>.

Author contributions BS conceived, setup, and ran experiments, collected data, primary analyzer of data, wrote the manuscript and prepared figures. TH conceived application of ANCOVAs. TH and KM contributed mightily to experimental design and statistical analysis. TH, KM, and JP reviewed the manuscript and gave informative advice that lead to constructive revisions.

Funding Open access funding provided by SCEL, Statewide California Electronic Library Consortium. Not Applicable.

Data availability All data was collected by Benjamin Schlau and data is available with the following link: <https://docs.google.com/spreadsheets>

ts/d/1r_yA4ZtozkPcgaabkXk_MWSzEqGleE_g/edit?usp=sharing&oid=109694860468006015664&rtpof=true&sd=true.

Declarations

Competing interests The authors declare no competing interests.

Ethical approval Not Applicable.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Bertness MD, Callaway R (1994) Positive interactions in communities. *Trends Ecol Evol* 9:191–193. [https://doi.org/10.1016/0169-5347\(94\)90088-4](https://doi.org/10.1016/0169-5347(94)90088-4)
- Bobbink R et al (2010) Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecol Appl* 20:30–59. <https://doi.org/10.1890/08-1140.1>
- Bowler P (2000) Ecological restoration of coastal sage scrub and its potential role in conservation plans. *Environ Manag* 26:85–S96. <https://doi.org/10.1007/s002670010064>
- Brooker R (2006) Plant–plant interactions and environmental change. *New Phytol* 171:271–289. <https://doi.org/10.1111/j.1469-8137.2006.01752.x>
- Chamberlain S, Bronstein J, Rudgers J (2014) How context dependent are species interactions? *Ecol Lett* 17:881–890. <https://doi.org/10.1111/ele.12279>
- Cione N, Padgett P, Allen E (2002) Restoration of native shrubland impacted by exotic grasses, frequent fire, and nitrogen deposition in Southern California. *Restor Ecol* 10:376–384. <https://doi.org/10.1046/j.1526-100X.2002.02038.x>
- D'Antonio C, Vitousek PM (1992) Biological invasions by exotic grasses, grass/fire cycle, and global change. *Annu Rev Ecol Syst* 23:63–87. <https://doi.org/10.1146/annurev.es.23.110192.000431>
- Eliason S, Allen E (1997) Exotic grass competition in suppressing native shrubland re-establishment. *Restor Ecol* 5:245–255. <https://doi.org/10.1046/j.1526-100X.1997.09729.x>
- Fuglsang A, Guo Y, Cuin T, Qiu Q, Song C et al. (2007) Arabidopsis protein kinase PKS5 inhibits the plasma membrane H⁺-ATPase by preventing interaction with 14-3-3 Protein. *Plant Cell* 19:1617–1634. <https://doi.org/10.1105/tpc.105.035626>
- Goldstein L, Suding K (2014) Intra-annual rainfall regime shifts competitive interactions between coastal sage scrub and invasive grasses. *Ecology* 95:425–435. <https://doi.org/10.1890/12-0651.1>
- Gray J (1982) Community structure and productivity in ceanothus chaparral and coastal sage scrub of southern California. *Ecol Mon* 52:415–434. <https://doi.org/10.2307/2937353>
- Halligan J (1973) Bare areas associated with shrub stands in grassland: the case of *Artemisia californica*. *Bioscience* 23:429–432. <https://doi.org/10.2307/1296544>
- Halligan J (1975) Toxic terpenes from *Artemisia californica*. *Ecology* 56:999–1003. <https://doi.org/10.2307/1936312>
- Hellmers H (1955) Root systems of some chaparral plants in southern California. *Ecol* 36: 667–678 doi: <https://doi.org/10.2307/1931305>
- Irvine Ranch Water District (2018) Water Quality Report. Irvine Ranch Water District. Irvine, CA
- Karban R (2007) Experimental clipping of sagebrush inhibits seed germination of neighbours. *Ecol Lett* 9:791–797. <https://doi.org/10.1111/j.1461-0248.2007.01068.x>
- Kummerow J, Krause D, Jow W (1977) Root systems of chaparral shrubs. *Oecologia* 29:163–177. <https://www.jstor.org/stable/4215454>
- Maron J, Marler M (2007) Native plant diversity resists invasion at both low and high resource levels. *Ecology* 88:2651–2661. <https://doi.org/10.1890/06-1993.1>
- Minnich RA, Dezzani RJ (1998) Historical decline of coastal sage scrub in the Riverside-Perris Plain, California. *Western Birds*. <https://doi.org/10.1890/06-1993.1>
- Oduor A, Kleunen M, Stift M (2020) Allelopathic effects of native and invasive *Brassica nigra* do not support the novel-weapons hypothesis. *Am J Bot* 107:1106–1113. <https://doi.org/10.1002/ajb2.1516>
- Palmer T, Stanton M, Young T (2003) Competition and coexistence: exploring mechanisms that restrict and maintain diversity within mutualist guilds. *Am Nat* 162:63–79. <https://doi.org/10.1086/378682>
- Rangkadilok N, Nicolas ME, Bennett RN, Premier RR, Eagling DR, Paul W, Taylor J (2002) Developmental changes of sinigrin and glucoraphanin in three Brassica species (*Brassica nigra*, *Brassica juncea* and *Brassica oleracea* var. *italica*). *Sci Hort* 96:11–26. [https://doi.org/10.1016/S0304-4238\(02\)00118-8](https://doi.org/10.1016/S0304-4238(02)00118-8)
- Rodríguez-Buritica S, Miriti M (2009) Biting the hand that feeds: the invasive grass *Schismus barbatus* (Poaceae) is facilitated by, but reduces establishment of, the native shrub *Ambrosia dumosa* (Asteraceae). *J Veg Sci* 20:241–250. <https://doi.org/10.1111/j.1654-1103.2009.05588.x>
- Schlau B, Huxman TE, Mooney KA, Pratt JD (2021) Facilitation at early growth stages results in spatial associations and stable coexistence in late growth stages of two long-lived, dominant shrubs. *Oikos* 130:2182–2190. <https://doi.org/10.1111/oik.08603>
- Schreiner P, Koide R (1993) Mustards, mustard oils and mycorrhizas. *New Phytol* 123:107–113. <https://doi.org/10.1111/j.1469-8137.1993.tb04536.x>
- Stylinski C, Allen E (1999) Lack of native species recovery following severe exotic disturbance in southern California shrublands. *J Appl Ecol* 36:1–12. <https://doi.org/10.1046/j.1365-2664.1999.00423.x>
- Strom L, Owen AG, Godbold DL, Jones DL (2005) Organic acid behaviour in a calcareous soil implications for rhizosphere nutrient cycling. *Soil Biol Biochem* 37:2046–2054. <https://doi.org/10.1016/j.soilbio.2005.03.009>
- Talluto M, Suding K (2008) Historical change in coastal sage scrub in southern California, USA in relation to fire frequency and air pollution. *Landsc Ecol* 23:803–815. <https://doi.org/10.1007/s10980-008-9238-3>
- Tawaha AM, Turk MA (2003) Allelopathic effect of black mustard (*Brassica nigra*) on wild barley (*Hordeum spontaneum*). *J Agron Crop Sci* 189:298–303. <https://doi.org/10.1046/j.1439-037X.2003.00047.x>
- Turk MA, Tawha AM (2003) Allelopathic effect of black mustard (*Brassica nigra*) on germination and growth of wild oat (*Avena fatua* L.). *Crop Prot* 22:673–677. <https://doi.org/10.1046/j.1439-037X.2003.00047.x>
- Violle C, Garnier E, Lecoœur J, Roument C, Pedeur C, Blanchard A, Navas ML (2009) Competition, traits and resource depletion in

- plant communities. *Oecologia* 160:747–755. <https://doi.org/10.1007/s00442-009-1333-x>
- Westman W (1981) Diversity relations and succession in Californian coastal sage scrub. *Ecology* 62:439–455. <https://doi.org/10.2307/1936680>
- Westoby M (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant Soil* 199:213–227. <https://doi.org/10.1023/A:1004327224729>
- Wootton J (1994) The nature and consequences of indirect effects in ecological communities. *Annu Rev Ecol Syst* 25:443–466. <https://doi.org/10.1146/annurev.es.25.110194.002303>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.