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Forest encroachment into a Californian grassland: examining the simultaneous effects of facilitation and competition on tree seedling recruitment

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Abstract Competition and facilitation are both considered major factors affecting the structure of plant assemblages, yet few studies have quantified positive, negative, and net effects simultaneously. In this study, we investigated the positive, negative, and net effects of tree saplings on the encroachment of two tree species, Douglas fir (*Pseudotsuga menziesii*) and tanoak (*Lithocarpus densiflora*), into a coastal California grassland. The study involved three components: sampling the spatial distributions of *P. menziesii* and *L. densiflora* in the grasslands, a field experiment examining seedling survival in different grassland environments, and a greenhouse experiment examining the effects of soil moisture on early seedling performance. The field experiment was conducted over a 2-year period, using *Pseudotsuga* in 2002 and both species in 2003. Seedlings were separated into four treatment groups: those planted in open grassland, in shaded grassland, under artificial (plastic) conifer saplings, and under natural *Pseudotsuga* saplings. Air temperature, relative humidity, soil moisture, incident radiation levels and fog water inputs were measured for each treatment group in 2003. In the greenhouse experiment, *Pseudotsuga* and *Lithocarpus* seedlings were grown for 13 weeks in watering treatments simulating the summer soil moisture conditions of the open grasslands and under *Pseudotsuga* saplings. Surveys of naturally established seedlings found that *Lithocarpus* occurred only under *Pseudotsuga* saplings, while most *Pseudotsuga* seedlings were located near but not directly under conspecific saplings. In the field experiment, positive effects of tree saplings were much larger than negative effects, resulting in strong net

facilitation of seedling establishment. Survival for both species was always higher under the plastic and live trees than in the open or shade plots. The primary mechanism facilitating seedling survival appeared to be increased soil moisture caused by input of fog precipitation coupled with reduced microsite evaporation. The greenhouse experiment further showed that soil moisture strongly affected seedling performance, with both species having much higher photosynthetic rates in the higher moisture treatment. In the lower moisture treatment, *Pseudotsuga* seedlings had higher photosynthetic rates and stomatal conductance than *Lithocarpus*, suggesting they may be able to better tolerate the environmental conditions found in the open grasslands. Our combined results suggest that rate and patterning of woody plant encroachment can be strongly influenced by facilitation and that fog precipitation may play a key role in plant interactions.

Keywords Facilitation · Fog precipitation · Grassland · *Lithocarpus densiflora* · *Pseudotsuga menziesii* · Seedling establishment · Woody plant encroachment

Introduction

Competition and facilitation are two factors that may strongly affect the dynamics of woody plant encroachment (Callaway et al. 1992; Scholes and Archer 1997). Numerous studies have demonstrated that both types of interactions occur simultaneously (see Callaway et al. 1991), indicating that it is critical to study competition and facilitation concurrently in order to understand the net effect of biotic interactions on plant assemblage structure (Callaway and Walker 1997; Holmgren et al. 1997; Brooker and Callaghan 1998). Previous studies examining woody plant establishment in grasslands have focused heavily on tree-grass competition, and less is known about competition among woody plant species

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(Scholes and Archer 1997). In contrast, several studies show that woody plants facilitate the establishment of other woody plants in abiotically stressful environments through a variety of different mechanisms (see Flores and Enrique 2003 for a recent review).

One of the most important mechanisms of facilitation in semi-arid environments is the amelioration of stressful micro-climatic conditions (Callaway 1995). Water is generally considered to be a major limiting factor in these systems, and the presence of a plant canopy generally lowers soil temperatures and increases relative humidity, which can reduce water loss via evaporation. Plants can also increase soil water content under their canopies through hydraulic lift (Richards and Caldwell 1987), but this phenomenon may have a relatively limited impact on plant neighborhood interactions in semi-arid environments due to a strong competition for water (Ludwig et al. 2004). Another way plants can increase water levels under their canopies is through the interception of fog precipitation (Azevedo and Morgan 1974). This phenomenon has been well documented in both the alpine tree-line and cloud forest ecosystems (Lovett et al. 1982; Holder 2004). Several studies have demonstrated that the uptake of fog water can be important for plants, particularly in Mediterranean environments, where summer rainfall is largely absent (Ingraham and Matthews 1995; Dawson 1998; Corbin et al. 2005). The drip of fog precipitation onto soil occurs when fog condenses on plant surfaces; since the amount of condensation is largely a function of surface area, large fog water inputs in mature forests is not surprising (e.g. Azevedo and Morgan 1974; Dawson 1998). It is less clear, however, whether smaller plants, such as tree saplings, are able to catch quantities of fog water large enough to significantly affect soil moisture conditions under their canopies [see Corbin et al. (2005) about fog precipitation and herbaceous vegetation].

At our research sites on Mount Tamalpais, California, there has been widespread tree encroachment into the grasslands over the past 50 years. The encroachment has been dominated by *Pseudotsuga menziesii* seedlings and saplings, but a number of *Lithocarpus densiflora* individuals are also present in the grasslands. While many factors may be contributing to the different rates of encroachment of *P. menziesii* and *L. densiflora*, seedling establishment in the grasslands is a major encroachment bottleneck for both species due to the prolonged period of water deficit during the summer months (Dunne and Parker 1999). Qualitative observations of the patterning of tree encroachment indicated that facilitation may play an important role, as *P. menziesii* and *L. densiflora* individuals appeared to be spatially aggregated in the grassland.

The objective of this study was to examine the simultaneous effects of facilitation and competition on tree encroachment into the grasslands. We first quantified natural distributions of seedlings and saplings of both species in the grasslands to evaluate our qualitative observation of intra- and inter-specific aggregation. We

then conducted a pair of related field and greenhouse experiments to test the effects of facilitation and competition on tree seedling survival and to identify the specific effects of soil moisture on early seedling performance. Based on the patterning of encroachment, we hypothesized that *P. menziesii* seedlings would be less dependent on facilitation from *P. menziesii* saplings than seedlings of *L. densiflora*. In addition, due to differing light requirements, we hypothesized that *P. menziesii* seedlings were more likely to be negatively affected by the presence of *P. menziesii* saplings than *L. densiflora* seedlings.

Methods

Study site

The study was conducted along Bolinas Ridge in the Marin Municipal Water District watershed on Mount Tamalpais (N 37°54', W 122°37') in southern Marin County, Calif. Within the watershed, the mixed evergreen forest is dominated by *P. menziesii* (hereafter referred to as *Pseudotsuga*) and *L. densiflora* (hereafter referred to as *Lithocarpus*) (Horton et al. 1999). The grasslands are a mixture of exotic annuals and native and exotic perennials dominated by *Danthonia californica*, *Bromus carinatus*, *Lolium multiflorum*, and *Briza major* (J. Corbin, personal communication). The study area is characterized by a Mediterranean climate with a seasonal summer dry period and average annual precipitation of 1250 mm (Dunne and Parker 1999). Soils in the grassland are classified as composites of the Saurin clay loam and Bonnydoon gravelly loam series (USDA 1990).

Spatial distribution sampling

In 2002, we assessed the spatial distributions of those *Pseudotsuga* and *Lithocarpus* individuals with a height exceeding 1 m in the grasslands (hereafter referred to as saplings). We established 100-m transects in areas of active forest encroachment on five arbitrarily selected north-facing slopes. We quantified the spatial pattern of all individuals by measuring the point-to-nearest-tree distance and then the tree-to-nearest neighbor distance (Krebs 1999). Both distances were measured at ten randomly located points along each transect. In 2004, we assessed the spatial distributions of those *Pseudotsuga* and *Lithocarpus* individuals with a height of less than 1 m in the grasslands. To determine if the younger *Pseudotsuga* and *Lithocarpus* were spatially associated with older saplings, we measured the distance from each young individual to the crown edge of the nearest older saplings along four of the same transects. Young individuals found underneath a sapling crown were assigned a distance of 0 m. It is important to note that all of the younger *Pseudotsuga* and *Lithocarpus* individuals

surveyed were not first-year seedlings; they appeared to be 2 years old or more.

Seedling field experiment

Experimental design

To determine the effects of initial tree colonists on subsequent tree recruitment in the grassland, we conducted field experiments in 2002 and 2003. The 2002 experiment tested the influence of *Pseudotsuga* saplings on the establishment and survival of *Pseudotsuga* seedlings. Because these seedlings were nursery-grown and maintained under different conditions than those in the field (see below), their mortality rates may have been higher than if they had been acclimated to the site before planting. To address this potentially confounding factor, we planted much younger seedlings of *Pseudotsuga* and *Lithocarpus* seedlings in 2003 and gave them sufficient time to acclimate to field conditions prior to the onset of the summer water deficit. During both years, the experiments were conducted on randomly selected north-facing slopes [with only one site (site 2) common between years]. In 2002, we randomly selected two sites along Bolinas Ridge that were separated by 0.3 km. In May, we purchased 120 *Pseudotsuga* seedlings from Cornflower Farms Nursery (Elk Grove, Calif.). The seedlings were grown from coastal Californian seed stock under moderate shade, fertilized, and well-watered conditions for 18 months. Seedlings were transplanted the day after purchase into one of four treatment groups: in open grassland, under shade cloth, under plastic conifer trees (hereafter plastic tree), under *Pseudotsuga* saplings (hereafter live tree). Seedlings in the latter two treatments were planted at the crown edge in the same grass matrix present in the open and shade treatments. For the shade treatment, we used a neutral-density shade cloth that was placed 15 cm above the ground. Preliminary field tests showed that photosynthetically active radiation (PAR) under the cloth was 40–80 μmol , which approximated the light levels under *Pseudotsuga* saplings in the grassland (P. Kennedy, unpublished data). For the plastic tree treatment, we used 2.5-m-tall artificial conifer trees ('Arctic Spruce'; Treeclassics, Lake Everton, Ill.), which simulated all of the above-ground effects of *Pseudotsuga* saplings but did not exploit any of the below-ground resources. To minimize transplantation stress and facilitate establishment, we hand-watered the seedlings with 2 l of water per week from 15 May to 10 June. Each seedling was also surrounded by a 15-cm-diameter cage constructed of 1-cm-diameter metal mesh (Pacific Steel and Supply, San Leandro, Calif.) to prevent herbivory. This procedure was cautionary; a subsequent study comparing caged and uncaged seedlings of both species in the grasslands found very little effect of herbivory on seedling survival (P. Kennedy, unpublished data). The 2002 experiment used a randomized block design, with one

replicate plot per treatment in each of five blocks at each site. Each block was centered around a randomly selected *Pseudotsuga* sapling, but the location of each treatment within a block was randomly assigned. Treatment plots were located approximately 2 m apart, and each plot contained three replicate seedlings planted approximately 25 cm apart. Seedling survival was monitored approximately every 2 weeks from 10 June to 20 September.

For the 2003 field experiments, we collected *Lithocarpus* acorns from forests adjacent to the grasslands along Bolinas Ridge in the fall of 2002. Viable acorns were surface sterilized in a 10% bleach solution for 5 min, air dried, and stored at 4°C prior to planting. *Pseudotsuga* seeds were collected from a coastal California forest and provided by the U.S. Forest Service, Placerville nursery. The seeds were surface-sterilized with hydrogen peroxide for 20 min, soaked in running water for 48 h, and stratified at 8°C for 2 months prior to planting. In the greenhouse, both species were planted into SC-10 super cell "cone-tainers" (Steuwe and Sons, Corvallis, Ore.) containing 150 ml of Supersoil potting mix (Supersoil, San Mateo, Calif.). After 2 months of growth under ambient, non-supplemented light conditions, the seedlings were transplanted into the field. Both species had similar root lengths when transplanted (approx. 15 cm); however, *Lithocarpus* seedlings had a large tap root while the roots of *Pseudotsuga* seedlings were more diffuse.

In February 2003, we randomly selected three sites separated by 0.5–1.4 km. At each site, seedlings were planted in the same four treatment groups as described above. Because there was no significant effect of blocking in 2002 (see Results), we set up four randomly located plots per species per site for each treatment. Within each plot, one seedling was transplanted and surrounded by a 15-cm-diameter mesh metal cage ($n=96$ total). No plots were within 2 m of each other. Seedling survival was monitored monthly until the end of May and then every 2 weeks through to 31 October.

A number of the abiotic conditions of each site and treatment were quantified in 2003. A radiation-shielded HOBO H8 Pro Series datalogger (Onset Computer, Bourne, Mass.) was placed 15 cm above the soil and continuously recorded air temperature (°C) and absolute humidity (% moisture) (one per treatment per site). The dataloggers were moved once a week between May and October to a randomly chosen replicate within each treatment plot at each site. Light levels in each treatment plot were quantified using gallium arsenide phosphide (GaAsP) sensors located 15 cm above the soil surface [Hamamatsu photonics, Hamamatsu City, Japan; see Percy et al. (1991) for details on the GaAsP sensors]. The sensors were attached to unlevelled stakes placed perpendicular to the soil surface and then connected to Campbell Scientific dataloggers (Campbell Scientific, Logan, Utah) at three dates between September and October. At each of the three sites, one plot of each treatment was randomly selected for light characteriza-

tion at each sampling date. The data from the GaAsP sensors was converted to PAR using a series of regressions of data collected from the GaAsP sensors and from Li-cor quantum light sensors (Campbell Scientific). Percentage soil moisture was calculated gravimetrically from 2.5-cm-diameter soil cores taken from the top 10 cm of soil within randomly selected plots of each treatment at each site. Cores were taken bi-weekly from 16 May through 30 October. Fog precipitation was collected using fog collectors similar to those used by Dawson (1998). The collectors were placed in a random location at each of the three sites from late May through the end of October and simultaneously checked and relocated approximately every 3 weeks to another position within the same site.

Statistical analyses

We analyzed the tree sapling distributions using Hopkin's test for spatial pattern (Krebs 1999). This test compares the distances from point-to-tree relative to tree-to-tree, so if the saplings are clumped, for example, point-to-tree distances are large relative to tree-to-tree distances. The ratio of the sums of the squared distances, h , can be tested with an F -distribution to determine if the distribution is clumped, random, or uniform (Krebs 1999). The spatial patterning of young *Pseudotsuga* and *Lithocarpus* distributions with respect to nearest saplings was sufficiently clear using descriptive statistics; so further inferential statistics were not required.

Since the design of the field experiment differed between years, seedling survival was analyzed separately each year. In 2002, we used a three-way mixed model analysis of variance (ANOVA), with site (random), treatment (fixed) and block (random) as predictor variables. In both years, seedling survival represented the number of days the seedlings were known alive; for example, if a seedling died during the 2 weeks between census dates, the number of days known alive was assigned as the previous census date. In 2002, we used the average survival from the three *Pseudotsuga* seedlings within each treatment plot. Since we lacked replicates of each treatment within each block, we assumed that the variance associated with the interaction between block and treatment was zero (see Underwood 1997, for discussion). For the 2003 experiment, we used a three-way mixed model ANOVA, with species (fixed), site (random), and treatment (fixed) as predictor variables. In both years, the variances were determined to be homogenous prior to performing the ANOVAs (Cochran's C test, $p > 0.05$), and Tukey HSD tests were used for a posteriori comparisons of means. We also separately compared seedling growth of *Pseudotsuga* and *Lithocarpus* in 2003 under the plastic and live trees using two-tailed t -tests. Growth was calculated as seedling height difference between 16 March and 16 November. Prior to running the t -tests, the variances were confirmed to be homogenous using Levene's test ($p > 0.05$).

Environmental data from the 2003 experiment were analyzed using a series of multiple-factor ANOVAs. PAR data were assessed as average values for four time periods within a day (8–10:59 a.m., 11–1:59 p.m., 2–4:59 p.m., and 5–6:59 p.m.) using a three-way mixed-model ANOVA, with treatment (fixed), site (random), and time of day (fixed) as predictor variables. Time of day was treated as a nested factor because each datum was derived from one sensor located within a single randomly selected plot of each treatment. We used two three-way mixed-model ANOVAs to analyze the air temperature and relative humidity data. In each case, the response variable was a weekly average within each time period ($n = 3$ per period), and predictor variables were treatment (fixed), site (random), and season (fixed). Season was divided into early (May–June), middle (July–August), and late (September–October) periods. We analyzed percentage soil moisture using a two-way fixed-factor ANOVA, with treatment and season as predictor variables. Here, we used sites as replicates for each sampling time because we only had one core per treatment per site. The variances of all abiotic variables were determined to be homogenous prior to running the ANOVAs (Cochran's C test, $p > 0.05$), and individual Tukey HSD tests were used for a posteriori comparisons of means.

To analyze the positive, negative, and net effects of *Pseudotsuga* saplings on seedling establishment, we used Hedges' d index to compare seedling survival in the open, plastic tree and live tree treatments (Gurevitch and Hedges 2001). Positive effects were calculated as the difference in seedling survival between the plastic tree and open treatments, negative effects as the difference between the plastic and live tree treatments, and net effects as the difference between the live tree and open treatments.

Seedling greenhouse experiment

Experimental design

Seeds of both species were collected and planted as described for the 2003 field experiment. In the greenhouse, seeds were first planted into SC-10 super cell "containers" (Steuwe and Sons) containing 150 ml of Supersoil potting mix (Supersoil). After 2 months, the seedlings were removed from the containers, gently rinsed to remove adhering soil, and bare-root transplanted into D40 Deepot pots (Steuwe and Sons). Each pot had 600 ml of a 5:3:2 mixture of field soil:Surface (Profile Products LLC, Buffalo Grove, Ill.):coarse sand. The soil mixture was used to improve soil aeration and provide uniform soil moisture conditions. Field soil was collected from the grassland adjacent to site 3 in the 2003 field experiment and pasteurized twice prior to mixing; the Surface and coarse sand were autoclaved on a 40-min wet-dry cycle at 250°C. After transplanting, the seedlings were grown for 30 days in well-watered

conditions prior to initiation of the watering treatment described below.

In April 2004, seedlings of each species were assigned to one of two watering treatments. The treatments were based on the mean soil water content in the open grasslands and underneath *Pseudotsuga* saplings as measured the previous summer at Mount Tamalpais (see above). To maintain the target moisture levels (8 and 14% soil moisture in the grassland and under the saplings, respectively) seedlings were given set water amounts three times per week ranging from 10 to 80 ml. We checked soil moisture once a week using a Campbell Scientific HydroSense refractometer (Logan), and water addition was adjusted accordingly. Prior to initiating the watering treatments, there were no significant differences in seedling height for either species (*Lithocarpus* $F_{1,38} = 1.653$, $p = 0.206$; *Pseudotsuga* $F_{1,38} = 0.576$, $p = 0.452$; $n = 40$ per species). Seedlings were grown for 13 weeks under ambient, non-supplemented light conditions (mean temperature: 22°C; relative humidity: 48%). Pots were haphazardly rearranged once a week to control for environmental variation within the greenhouse.

After 13 weeks, we measured the gas exchange rates of seedlings using a Li-6400 portable gas exchange system (Li-Cor, Lincoln, Neb.). For *Lithocarpus* seedlings, measurements were made on one mature leaf per seedling. The leaves were all large enough to fill the entire area of the measurement chamber. For *Pseudotsuga* seedlings, measurements were made on a mature portion of the shoot that was placed into the measurement chamber. To calculate the leaf area of *Pseudotsuga* in the chamber, we harvested the portion of the shoot that was contained in the chamber. Needles were removed from the stem, and their total two-dimensional area was calculated using Image-Pro Plus 4.5 (Media Cybernetics, Silver Spring, Md.). The gas exchange measurements were then recalculated to correct for differences in leaf areas in the measurement chamber using the Li-Cor simulator computer program (Li-Cor). All measurements were made holding the photon flux density at $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$, the reference carbon dioxide concentration at $370 \mu\text{l l}^{-1}$, leaf temperature at 25°C, and flow rate at 500mmol s^{-1} . Seedlings were harvested immediately after gas exchange measurements; shoots and roots were dried at 80°C for 48 h and then weighed separately. There were a total of 80 seedlings; 40 *Lithocarpus* seedlings and 40 *Pseudotsuga* seedlings ($n = 20$ replicates per treatment).

We also measured the carbon isotope composition of leaves from both species (given as $\delta^{13}\text{C}$). Unlike gas exchange rates measured at a single-time point, $\delta^{13}\text{C}$ gives a measure of plant carbon and water relations over the life of the leaf, which provides a time-integrated index of water-use efficiency (WUE) (Farquhar et al. 1989). A randomly selected subsample of the dried leaves from each individual was placed into a separate screw-type security capsule with a stainless steel ball-bearing. These samples were ground to a fine powder for

2 min in a Wig-L-Bug Crescent Amalgamator (Dentsply Int, Surrey, UK). A 4- μg aliquot of powder from each sample was placed in a tin capsule (COSTEK, Valencia, Calif.) and analyzed for isotope ratios with an ANCA/SL elemental analyzer coupled with a PDZ Europa Scientific 20/20 Mass Spectrometer at the Center for Stable Isotope Biogeochemistry, University of California, Berkeley. The isotope composition is expressed in “delta” notation (‰), and is calculated as $\delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$. R_{sample} and R_{standard} represent the molecular ratios of [^{13}C] to [^{12}C] in the sample and standard (in this case V-PDB), respectively.

Statistical analyses

We used a series of two-way fixed-factor ANOVAs to analyze seedling photosynthetic rate, stomatal conductance, instantaneous WUE (calculated as photosynthetic rate/stomatal conductance), and $\delta^{13}\text{C}$. Prior to running the ANOVAs, stomatal conductance was $\log(X+1)$ transformed to reduce heterogeneity in variances, while the variances of photosynthetic rate, instantaneous WUE, and $\delta^{13}\text{C}$ were determined to be homogenous without transformation (Cochran’s C test, $p > 0.05$). Tukey HSD tests were used for a posteriori comparisons of means. All statistical tests were performed using SYSTAT 10.2 (SYSTAT Software, Richmond, Calif.) and considered to be significant at $p \leq 0.05$.

Results

Spatial distribution sampling

Across all transects sampled, we encountered only saplings of *Pseudotsuga*. Their densities varied considerably across sites, ranging from 95 to 413 stems/ha. However, at all sites, saplings had a significantly clumped distribution (Hopkin’s test, $p < 0.05$). The vast majority of younger trees (94%) sampled were also *Pseudotsuga*. The location of younger trees with respect to saplings varied between species (Table 1). *Lithocarpus* were found only under saplings, while most *Pseudotsuga* were located near but not directly under the saplings.

Seedling field experiment

In 2002, there were significant differences in survival among treatments ($F_{3,31} = 45.612$, $p < 0.001$) and sites ($F_{1,31} = 25.951$, $p < 0.001$), but not among blocks ($F_{4,31} = 2.390$, $p = 0.072$). Seedling survival (i.e. mean days known live) was significantly higher under plastic and live trees than in the open or shade plots (Tukey HSD test, $p < 0.05$) (Fig. 1). Mortality occurred more quickly in the open and shade plots, with approximately 80% of the seedlings dying within 30 days (Fig. 1a). By

Table 1 Location of young individuals (> 1 m tall) of *Pseudotsuga menziesii* and *Lithocarpus densiflora* at four grassland sites on Mount Tamalpais, California

Species	Location (no. of individuals)		Distance ^a	
	Under tree	Away	Median	Standard error
<i>Lithocarpus</i>	11	0	0	0
<i>Pseudotsuga</i>	26	125	1.6	0.4

^aDistance is calculated from the sapling crown edge to the young individual crown edge, with zero values given for young individuals located directly under sapling crowns

the end of the experiment (20 September), all of the seedlings in the open plots had died, and only two individuals in the shade plots were still alive. In contrast, seedling mortality was more gradual under the plastic and live trees and strongly site-dependent. On 20 September, all of the seedlings under live trees had died at site 1, and there were only two surviving individuals under plastic trees. However, at site 2, $51 \pm 7\%$ (mean \pm SE) and $29 \pm 10\%$ of the seedlings growing under plastic and live trees, respectively, were still alive.

In 2003, there were significant differences in seedling survival among treatments ($F_{3,72} = 14.623$, $p < 0.001$) and sites ($F_{2,72} = 5.491$, $p = 0.006$) but not between species ($F_{1,72} = 0.396$, $p = 0.531$). Survival was significantly lower for seedlings in the open plots than under plastic or live trees and intermediate in the shade plots (Tukey HSD test, $p < 0.05$) (Fig. 1b, c). Mortality in the open plots started in June for *Pseudotsuga* and increased gradually until the end of the experiment. In contrast, *Lithocarpus* mortality in the open plots was concentrated between late July and early August, with no further mortality after that period. The two species responded somewhat differently in the shade plots, with *Lithocarpus* having a higher survival rate than *Pseudotsuga*. Survival variation between sites occurred mostly between the open and shade plots, with seedlings living longer at site 3 [241 ± 10 days (mean across treatments)] than at sites 1 and 2 (211 ± 8 and 217 ± 6 days, respectively). Neither species exhibited differential growth under plastic versus live trees (*Pseudotsuga* $t = -0.695$, $df = 19$, $p = 0.493$; *Lithocarpus* $t = -1.317$, $df = 13$, $p = 0.211$), and none of the higher-order interactions of seedling survival were significant.

PAR, air temperature, relative humidity, and soil moisture all varied considerably among treatments and throughout the year in 2003 (Table 2; Fig. 2a). Site had no effect except on soil moisture, which was slightly but significantly higher at site 2 than at sites 1 and 3 [$F_{2,72} = 3.857$, $p = 0.026$; site 1 = 11.5 ± 1.2 , site 2 = 12.4 ± 0.9 , site 3 = 11.1 ± 1.0 (mean \pm SE %soil moisture)]. PAR was significantly higher in the open plots than in the other treatments, but there was also much greater variation in open plots throughout the day (treatment \times time of day interaction: $F_{9,72} = 8.043$,

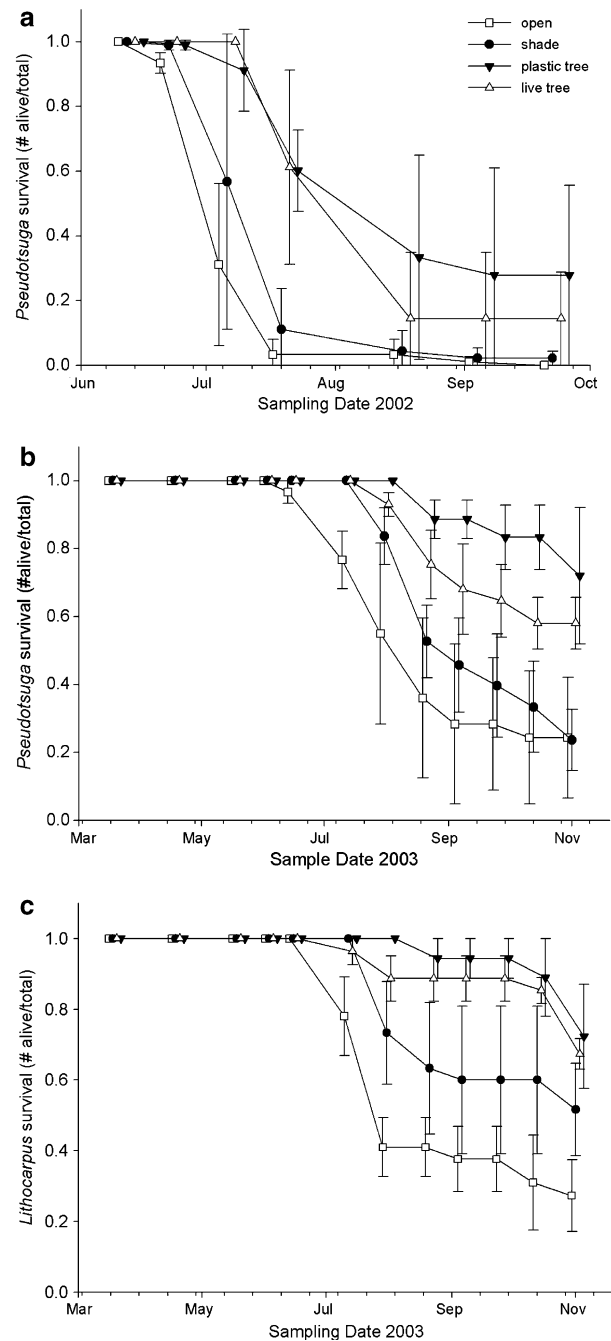


Fig. 1 Survival of *Pseudotsuga menziesii* (a, b) and *Lithocarpus densiflora* (c) seedlings in the field experiment in 2002 and 2003. Proportions are calculated as the mean survival \pm 1 SE across sites within the four experimental treatment groups. Note that at each census date, each treatment has been slightly offset to eliminate the overlap in error bars

$p < 0.001$). Air temperatures were significantly lower under the plastic and live trees than in the open and shade plots from May through August, but not during September and October (treatment \times season interaction: $F_{6,12} = 9.238$, $p = 0.001$). Conversely, relative humidity was significantly higher under the plastic and live trees than in the open plots, with the shade plots being

Table 2 Photosynthetically active radiation (PAR), air temperature, and relative humidity across treatments

Time of day	PAR ^a (μmol)			Air temperature ^b (°C)				Relative humidity (%)			
	Treatment	Mean	SE	Season	Treatment	Mean	SE	Season	Treatment	Mean	SE
8–11 a.m.	Open	461	72	Early (May–June)	Open	22.6	1.4	Early (May–June)	Open	59	6
	Shade	39	9		Shade	22.4	1.2		Shade	58	5
	Plastic tree	65	13		Plastic tree	18.4	1.0		Plastic tree	69	4
	Live tree	49	11		Live tree	18.0	0.9		Live tree	68	5
11 a.m.–1 p.m.	Open	1276	122	Middle (July–Aug)	Open	27.7	0.9	Middle (July–Aug)	Open	33	3
	Shade	116	22		Shade	28.7	0.9		Shade	37	2
	Plastic tree	48	12		Plastic tree	24.3	0.6		Plastic tree	46	2
	Live tree	34	8		Live tree	23.7	0.7		Live tree	47	2
2–5 p.m.	Open	1017	153	Late (Sept–Oct)	Open	16.3	11	Late (Sept–Oct)	Open	55	6
	Shade	104	30		Shade	16.2	11		Shade	69	3
	Plastic tree	64	24		Plastic tree	16.2	0.7		Plastic tree	70	3
	Live tree	36	8		Live tree	16.1	0.5		Live tree	70	3
5–7 p.m.	Open	168	71								
	Shade	18	6								
	Plastic tree	74	43								
	Live tree	23	5								

^aThe light measurements were taken on three dates between September and October 2003

^bDaily average temperature and relative humidity measurements were taken during three time periods in 2003

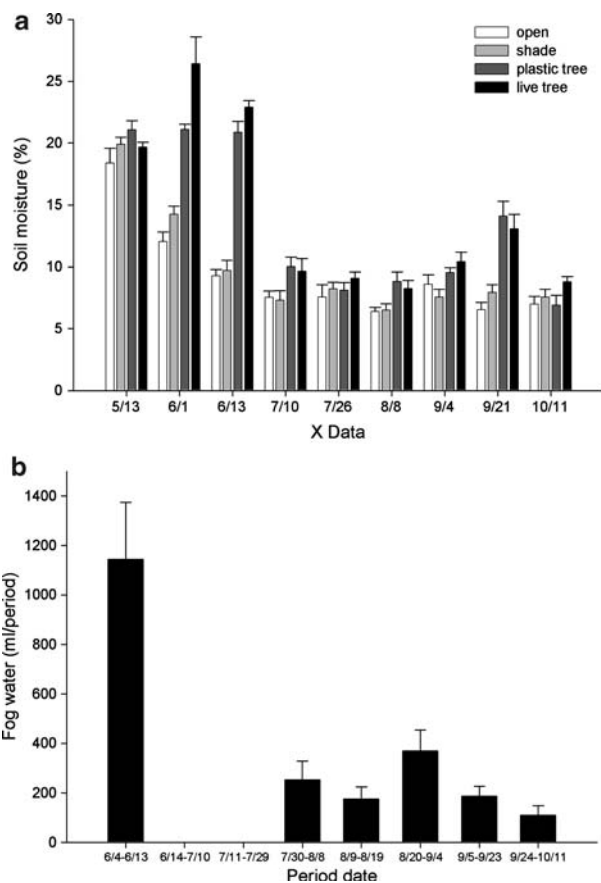


Fig. 2 Water conditions on Mount Tamalpais in 2003. **a** Mean percentage of soil moisture conditions + 1 SE in the top 10 cm across sites in the four experimental treatments, **b** mean fog water input + 1 SE error across sites. Note that between mid-May and early November there was no rainfall, so fog water was the only water input

intermediate (treatment $F_{3,6} = 11.236$, $p = 0.007$). Relative humidity was also significantly lower in July and August than during the earlier and later periods (season: $F_{2,6} = 178.323$, $p < 0.001$). Finally, soil moisture was significantly higher under the plastic and live trees than in the open and shade plots (treatment $F_{3,72} = 18.096$, $p < 0.001$). These differences among treatments appeared to be strongly influenced by fog water inputs, since fog water was the only water input between late May and early November 2003 (Fig. 2b). Soil moisture was also significantly higher early in the growth season, particularly under plastic and live trees when fog was most abundant (season $F_{2,72} = 108.834$, $p < 0.001$). None of the other main effects or higher-order interactions were significant.

Positive effects of tree saplings on seedling survival were larger than negative effects at all sites in both years, resulting in a net facilitation for both species (Table 3). Despite the general net positive effect of saplings on seedling survival, there was considerable variation in the magnitude of facilitation between sites, with d values ranging from 7.42 (very strong) to 0.49 (relatively weak). In addition, sites with the highest positive effects were not the same between species (site 1 for *Lithocarpus* in 2003 and site 2 for *Pseudotsuga* in 2002 and 2003).

Seedling greenhouse experiment

The soil moisture levels in the greenhouse were very similar to the mean percentage of soil moisture in the open and live tree plots measured in the field in 2003. Soil moisture in the higher moisture treatment was $14.9 \pm 0.4\%$ (mean \pm SE) and $13.8 \pm 0.6\%$ for *Pseudotsuga* and *Lithocarpus*, respectively, and $7.4 \pm 0.1\%$ and

Table 3 Positive, negative, and net effects of tree saplings on seedling survival

Species	Year	Effect					
		Positive (plastic tree-open)		Negative (live tree-plastic tree)		Net ^a (live tree-open)	
		Mean	Range	Mean	Range	Mean	Range
<i>Pseudotsuga</i>	2002	3.98	3.75–4.21	–0.77	–1.04–0.50	2.98	2.51–3.46
<i>Pseudotsuga</i>	2003	2.33	0.61–5.51	–0.35	–0.62–0.09	1.64	0.82–3.56
<i>Lithocarpus</i>	2003	2.86	1.25–5.75	–0.23	–0.62–0.27	3.00	0.49–7.42

^aAll values represent d values calculated using Hedges' d index, which standardizes the effect size between samples with different levels of replication

7.2 ± 0.2% in the lower moisture treatment. Photosynthetic rates were similar between species in the higher moisture treatment but significantly lower for *Lithocarpus* than *Pseudotsuga* in the lower moisture treatment (species × water treatment interaction: $F_{1,75} = 4.757$, $p = 0.032$) (Fig. 3a). Stomatal conductance was significantly lower for both species in the lower moisture treatment (water treatment $F_{1,75} = 171.771$, $p < 0.001$) (Fig. 3b) and at both moisture levels, *Pseudotsuga* had significantly higher conductances than *Lithocarpus* (species $F_{1,75} = 38.267$, $p < 0.001$). Differences in physiology were also apparent in instantaneous WUE and $\delta^{13}\text{C}$ (Fig. 3c, d). For both variables, *Lithocarpus* had higher WUE and $\delta^{13}\text{C}$ values than *Pseudotsuga* regardless of water treatment. However, *Lithocarpus* had much higher WUE and $\delta^{13}\text{C}$ values than *Pseudotsuga* in conditions of low soil moisture compared to conditions of higher soil moisture (species × water treatment interaction: WUE, $F_{1,75} = 14.042$, $p < 0.001$; $\delta^{13}\text{C}$, $F_{1,71} = 58.845$, $p < 0.001$).

Discussion

Facilitation of seedling recruitment by previously established saplings appears to be a major factor affecting the encroachment of both *Pseudotsuga* and *Lithocarpus* seedlings into the grasslands on Mount Tamalpais. Although there was considerable variation across sites, seedling survival was always the highest under plastic or live trees. The positive effects of the saplings were much stronger than negative effects in both years, and their magnitude was similar to those observed by Holzapfel and Mahall (1999) and Maestre et al. (2003). We also found that the positive effects of saplings were generally comparable between *Lithocarpus* and *Pseudotsuga*, which did not support our hypothesis that *Lithocarpus* would benefit more from sapling facilitation than *Pseudotsuga*. The very small competitive effects that we observed do not imply that competition does not occur in this system, rather it is likely that early seedling establishment is dominated by facilitation while later life stages may be more adversely affected by tree sapling competition (Anderson et al. 2001). Despite the beneficial effects of saplings on early seedling recruit-

ment, most of the naturally occurring young *Pseudotsuga* individuals were located near but not under the canopy of conspecific saplings. We believe this may reflect the fact that *Pseudotsuga* grows poorly under conditions of low light (Hermann and Lavender 1990) and may need to establish outside the canopy to grow beyond the first-year seedling stage. It is not clear how far from the sapling canopies the effects of fog precipitation are present, but in establishing near – but not directly under – saplings, young *Pseudotsuga* individuals may balance the trade-off between facilitation and competition with previously established individuals. In contrast, *Lithocarpus* typically inhabits forest understories and appears to be better able to tolerate the decreased light under saplings due to a lower light compensation point than *Pseudotsuga*.

Our experimental design isolated a number of environmental factors that could potentially explain the increase in survival under plastic and live trees. The shade treatment decreased light levels, moderately decreased relative humidity and air temperatures, but did not increase soil moisture conditions relative to open plots. Decreased seedling survival in the shade plots relative to under the plastic and live trees suggests that shade alone is not sufficient for survival, particularly for *Pseudotsuga*. Larger trees have been shown to facilitate seedling establishment by increasing nutrient availability under their canopy through a variety of different mechanisms (see Scholes and Archer 1997 for review). If this factor were driving the observed facilitation, we would have expected higher survival and growth under the live trees than under the plastic trees, but there was no difference in either variable for both species. It is also possible that the saplings established in areas of the grasslands that are inherently better for seedling establishment than areas without saplings. To control for this potential confounding effect, one would need either to transplant or to have planted the saplings at random locations in the grasslands. Although manipulating the location of the saplings was not feasible, the random placement of the plastic tree treatment and the equal seedling growth and survival under the plastic and live trees suggest that potential microsite effects were likely not to be present. The major outstanding differences between the plastic/live tree and shade/open treatments were increased soil

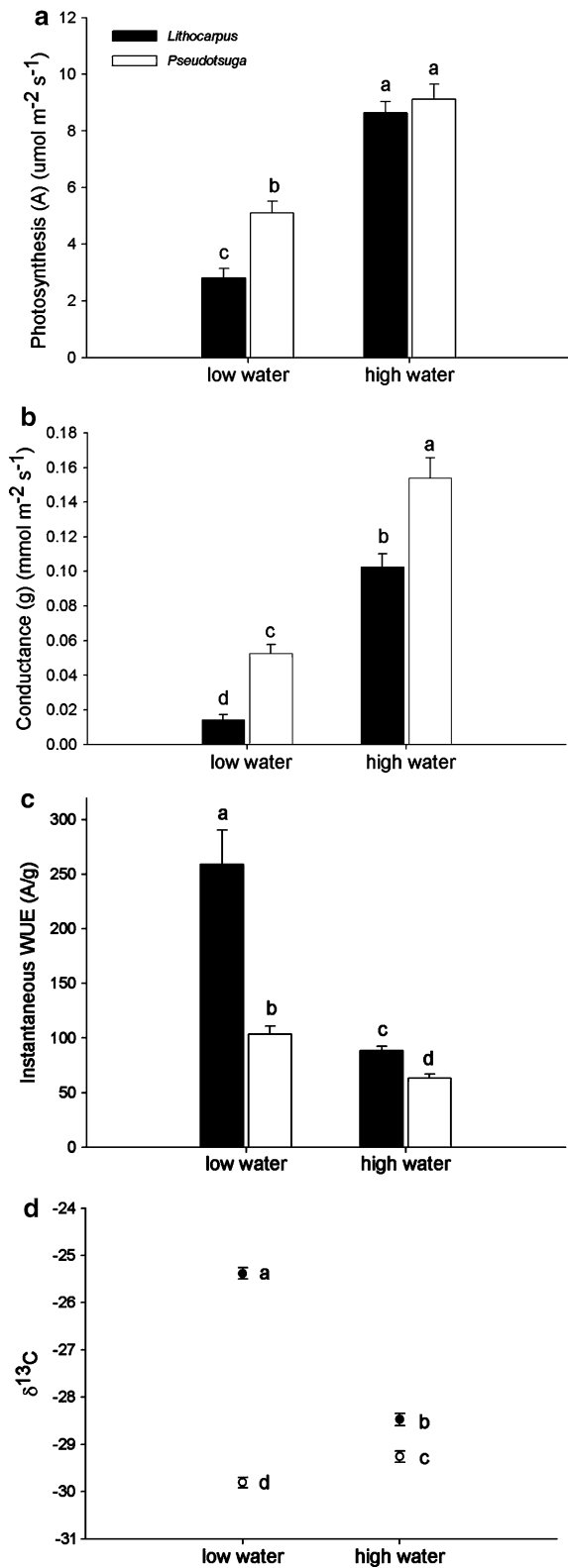


Fig. 3 Photosynthetic rate (a), stomatal conductance (b), instantaneous water-use efficiency (WUE) (c), $\delta^{13}\text{C}$ (mean + 1 SE) (d) of *P. menziesii* and *L. densiflora* seedlings grown for 13 weeks under lower (7%) and higher (14%) soil moisture conditions in the greenhouse. Different letters indicate significant differences at $p < 0.05$

moisture, lower air temperatures, and higher relative humidity. While we were not able to separate these factors with our experimental design, we believe that their effects are synergistic: lower air temperatures and higher relative humidity complement the effects of fog precipitation by lowering the evaporation of water from the soil. Lower soil evaporation is significant because even if seedlings in the open grasslands and underneath trees experience the same evaporative demand (i.e. leaf-to-air vapor pressure deficits), seedlings with greater access to soil moisture under the trees would be less negatively affected by equivalent vapor pressure deficits. In addition, fog precipitation increases soil moisture during the critical period when there is little-to-no rainfall. Although we did not measure seedling fog water uptake directly, Ingraham and Matthews (1995) showed that *Pinus muricata* and *Cupressus macrocarpa* used considerable quantities of fog water during the summer months in Point Reyes National Seashore (located 20 km north of Mount Tamalpais), and other studies such as Dawson (1998) have shown that fog precipitation can have a major impact on the water status of understory plants.

In the greenhouse experiment, we found that *Pseudotsuga* seedlings had a greater water use (i.e. higher stomatal conductance) than *Lithocarpus* but also higher carbon fixation, which resulted in a decrease in $\delta^{13}\text{C}$ values. *Lithocarpus* was much more physiologically limited under conditions of low water, as indicated by much lower photosynthesis and conductance and higher instantaneous WUE and $\delta^{13}\text{C}$ values. These differing physiological responses suggest that *Lithocarpus* is less tolerant of low water conditions and that *Pseudotsuga* seedlings may be better suited to endure the stressful water conditions present in the open grasslands over the summer months. The much higher density of *Pseudotsuga* saplings as well as the presence of young *Pseudotsuga* individuals out in the open grasslands are consistent with this idea. Surprisingly, however, we did not observe differences in the survival of the two species in the open grasslands in the field experiment. We suggest these discordant results may be due to differential survival between species only being observed during drier years (annual rainfall in 2003 was slightly above average). The disparity in the relative abundances of *Pseudotsuga* and *Lithocarpus* in the open grassland may also reflect differences in seed supply. In an earlier study, Kennedy and Diaz (2005) found that the dispersal of *Lithocarpus* acorns into grasslands was extremely low while the dispersal of wind-dispersed *Pseudotsuga* was likely to be quite high. The exclusive occurrence of *Lithocarpus* seedlings under *Pseudotsuga* saplings may thus reflect patterns of dispersal as well as differences in seedling physiology and annual climatic conditions.

To our knowledge, this study is the first to use artificial trees in an investigation of the simultaneous effects of competition and facilitation. Many studies of positive and/or negative interactions compare plots with and

without neighbors and/or measure differences in plant performance. Although these methods indicate whether competition or facilitation is occurring, they do not allow one to determine the mechanism by which positive or negative effects occur because there are multiple differences between plots (e.g., light, temperature, disturbance, nutrients). The second method uses artificial structures to simulate the presence of the existing vegetation. While this method helps control for a priori differences in the abiotic and biotic conditions between plots, artificial structures typically simulate only certain aspects of the existing vegetation. In our investigation, we found that a shade cloth treatment did not capture the important effects of fog precipitation, and while we recognize that this particular phenomenon is not a major factor in most ecosystems, we believe that the use of more realistic artificial structures, such as plastic trees, will better represent the multitude of different abiotic effects that existing vegetation can have under its canopy (see Holzapfel and Mahall 1999 and Maestre et al. 2003 for alternative approach to mimicking plant effects).

Species' interactions can have a large impact on the trajectory and rate of succession (Connell and Slatyer 1977). We found that the early stages of grassland to forest succession are driven primarily by facilitation. A number of other studies have also shown that positive interactions can facilitate succession, although most of these have focused on areas undergoing primary succession (e.g., Rubio-Casal et al. 2001; Walker et al. 2003). In tree seedling facilitation by other woody plants, it appears that competitive effects may often overwhelm the beneficial effects of facilitation (Meiners and Gorchoff 1998; Rousset and Lepart 2000). The balance between these factors, however, often depends on the harshness of the environment, with facilitation having a greater importance in more stressful conditions (Callaway et al. 2002). In our system, the grasslands typically represent a very stressful environment for seedling establishment, and this is likely the reason why facilitation was so important during the early stages of succession. However, studies examining a broader range of life history stages (i.e., germination to sapling) and incorporating longer temporal intervals are needed in order to fully assess the balance between facilitation and competition in forest encroachment.

We realize that the controls on forest encroachment are complex and can simultaneously depend on multiple biotic and abiotic variables (Terwilliger and Pastor 1999; House et al. 2003). In two other studies, we have found that differential seed dispersal and changes in mycorrhizal fungi may also affect the encroachment of *Pseudotsuga* and *Lithocarpus* into these grasslands (Kennedy and Diaz 2005; Kennedy and Bruns, in review). The results of the present study suggest that facilitation of seedling establishment by *Pseudotsuga* saplings was primarily driven by the amelioration of soil moisture conditions caused by the input of fog precipi-

tation and lowered microsite evaporation. Future studies employing isotopic techniques to directly demonstrate and quantify seedling fog water uptake are necessary for a more complete understanding of the role of fog precipitation in woody plant encroachment.

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