

## ORIGINAL ARTICLE



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# Seed germination and plant fitness response of a narrowly endemic, rare winter annual to spatial heterogeneity in microenvironment

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## Abstract

Environmental heterogeneity affects distributions of plant species, although the effects of fine-scale heterogeneity on plant demographies are not widely studied. Diminutive winter annuals, especially rare taxa, can be sensitive to spatial variation in microenvironment as a consequence of their small stature above and belowground. To address whether spatial environmental heterogeneity affects demography, germination and fitness of *Chorizanthe orcuttiana*, an endangered winter annual distributed in distinct easterly and westerly microhabitats within an exceedingly narrow niche in California, we performed multiyear observational and empirical studies. We manipulated after-ripening environment, soil moisture and ambient light at both aspects, and profiled microclimate, soil physicochemistry and soil microbiomes at all sites. We show that easterly aspects host larger plants in larger populations, and have lower air temperatures combined with higher soil moisture in comparison to the west-facing sites. Yet, soil physicochemistry and microbiomes were similar across all sites. Manipulations of after-ripening conditions showed that seeds exposed to low humidity (17%) during dormancy and sown at easterly aspects exhibited the highest germination percentages, whereas seeds incubated *in situ* and subsequently sown at westerly aspects yielded the lowest germination. Simultaneous manipulations of soil moisture and light showed that at both aspects higher moisture combined with shade led to higher germination, whereas ambient soil moisture combined with shade yielded the lowest germination. Altogether, our studies show that the diminutive, rare winter annual *C. orcuttiana* exhibits higher germination and plant fitness under cooler soil conditions with higher soil moisture while preferring drier environments during after-ripening.

## KEYWORDS

after-ripening, aspect, microclimate, microhabitat, soil moisture

## 1 | INTRODUCTION

Climatic and edaphic variability over small spatial scales can have profound effects on plant populations (Menges, McIntyre, Finer, Goss, & Yahr, 1999; Scherrer & Körner, 2011). Terrain variability within a kilometer brings changes in thermal properties, water and light availability that allow wide variation within the regional climate (Ashcroft, French, & Chisholm, 2011; Dobrowski, Abatzoglou, Greenberg, & Schladow, 2009; Fridley, 2009). Narrow-niche endemic species may depend upon this variation for their persistence. For example, many endemic plants exhibit small-scale variability in germination or fitness based on light (Castellanos, Tinoco-Ojanguren, & Molina-Freaner, 1999; Kluse & Doak, 1999), soil moisture (Colling, Matthies, & Reckinger, 2002; Diez, 2007), soil temperature (Choi, Jeong, & Kim, 2019; Franco & Nobel, 1989), litter (Kalliovirta, Rytteri, & Heikkinen, 2006), or soil chemical composition (Baskin & Baskin, 1988). In addition, the spatial variability within these variables also affects the after-ripening (alleviation of dormancy under warm and dry conditions) process of species that undergo periods of dormancy before germinating (Dwyer & Erickson, 2016). For instance, seeds of winter annuals experience a 5–6-month dormancy during summer when conditions are unfavorable for germination; these after-ripening conditions may vary spatiotemporally and thus shape their demography in the subsequent growing season (Dwyer & Erickson, 2016). Further, exposure to soil-borne microbial pathogens during the after-ripening period can reduce seed viability (Leishman, Masters, Clarke, & Brown, 2000). Although rarely documented, small winter annuals can also exhibit edaphic endemism by colonizing nutrient-poor substrates to avoid competition from other plants or to avoid soil pathogens (Leishman et al., 2000; Martínez-Berdeja, Torres, Altshuler, & Ezcurra, 2014). Such local niche variability acts as a driver for adaptive divergence and formation of species or ecotypes (Edwards & Donoghue, 2013).

Although local adaptations can increase functional or taxonomic diversity in widely distributed species (Opedal, Armbruster, & Graae, 2015; Stark, Lehman, Crawford, Enquist, & Blonder, 2017), extreme specificity to micro-niches can make narrowly endemic species susceptible to local extinctions, reducing intraspecific diversity and potentially leading to local extinctions (Jones, Smith, & Menges, 2017; Myers, Mittermeier, Mittermeier, DaFonessa, & Kent, 2000). Accordingly, local extinction events are generally more frequent in endemic species occurring within highly biodiverse, heterogeneous and physiographically complex ecosystems (Brooks et al., 2002; Gomez & Lunt, 2007; Stebbins & Major, 1965). This

pattern has been documented in one of the world's most threatened biodiversity hotspots, the California Floristic Province, where approximately 50% of the >2000 endemic plant taxa are either extinct or threatened with extinction (Brooks et al., 2002; Myers et al., 2000). The state of California, which represents 70% of the land area of the California Floristic Province, also hosts 93% of its endemic flora (Raven & Axelrod, 1978; Rissler, Hijmans, Graham, Moritz, & Wake, 2006). A majority of the at-risk species with restricted geographic ranges and small populations have narrow niches (Fiedler, 1995), resulting in approximately 66% of the flora endemic to California at risk of complete loss (Loarie et al., 2008; Ulrey, Quintana-Ascencio, Kauffman, Smith, & Menges, 2016).

Along with restricted ranges and low local abundances, small stature, herbaceous growth form and an annual or biennial life cycle are some of the traits frequently associated with rare plants (Harper, 1979; Kunin & Gaston, 1993; Lloyd, Wilson, & Lee, 2003; Murray, Thrall, Gill, & Nicotra, 2002). For example, of the 34 *Chorizanthe* (Polygonaceae) species that occur in California, all are small-statured herbaceous annuals and 20 of the 25 species endemic to the state are protected as rare, occurring only in one or two counties (Calflora, 2018; CNPS, 2018; Morgan, Styler, & Reveal, 2014). *Chorizanthe* species generally inhabit coastal sage scrub communities that also host many other rare, endemic and protected taxa, which are threatened by disturbances caused by urban development and land use (Bell, Allen, Weathers, & McGiffen, 2016). Higher frequency of droughts and other environmental stochasticities that are likely to occur in this habitat introduce additional bottlenecks for sensitive, diminutive, herbaceous species, such as winter annuals. Seeds of winter annuals receive germination cues from the concurrence of autumn rain and cool temperatures (Commander, Merritt, Rokich, & Dixon, 2009; Dwyer & Erickson, 2016), which induce demographic responses based on the differences in inter-annual rainfall (Cowling, Ojeda, Lamont, Rundel, & Lechmere-Oertel, 2005; Venable, 2007) or temperature (Huxman et al., 2013). To date, only four narrowly endemic *Chorizanthe* species have been studied to identify their ecological constraints (Baron & Bros, 2005; Fox, 2007; Fox, Steele, Holl, & Fusari, 2006; Jones et al., 2010; Kluse & Doak, 1999; Martínez-Berdeja, Pietrasiak, Tamase, Ezcurra, & Allen, 2013; McGraw & Levin, 1998), mirroring the global pattern of scarcity of information on ecological determinants of species distributions or plant fitness at small spatial scales.

Our study species, *Chorizanthe orcuttiana* Parry (San Diego spineflower), ideally represents small winter annual plants within the coastal sage scrub that have extremely narrow distributions and extreme niche

specificity across a small geographic space. *C. orcuttiana* is protected under the U.S. Endangered Species Act (U.S. Fish and Wildlife Service, 2007), which enables establishment of long-term studies at protected locations in a region where natural areas are otherwise threatened by ongoing land development. When we initiated our studies, only four small and disjunct populations were known from within an area of 150 km<sup>2</sup> in San Diego County, CA. We discovered that populations at sites with westerly aspects within 500 m of the Pacific Ocean are smaller, and these sites are drier with sparser, shorter shrub cover in comparison to the sites with an easterly aspect that are located on the bay-side of the peninsula approximately 1 km from the Pacific Ocean coastline. Populations of *C. orcuttiana* occurring on easterly aspects (bay-side) are consistently larger and occur among taller shrubs. Considering these differences, it is reasonable to expect that the sites have unique microhabitats. In fact, the effect of aspect on climate and edaphic characteristics is expected because the quantity and quality of solar radiation regulates air temperature, relative humidity, soil moisture and soil chemistry (Desta, Colbert, Rentch, & Gottschalk, 2004; Smith, 1977). Southwesterly aspects, which receive higher solar radiation, are generally hotter and drier than northeasterly aspects, and thus present unique microenvironments for after-ripening, seed germination, seedling development and plant growth (Desta et al., 2004). Being a winter annual, *C. orcuttiana* maintains a seed bank that incubates in soil during the dormant season (summer). It is likely that climatic variability during this period is linked to variability in germination, and possibly the fitness response in subsequent growing seasons (Dwyer & Erickson, 2016). The effect of after-ripening on plant fitness could be mediated by the timing of germination of a seed, where early germinants have potentially higher survival rates and higher fitness in comparison with the late germinants (Donohue, 2005). Remarkably, the microclimatic differences across *C. orcuttiana* populations are detectable even within <1 km<sup>2</sup>, leading us to question: first, whether *C. orcuttiana* exhibits variation in population size and plant fitness across its apparently distinct microhabitats; and second, if germination and plant fitness can be increased by manipulating after-ripening conditions and/or microhabitat conditions.

To address our questions, we used four natural populations and an additional four experimental locations over multiple years. We predicted that: (a) plant fitness will be higher at sites with easterly aspects in comparison to the west-facing sites; (b) seeds exposed to a combination of drier after-ripening during the dormant season and east-facing microhabitat during the growing season will show higher germination and fitness in comparison to all other manipulations. Simultaneously, we

expected germination and fitness to be lowest in seeds treated to ambient *in situ* after-ripening conditions and germinated at sites with western aspects; and (c) seeds exposed to a combination of supplemental soil moisture, reduced irradiance and easterly aspect will exhibit the highest seed germination and plant fitness. Besides, we also characterized biotic and abiotic niches of the species across eastern and western aspects to identify the predictors that were most strongly linked to germination and plant fitness over multiple years.

## 2 | MATERIAL AND METHODS

### 2.1 | Study species

*C. orcuttiana* is an annual allopolyploid species restricted to a few locations within San Diego County in southern California (Hardham, 1989). Plants occur in sandy soils derived from sandstones and sedimentary rocks that underlie the coastal plains of coastal sage scrub habitat. Seed germination occurs between late December and January after autumn rainfall, and plants can be observed aboveground from mid to late January. Anthesis begins in February or March, when light yellow flowers enclosed within involucre can be observed (Kaur, Sharma, & Markovchick, 2016). After pollination, involucre matures, leading to plant senescence in May and June. The California Natural Diversity Database lists 14 historic Element Occurrences (EOs; hereafter referred to as populations) of *C. orcuttiana* (California Department of Fish and Wildlife—Natural Diversity Database [CNDDB], 2014); however, at present, only seven extant locations are known, three of which were relocated after our studies were underway.

### 2.2 | Observational studies

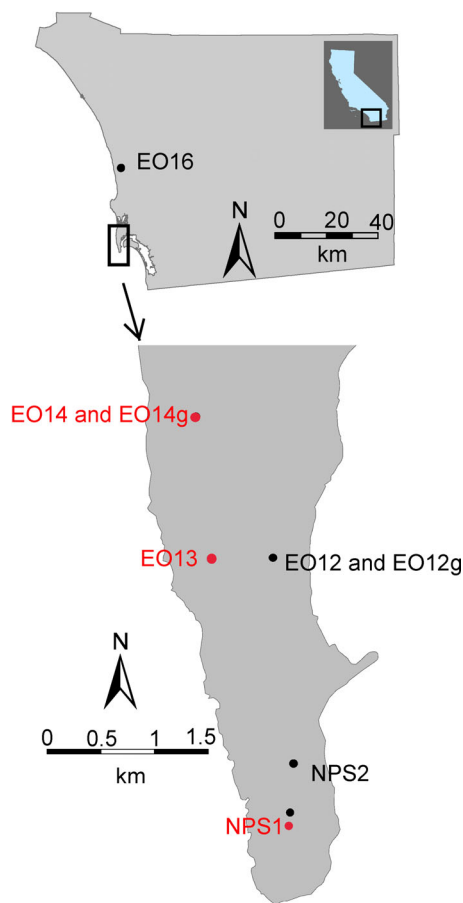
#### 2.2.1 | Spatial variation in population size and plant fitness

To document the population and plant fitness variability among naturally occurring populations of *C. orcuttiana*, we used four populations to record population size, plant fitness and biotic as well as abiotic variables described below. Two populations (EO12 and EO13) are separated by 0.5 km on the Point Loma peninsula, whereby EO12 occurs on the easterly slopes and within 1 km from the Pacific Coast, whereas EO13 occurs on the westerly slopes and within 500 m of the Pacific Coast. The third population (EO14), occurring on a west slope and within 500 m of the Pacific Coast, is located approximately 1.5–2

km from EO13 and EO12 (Figure 1). The fourth population (EO16), with an easterly aspect, lies within Torrey Pines State Natural Reserve Extension, approximately 25 km north of the Point Loma peninsula (Figure 1) and <1 km from the Pacific coast. Populations EO12 and EO16 occur at 90 m elevation, whereas EO13 and EO14 are situated slightly higher at 100 m. Plants of *C. orcuttiana* occur within the coastal sage scrub communities in the mild maritime climates of southern California, where it is located in open, sandy areas among other herbaceous species such as *Crassula connata*, *Camissoniopsis bistorta*, *Mucronia californica*, *Cardionema ramosissimum* and *Dudleya* spp. The larger herbaceous species and shrubs include *Encelia californica*, *Isocoma*

*menziesii*, *Artemisia californica*, *Corethrogyne filaginifolia*, *Salvia mellifera*, *S. apiana*, *Adenostoma fasciculatum*, *Eriogonum fasciculatum*, *Rhus integrifolia*, *Ceanothus verrucosus* and *Cneoridium dumosum*. Notably, the northern *C. orcuttiana* population (EO16) uniquely features a congeneric, *C. procumbens*, and mature trees of the rare *Pinus torreyana*.

In each of the years from 2014 to 2018, we conducted a complete census by counting individual plants at EO12, EO13 and EO14. Population size estimates for EO16 were obtained from a secondary source (Margaret Fillius, Torrey Pines Association member), when available. We collected plant growth data at each site between 2015 and 2018. We haphazardly selected 30 plants from each population in each growing season, and individually marked them with temporary markers. Because plants typically occur in patches that are  $\leq 5 \times 5$  m in size, the 30 plants selected for collecting growth data represented at least a  $3 \times 3$  m area. This area also hosted all environmental monitoring instrumentation (described below) at each site. Plant width at the widest point (cm) and number of stems were recorded for the selected individuals as the measure of vegetative success, whereas the number of involucre was recorded as the measure of reproductive success of the same experimental units (Figure S1a). Data were collected at a monthly interval between February and April.



**FIGURE 1** A map of San Diego County and an enlarged map of the Point Loma Peninsula in California, USA, showing study locations. Four natural populations of *Chorizanthe orcuttiana* were monitored at three peninsular locations (EO12, EO13 and EO14) and one location within Torrey Pines State Natural Reserve (EO16). Four experimental populations were established, including one each at EO12 and EO14 (EO12g and EO14g, respectively) and two (NPS1 and NPS2) at foreign sites approximately 2 km south of EO12 at Cabrillo National Monument. EO16, EO12 and NPS2 (black circles) represent eastern sites, whereas EO14, EO13 and NPS1 (red circles) represent western sites

### 2.2.2 | Characterization of spatial variation in microclimate, soil physicochemical properties and microbial communities

To document microhabitat variability among naturally occurring populations and experimental sites, we installed instrumentation at three easterly aspect sites (EO12, EO16 and NPS2) and three westerly aspect sites (EO13, EO14 and NPS1) to measure all or some of the variables listed herein. The experimental sites NPS1 and NPS2 are located approximately 2 km south of EO12 within Cabrillo National Monument (US National Park Service) toward the southern tip of the peninsula (Figure 1). Both NPS1 and NPS2 do not host *C. orcuttiana* but have a similar microclimate to their westerly (EO13 and EO14) and easterly (EO12 and EO16) counterpart sites, respectively, where *C. orcuttiana* occurs naturally. Air temperature ( $^{\circ}\text{C}$ ), and relative humidity (%) were recorded approximately 20 cm above the soil surface with a Hygrochron DS1923 (Maxim Integrated, San Jose, CA, USA), whereas soil temperature ( $^{\circ}\text{C}$ ) was recorded at a root depth between 5 and 7 cm with a Thermochron DS1921 (Maxim Integrated) and soil moisture ( $\text{m}^3/\text{m}^3$ )



WVC %) was recorded at 5–10 cm depth with an EC-5 sensor (Decagon Devices, Pullman, WA, USA). We used an ECRN-100 sensor (Decagon Devices) to measure precipitation (mm) and a QSO-S (Decagon Devices) photosynthetically active radiation (PAR) ( $\mu\text{mol}/\text{m}^2\text{s}$ ) sensor to record photosynthetically active radiation. All environmental sensors were programmed to record data at a 4-hr frequency. Based on the phenology of the species, we categorized the annual time series data into two distinct periods: (a) pre-germination conditions were represented by the time period including the months of October, November and December, and (b) the recruitment and plant growth conditions were represented by the time period including the months of January, February and March.

To characterize soil, we collected up to 500 g of soil by aggregating three individual soil cores from 0 to 7-cm soil depth at EO12, EO14, EO16, NPS1, NPS2, and at the experimental locations at EO12 (EO12g) and EO14 (EO14g). Soil was sampled in February and in April across 3 years (2015, 2016 and 2017), and each sample was characterized for pH, organic matter, soluble salts, cation exchange capacity, micronutrients, macronutrients and texture. Sample collection methods for microbial community characterization are described in the Supporting Information.

## 2.3 | Manipulative experiments

### 2.3.1 | Combined effect of after-ripening conditions and microhabitat on seed germination and plant fitness

To test the combined effects of after-ripening and microhabitat on seed germination and plant fitness of *C. orcuttiana*, we used a factorial arrangement of three after-ripening conditions and two distinct microhabitat types. The experiment was conducted in two consecutive years by using seeds collected in the respective years.

To apply the after-ripening experimental treatments, we used a homogenized mix of approximately 1,500 involucre collected from EO12 in May 2015 and again in June 2016. Five hundred seeds enclosed within a mesh bag were assigned to each of the three after-ripening treatments by placing them: (a) at approximately 2 cm depth in soil at EO12; (b) in a laboratory at 22°C and ambient relative humidity ( $\sim 45\%$ ); and (c) in a laboratory at 22°C with modified relative humidity ( $\sim 17\%$ ). Involucres were removed from their respective after-ripening treatment conditions in October of each involucre collection year to initiate the experiments. To enable recovering the ungerminated seeds, and to make certain that the

emerging plants indeed belonged to the experimental treatments, we constructed  $10 \times 15$ -cm nylon mesh (0.5 mm) bags to enclose the involucre prior to their placement at each of the four sites. One nylon mesh bag containing 20 involucre served as an experimental unit in the randomized complete block design (described below).

We used four experimental sites to represent two unique microhabitats; two of these (EO12g and NPS2) were east-facing, whereas the other two (EO14g and NPS1) faced west (Figure 1). At each of the four sites, the experiments were conducted in a  $3 \times 2$ -m area. To remove *C. orcuttiana* involucre that might have been present naturally in the soil, we sieved the top 7–10 cm of the soil at each experimental location prior to installing the experimental units. A randomized complete block design was subsequently established with nine blocks (i.e., rows), and one replicate (a mesh bag containing 20 seeds of *C. orcuttiana*) from each after-ripening treatment was randomly assigned to each of the nine rows. Seed bags were placed at the depth of 1.5 cm and covered with native soil. Experimental sites were inspected regularly to record data on plant emergence, plant width, number of stems, and number of involucre between February and April. At the end of each growing season (June), we excavated each experimental unit to assess the condition of ungerminated seeds (data not presented).

### 2.3.2 | Combined effect of light, soil moisture and microhabitat on seed germination and plant fitness

To determine the combined effect of light, soil moisture and microhabitat on seed germination and plant fitness, we used a factorial arrangement of two light treatments (reduced light conditions and ambient), two moisture treatments (supplemental soil moisture and ambient) and two microhabitats (easterly aspect and westerly aspect). We used the same experimental design to repeat the experiment twice by using seeds collected in the respective years. Seeds were installed in experimental areas in late October of each respective year.

To represent two microhabitat types, we paired an east-facing site (EO12g) with a west-facing site (EO14g) (Figure 1). At each site, the treatments were applied within a  $3 \times 2$ -m area with a randomized complete block design with three blocks. Each light and soil moisture treatment combination was replicated twice within each block. To manipulate ambient light, we constructed mesh screen structures ( $15 \text{ cm} \times 15 \text{ cm} \times 15 \text{ cm}$ ) to reduce the average photosynthetically active radiation (PAR) by 54%. Supplemental moisture treatment was applied with

a drip irrigation kit (Dripworks, Willits, CA, USA), programmed to deliver 65 mL water per minute at a 72-hr interval from October to March.

A single nylon mesh bag (10 × 15 cm) containing 20 involucre of *C. orcuttiana* served as a single experimental unit, and two replicates were used per block to represent each of the four combinations of light and soil moisture. Site preparation and seed bag burial methods were the same as described above in B.1 and occurred in late October. Germination data were collected in both experimental years by using the same methods as those described above in B.1, whereas plant fitness data were collected only in one year. At each site, soil moisture and PAR were measured both for ambient and for experimentally manipulated conditions as described in Section C below.

## 2.4 | Statistical methods and data analyses

### 2.4.1 | Spatial variation in population size and plant fitness

All statistical analyses were carried out in R version 3.5.2 (R Core Team, 2019). We first determined the variation between population sizes of easterly and westerly aspects by using the Kruskal-Wallis test. To determine the aspect-associated spatial variation in plant fitness of *C. orcuttiana* in naturally occurring populations, we first identified the best measure of plant fitness by determining the pairwise Spearman correlation between plant width, number of stems and number of involucre to eliminate the covariates. As a result, we selected plant width as the measure of plant fitness because it was strongly and positively correlated (with  $\alpha = 0.05$ ) with number of stems ( $n = 270$ ,  $r^2 = 0.83$ ; Figure S1b) and number of involucre ( $n = 180$ ,  $r^2 = 0.89$ ; Figure S1c). We then compared the highest measure of width for each experimental unit between February and April across the three sites by using a linear regression model.

### 2.4.2 | Characterization of spatial variation in microclimate, soil physicochemical properties and microbial communities

Spatial variation in the microclimate was captured by running a linear mixed-effect model on the weekly means of microclimatic variables, except for precipitation, which was analyzed by using the total amount of weekly precipitation, from October to March (period of seed

germination, seedling establishment and plant growth) in 2015 to 2018. Variation in each variable was determined in response to the fixed effects of aspect, whereas weekly averages nested within month and years served as the random effect. To determine the variability in plant width in response to the spatial variability in microclimate, we used a linear regression between annual mean plant width and mean air temperature between January and March (plant growth period) recorded over 4 years. Although air temperature, soil temperature and soil moisture are most relevant for the diminutive, prostrate habit of *C. orcuttiana*, air temperature was selected as the representative variable because it was significantly positively correlated (at  $\alpha = 0.05$ ) with soil temperature ( $n = 374$ ,  $r^2 = 0.87$ ) and negatively correlated with soil moisture ( $n = 327$ ,  $r^2 = -0.51$ ). Air temperature also showed a significant correlation with relative humidity ( $n = 353$ ,  $r^2 = -0.29$ ), PAR ( $n = 213$ ,  $r^2 = 0.54$ ) and precipitation ( $n = 286$ ,  $r^2 = -0.30$ ).

To visualize the variation in physicochemical edaphic variables measured at seven sites, we conducted a principal component analysis (PCA). Each variable was centered and standardized prior to analyses. Only two major principal components (PC1 and PC2) that explained approximately 50% variance in the dataset were selected. The percent contribution of each individual variable and each sample to a given principal component was calculated with the `fviz_contrib()` function in the *factoextra* package (Kassambara & Mundt, 2017). The workflow for the analyses of soil bacterial and fungal community data is described in the Supporting Information.

### 2.4.3 | Combined effect of after-ripening conditions and microhabitat on seed germination and plant fitness

For this experiment, the germination response was tested with a generalized linear mixed-effects model (GLMM) by using the *afex* package (Singmann et al., 2016), which uses the *glmer* function from the *lme4* package (Bates, Maechler, Bolker, & Walker, 2015). For each treatment combination, the highest number of germinants recorded across the data collection events was used as the response variable. Four individual experimental locations (EO12g, EO14g, NPS1 and NPS2) and nine blocks at each location were included as random effects. Because the germination data were binary (0/1), we modeled them with a binomial distribution with logit link. Differences among treatment combinations ( $\alpha = 0.05$ ) were assessed by using parametric bootstrapping with 10,000 simulations with the *afex* package that uses functions from the *pbrtest* package (Halekoh & Højsgaard, 2014).

## 2.4.4 | Combined effect of light, soil moisture and microhabitat on seed germination and plant fitness

Seed germination data were treated as described above with a GLMM, except the random effects included three blocks and two replicates. We assessed the significance of predictor variables by using the methods described above for seed germination.

## 3 | RESULTS

### 3.1 | Observational studies

#### 3.1.1 | Spatial variation in population size and plant fitness

We observed that sites with easterly aspects hosted larger populations in comparison to the sites with a westerly aspect ( $p = .002$ , Table 1). We also detected aspect-associated spatial variation in plant width (the selected measure of plant fitness) across the four natural populations ( $F = 6.24$ ,  $p = .013$ ; Figure 2a, Table 2). Plants at east-facing sites, which also host larger populations, exhibited the larger mean width (4.6 cm) in comparison to westerly sites (3.6 cm; Figure 2a).

#### 3.1.2 | Characterization of spatial variation in microclimate (and its link to plant fitness), soil physicochemical properties and microbial communities

During the pre-germination/germination (between October and December) and plant growth periods (between January and March), we observed aspect-associated variability (with  $\alpha = 0.05$ ) in air temperature ( $F = 137.08$ ), soil temperature ( $F = 142.78$ ), soil moisture ( $F = 26.75$ ) and PAR ( $F = 88.15$ ) across 4 years (Figure 3). Sites with easterly aspects experienced lower air and soil temperature along with higher soil moisture in comparison to the sites exposed to a westerly aspect that received higher PAR (Figure 3, Table S1). Relative humidity ( $F = 2.00$ ,  $p = .16$ ) and precipitation ( $F = 0.02$ ,  $p = .89$ ) values, however, showed an overlap among sites with easterly and westerly aspects during the same time period (Figure 3, Table S1). Variation in air temperature also affected plant width ( $F = 124.89$ ,  $p < .001$ ), whereby lower air temperature yielded higher plant width and vice versa (Figure 2b).

Principal component analysis of soil physicochemical variables across seven sites revealed spatial variation,

**TABLE 1** Spatial variation in population size (number of individuals) of *Chorizanthe orcuttiana*. Population size of *C. orcuttiana* was recorded at EO12 and EO14 between 2014 and 2018, and at EO16 in 2014 and 2015. Populations EO12 and EO16 represent easterly aspects while EO13 and EO14 represent westerly microhabitats.

Site	2014	2015	2016	2017	2018
EO12	246	1,102	622	2,490	3,629
EO13	0	61	42	50	291
EO14	0	171	76	219	178
EO16	211	820			

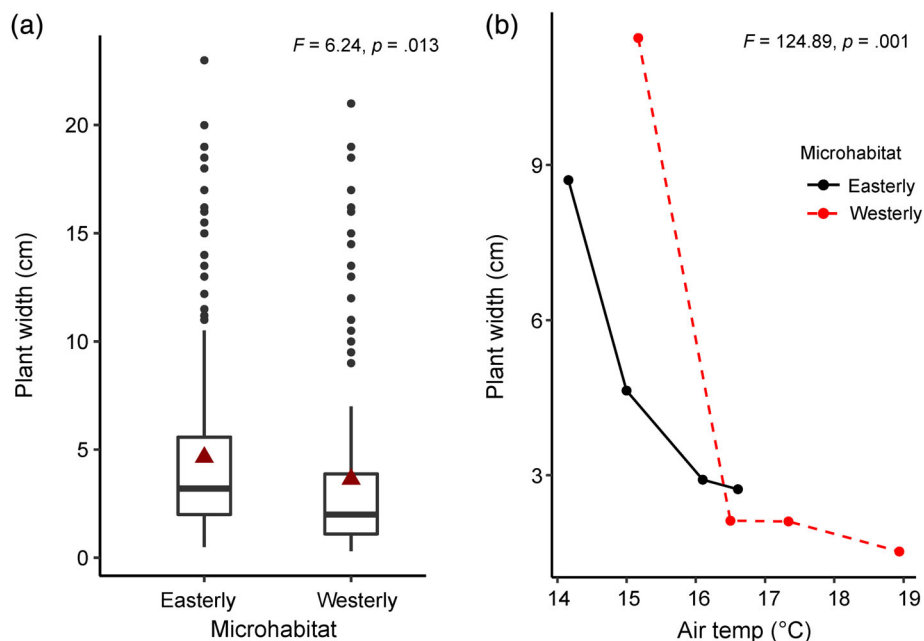
although we did not observe clustering of sites based on their aspect (Figure 4). Principal components (PCs) 1 and 2 explained 32% and 17% variation, respectively. Although Mg, CEC, Na,  $\text{NO}_3$ , salinity, OM, K, sand and Mn contributed in decreasing order to the variation explained by PC1, the variation in PC2 was explained by Ca, P1, P2 and pH in decreasing order. With respect to PC1 and PC2 that explained 50% of the variation, individual soil samples representing an easterly aspect exhibited more soil physicochemical variability in comparison to a westerly aspect; however, there was considerable overlap between the soil physicochemical properties of easterly and westerly aspects (Figure 4, Table S2). Both soil bacterial and fungal communities (Figure S1 and S2, respectively) showed spatial variability; however, when grouped according to the aspect position, microbial communities at easterly and westerly aspects were indistinguishable from each other (Figure S2 and S3). Detailed results of soil microbial community analyses are presented in the Supporting Information.

### 3.2 | Manipulative experiments

#### 3.2.1 | Combined effect of after-ripening environment and microhabitat on seed germination and plant fitness

We identified a strong interaction of after-ripening conditions during dormancy and microhabitat during the germination period that influenced seed germination (Figure 5, Tables 3 and S3). The germination response to after-ripening conditions differed in two microhabitats ( $\chi^2 = 25.2$ ,  $p < .001$ ). Germination percentage was highest (2.2%) for the seeds after-ripened at 22°C and 17% relative humidity and subsequently exposed to the east-facing microhabitats, whereas the combination of after-ripening in native soil and the west-facing microhabitats yielded the lowest germination (0.14%; Figure 5,

**FIGURE 2** Spatial variation in plant width, the indicator of plant fitness, of *Chorizanthe orcuttiana*. (a) Box plots showing plant width of *C. orcuttiana* for 60 individuals at easterly (represented by populations EO16 and EO12) and westerly aspects (represented by populations EO14 and EO13) collected each year across 2015–2018. Triangles represent mean plant width. (b) Plant width as a function of air temperature at easterly and westerly microhabitats. Individual points represent mean air temperature and mean plant width during the plant growth period between January and March over 4 years (2015–2018). The solid and dashed lines represent easterly and westerly aspects, respectively



**TABLE 2** Results of an analysis of variance (ANOVA) from a linear regression model to test the effect of microhabitat aspect on plant fitness of *Chorizanthe orcuttiana*

	df	Sum Sq	Mean Sq	F	p
Microhabitat	1	114	114	6.24	.013
Residuals	448	8,211	18		

Note: Plant fitness data were collected across 4 years between 2015 and 2018 from natural populations. Microhabitats were represented by two easterly populations (EO12 and EO16) and two westerly populations (EO13 and EO14). df, degree of freedom.

Tables 3 and S3). Plant width (though not tested statistically) also showed variation in absolute number across the interactions of after-ripening treatments and microhabitat conditions (Figure 6). After-ripening at 22°C with 45% relative humidity yielded the highest plant width (7.8 cm) on westerly aspects, whereas in easterly aspect microhabitats, *in situ* soil after-ripening yielded the highest plant width (14.5 cm).

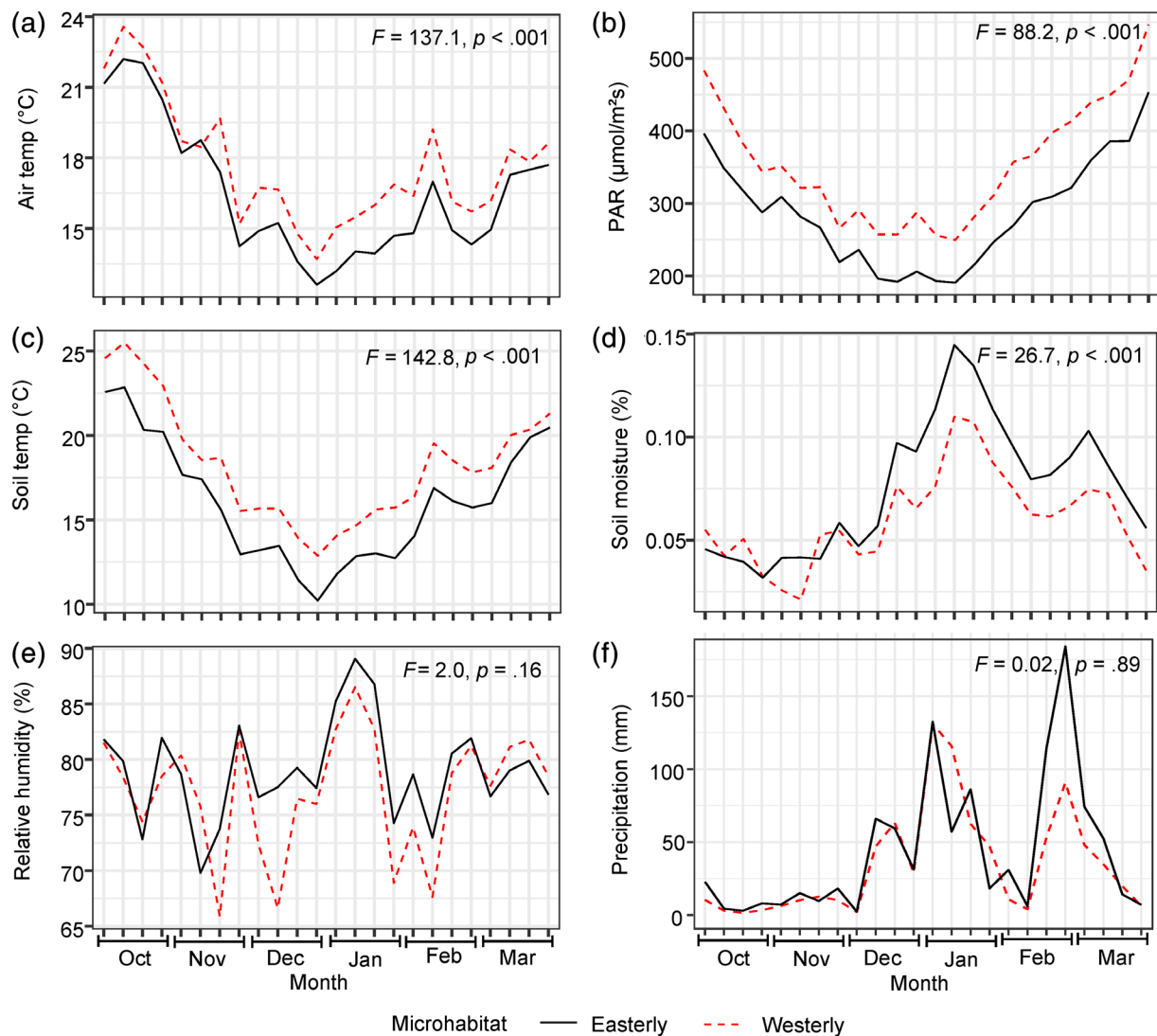
When considering only the main effects of the three after-ripening treatments, seeds exposed to 22°C and 17% relative humidity had the highest germination (1.9%;  $\chi^2 = 32.8$ ,  $p < .001$ ) in comparison to seeds after-ripened at 22°C and 45% relative humidity (1.2%) or in soil at a native site (0.3%). Plant width, however, was highest for the soil after-ripening treatment (11.6 cm) when compared to the after-ripening at 22°C with either 17% relative humidity (4.0 cm) or 45% relative humidity (5.9 cm). Germination did not respond variably to microhabitat alone in this experiment (easterly = 1.34% and westerly

= 0.97%, Table 3). Plant width (not tested statistically) also showed a slight difference between easterly (4.9 cm) and westerly (6.0 cm) aspect microhabitats.

### 3.2.2 | Combined effect of light, soil moisture and microhabitat on seed germination and plant fitness

We detected a significant three-way interaction ( $\chi^2 = 6.5$ ,  $p = .021$ ) of light, soil moisture and microhabitat on seed germination (Figure 7, Tables 4 and S4). All treatment combinations, including ambient light and ambient soil moisture, resulted in higher germination at the easterly site than the westerly site (Figure 7). The highest germination (7.0 to 8.0%) occurred under increased soil moisture at EO12g regardless of light modification (Figure 7). Lower germination percentages (from 0.0 to 0.4%) were observed at EO14g in the presence of either ambient light or ambient soil moisture, but when ambient light and soil moisture were combined, the response increased to 1.7% (Figure 7). The combination of modified light and soil moisture at the westerly aspect showed the highest germination response (2.1%) in comparison to all other combinations at this site. Although not tested statistically, plant width also exhibited variation across a two-way interaction of light and soil moisture, whereby width was highest (9.0 cm, Figure 8) when ambient soil moisture and ambient light were combined, followed by manipulated light and manipulated moisture (7.4 cm, Figure 8). When considering only the main effects, the easterly site hosted a higher cumulative percentage of germination





**FIGURE 3** Characterization of microclimate at easterly and westerly study sites hosting *Chorizanthe orcuttiana*. Weekly means of (a) air temperature, (b) photosynthetically active radiation (PAR), (c) soil temperature, (d) soil moisture, (e) relative humidity and (f) total precipitation during the growing season spanning October through March across 4 years (2015–2018). Three populations (EO16, EO12 and NPS2) represented eastern aspects and another three populations (EO14, EO13 and NPS1) represented western aspects

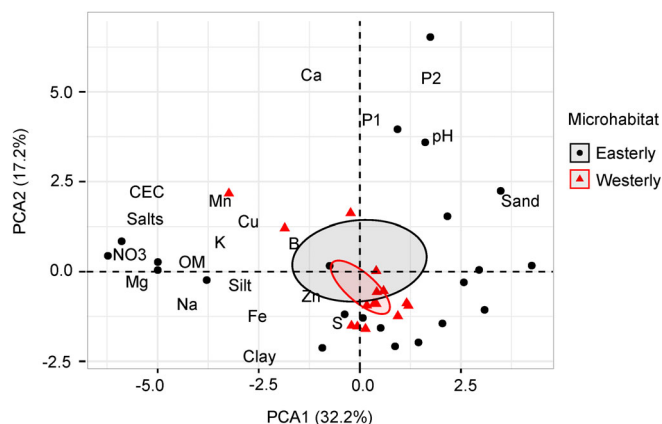
(6.0%;  $\chi^2 = 46.0$ ,  $p < .001$ ; Table 4) in comparison to the westerly site (1.0%). The main effects of light or soil moisture on seed germination were not detected ( $\chi^2 = 1.09$ ,  $p = .38$ ;  $\chi^2 = 2.40$ ,  $p = .12$ , respectively).

## 4 | DISCUSSION

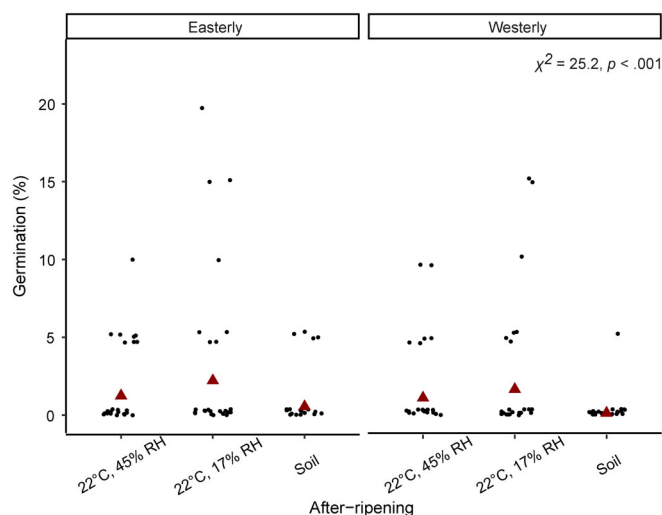
We report consistent natural variation in populat size, plant fitness and microclimatic conditions over 4 years across three disjunct populations of a narrowly endemic winter annual plant species, *C. orcuttiana*, that are variably exposed to easterly or westerly aspects while being situated within 1 to 25 km from each other. Coupled with

the results from the observational studies, our multi-year empirical investigations establish that microhabitat differences (eastern vs. western aspects) explain spatial variation in seed germination and plant fitness. Germination and fitness responses were further nuanced by interactions with seed after-ripening conditions during dormancy, and with soil moisture and light during the growing season.

The observational studies revealed that sites with an easterly aspect exhibited larger population size and higher plant fitness, and comparisons of microclimates at these sites suggested that sites with an easterly aspect experienced cooler air and soil temperature along with higher soil moisture in comparison to the sites with



**FIGURE 4** Principle component analysis (PCA) showing the clustering of study sites hosting *Chorisanthus orcuttiana* with respect to soil physicochemical variables. Four sites (EO16, EO12, EO12g and NPS2) represented eastern aspects and three sites (EO14, EO14g and NPS1) represented western aspects. PCA components 1 and 2 show the clustering of easterly and westerly sites. Soil was characterized for organic matter (OM), pH, nitrate ( $\text{NO}_3$ ), phosphorus (P1 [readily available to plants] and P2 [readily available to plants + active reserve]), potassium (K), magnesium (Mg), sodium (Na), calcium (Ca), zinc (Zn), manganese (Mn), iron (Fe), copper (Cu), boron (B), soluble salts (Salts), cation exchange capacity (CEC), sand, silt and clay contents. Multiple soil cores were collected at each study site in February and April across 3 years

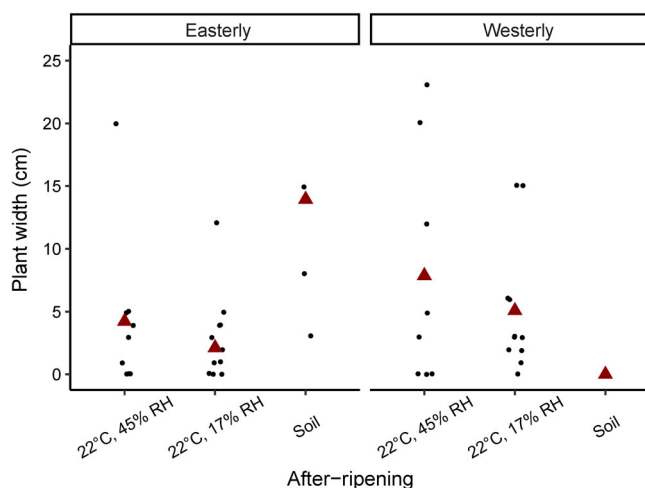


**FIGURE 5** Germination response in *Chorisanthus orcuttiana* seeds exposed to combinations of after-ripening conditions and microhabitats. The three after-ripening treatments included 22°C with 45% relative humidity (RH), 22°C with 17%, and storage in native soil (Soil); and microhabitat conditions included easterly and westerly aspects. The experiment was conducted twice across 3 years (2015–2017). Circles represent germination from a single replicate, whereas the triangles show the mean response

**TABLE 3** Results of an analysis of variance (ANOVA) from a generalized linear mixed model to present the significance for the fixed effects of after-ripening treatments (AR), microhabitat types (microhabitat) and their interaction in explaining the germination response of *Chorisanthus orcuttiana* seeds

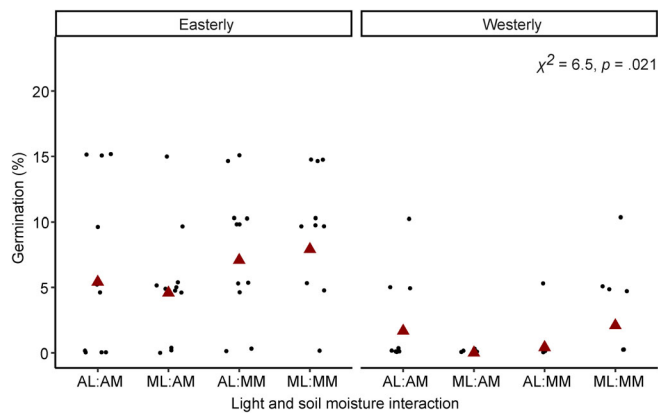
	df	$\chi^2$	p
AR	2	32.85	<.001
Microhabitat	1	3.05	.240
AR: microhabitat	2	25.19	<.001

*Note:* The model included four experimental locations (EO12g, EO14g, NPS1 and NPS2) and nine blocks at each location as random effects. The experiment was conducted twice between 2015 and 2017. The chi-squared value presents the difference in likelihood of full and restricted models. The *p*-values were generated with parametric bootstrapping by using 10,000 simulations. The coefficients of the model are presented in Table S3. *df*, degree of freedom.



**FIGURE 6** Plant width in *Chorisanthus orcuttiana* in response to combinations of after-ripening conditions and microhabitats. The three after-ripening treatments included 22°C with 45% relative humidity (RH), 22°C with 17%, and storage in native soil (Soil); and microhabitat conditions included easterly and westerly aspects. The experiment was conducted twice across 3 years (2015–2017). Circles represent germination from a single replicate, whereas the triangles show treatment means

westerly aspects. Precipitation, ambient relative humidity, edaphic characteristics or microbial profiles did not segregate sites based on the aspect of microhabitat. The demographic variability in winter annuals has been associated with total precipitation in the growing season (Angert, Huxman, Barron-Gafford, Gerst, & Venable, 2007; Fox et al., 2006; Venable, 2007). However, precipitation is unlikely to affect population size and plant fitness of *C. orcuttiana* where populations occurring in close proximity differ with respect to temperature (air and soil),



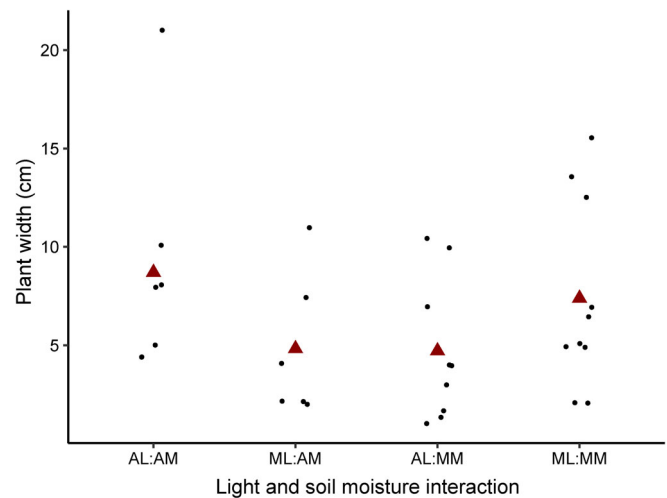
**FIGURE 7** Germination response in *Chorizanthe orcuttiana* seeds exposed to combined manipulations of soil moisture, ambient light and microhabitats. Soil moisture treatments included ambient soil moisture (AM) and supplemental moisture (MM); light manipulations included ambient light (AL) and reduced light (ML); and microhabitat treatments included easterly and westerly aspects. The experiment was conducted twice across 4 years (2014–2017). Circles represent germination from a single replicate, whereas the triangles show treatment means

**TABLE 4** Results of an analysis of variance (ANOVA) from a generalized linear mixed model to present the significance for the fixed effects of microhabitat types (microhabitat), light, soil moisture (moisture) and their interaction in explaining the germination response of *Chorizanthe orcuttiana* seeds

	<i>df</i>	$\chi^2$	<i>p</i>
Microhabitat	1	45.96	<.001
Light	1	1.09	.380
Moisture	1	2.40	.130
Microhabitat:light	1	1.00	.400
Microhabitat:moisture	1	0.77	.440
Light:moisture	1	8.57	.007
Microhabitat:light:moisture	1	6.54	.021

*Note:* The model included three blocks and two replicates as the random effects. The experiment was conducted twice between 2014 and 2017. The chi-squared value presents the difference in likelihood of full and restricted models. The *p*-values were generated with parametric bootstrapping by using 10,000 simulations. The coefficients of the model are provided in Table S4. *df*, degree of freedom.

soil moisture and PAR, but not precipitation. Levine, McEachern, and Cowan (2008) also showed that although the population size of three small rare annual species native to the Channel Islands in California showed a negative response to increasing air temperature, it did not respond to variability in total precipitation. However, a decrease in the abundances of multiple winter annual



**FIGURE 8** Plant width in *Chorizanthe orcuttiana* in response to combined manipulations of soil moisture, ambient light and microhabitats. Soil moisture treatments included ambient soil moisture (AM) and supplemental moisture (MM); light manipulations included ambient light (AL) and reduced light (ML); and microhabitat treatments included easterly and westerly aspects. The experiment was conducted twice across 4 years (2014–2017). Circles represent germination from a single replicate, whereas the triangles show treatment means

species was reported in response to decreasing precipitation (and increasing air temperature) in the Sonoran Desert over 25 years (Huxman et al., 2013; Kimball, Angert, Huxman, & Venable, 2010). Clearly, demographic and fitness responses can operate variably in species with similar life histories based on their niche specificity and local distributions. These observations cast a doubt on broad generalizations, especially for taxa that may be peculiar in their ecological preferences that render them endemic to the specific regions (Fox, 2007).

Besides microclimate, edaphic characteristics also affect plant endemism (McGraw & Levin, 1998). Considering that the results of our observational studies did not show segregation of soils at the easterly and westerly sites, small-scale variations in soil type may not be a leading driver of plant or population performance in *C. orcuttiana*. Soil physicochemistry, as opposed to microclimate, also did not affect survivorship and fecundity in a congeneric species, *C. pungens* var. *hartwegiana* (McGraw & Levin, 1998). Similarly, the microbial profiles across the seven test locations did not segregate based on aspect although spatial differences in taxonomic and functional diversity were observed. One of the functional groups of concern to seed banks is that of pathogenic fungi. Although others have reported a decrease in seed viability in the presence of pathogenic fungi in soil (Crist & Friese, 1993; Gilbert, 2002; Mordecai, 2015), we did not detect appreciable spatial differences in

abundances of pathogenic fungi. Further, when the ungerminated seeds from our manipulative experiments were tested in a common garden (data not shown), we did not observe variation in germination percentages among seeds incubated at eastern and western sites, suggesting that seed degradation was not likely to be influenced by microhabitat conditions during the study period.

When we manipulated after-ripening conditions during seed dormancy, and light and soil moisture conditions during seed germination at both the easterly and westerly aspects, our results paralleled the observational studies in that germination and fitness were generally highest at the east-facing sites. The overall low germination rates across the two manipulative experiments are likely to be due to the bet-hedging strategy in winter annuals whereby germination is delayed temporally to maximize reproductive fitness under variable annual environments (Clauss & Venable, 2000). Throughout our experiments, survival of germinated seedlings varied and typically yielded low sample sizes that precluded strong comparisons of plant fitness across treatments.

The difference in mean germination in response to 17% and 45% relative humidity during after-ripening was slightly lower at the westerly sites when compared to easterly sites, which could possibly be because of excessive drying of the seeds in westerly microhabitat conditions that offer lower soil moisture and higher air and soil temperature. Consequently, seeds may have entered secondary dormancy (Baskin & Baskin, 1979; Finch-Savage, Cadman, Toorop, Lynn, & Hilhorst, 2006). We used seeds from the easterly site (EO12) for all experimentation because it is the largest population that yielded a sufficient number of seeds for this purpose; it is, thus, likely that seeds from the easterly sites perform differently when exposed to the westerly conditions and vice versa. Reciprocal experiments with seeds representing both aspects may clarify such variation.

Ambient relative humidity is known to modulate transcriptional and translational signals during after-ripening that prompt dormancy release and germination, although the optimal levels are species specific (Finch-Savage et al., 2006; Holdsworth, Bentsink, & Soppe, 2008; Née, Xiang, & Soppe, 2017). For example, in a weedy winter annual, *Draba verna*, relative humidity between 30 and 60% during *in vitro* after-ripening led to higher germination in comparison to after-ripening at <20% relative humidity, whereas  $\geq 60\%$  relative humidity resulted in seed degradation (Baskin & Baskin, 1979). Similarly, 40–50% relative humidity during *in vitro* after-ripening resulted in higher seed germination compared to 70% relative humidity in an invasive perennial grass, *Cenchrus ciliaris* (Sharif-Zadeh & Murdoch, 2001). Relative

humidity during after-ripening may also interact with temperature to influence germination; for instance, seeds of *Nicotiana plumbaginifolia* exposed to after-ripening at 24°C and 50% relative humidity showed higher dormancy release and degradation of dormancy-promoting mRNAs when compared to seeds stored at 4°C and 40% relative humidity (Bove et al., 2005). It is, again, clear that ideal or required environmental after-ripening conditions may not be generalized across taxa. Depending on the species, mechanisms driving this variation may span the range from cell membrane damage due to high relative humidity to desiccation under low-humidity conditions (Baskin & Baskin, 1979; Meimoun et al., 2014). Nonetheless, apart from our present study, the influence of relative humidity during after-ripening on *in situ* or *in vitro* seed germination in winter annuals from the California Floristic province is currently unknown although many winter annuals from this arid environment are endemic and rare. *Ex situ* seed storage is a common practice in rare species conservation programs, although optimal storage conditions are not often identified for imperiled taxa. Although we focused on quantifying the germination response in *C. orcuttiana* to manipulated relative humidity, its interactions with seed collection time, storage temperature and storage duration may refine our understanding of how the after-ripening environment governs dormancy release, germination and plant fitness.

We also observed interactive effects of light, moisture and microhabitat aspect on seed germination and plant fitness in *C. orcuttiana*. Although other studies examining the combined effects of aspect position, soil moisture and light manipulations on seed germination of winter annuals are not available, individual effects of similar predictors on recruitment or reproductive success have been reported. For example, Fox (2007) showed a positive relationship of soil moisture with seedling survival and seed set in *C. pungens* var. *pungens* in California's Central Coast, whereas similar patterns were not observed in a co-occurring species, *Gilia tenuiflora* var. *arenari*. Further, a rare annual, *C. pungens* var. *hartwegiana*, native to central coastal California showed higher plant survival and reproduction under intermediate shade in a common garden experiment in comparison to no-shade and high-shade treatments (McGraw & Levin, 1998). Similarly, Kluse and Doak (1999) reported higher vital rates of *C. pungens* var. *hartwegiana* when introduced into a habitat shaded with a sparse pine canopy compared to its native open sandy habitat. Our results on seed germination of *C. orcuttiana* are congruent with previous observations, whereby a combination of eastern aspect and supplemental soil moisture enhanced seed germination regardless of the light treatment, whereas a combination of shade and supplemental moisture was the most



effective in the otherwise drier and hotter west-facing microhabitats. It is likely that supplemental soil moisture coupled with shade resulted in higher soil moisture retention by soil and consequently enhanced seed germination in both microhabitats.

Collectively, our studies are unique in establishing spatial variation in microhabitat conditions, and in testing and identifying the interactive nuances of variable germination conditions (microhabitats), environment during seed dormancy, supplemental water and shade on *in situ* germination of a narrowly endemic annual plant threatened with extinction. The higher success of *C. orcuttiana* in east-facing environments was supported by both the observational and experimental data. Although larger (spatially and temporally extensive) edaphic and microclimate datasets are necessary to definitively establish linkages of these variables with seed germination and plant fitness, early patterns are aligned with the patterns suggested by the experimental studies, in that population size and fitness are larger in easterly populations with cooler and more moist soil conditions. Our frequent observations of dried, wilted and dead plants under hotter, drier westerly conditions at EO13, EO14 and NPS1 also lend support to the potentially strong link between fine-scale microclimatic variation and plant fitness in this species. The affinity of *C. orcuttiana* toward cooler microhabitats is likely to affect its persistence in populations situated at the hotter, westerly aspects. Simultaneously, it is possible that persistence of populations in less than optimal conditions indicates an adaptive genetic divergence. Putative genetic differences among populations cannot be ruled out as a source of variation in plant and population performance, especially considering that *C. orcuttiana* is a known polyploid (Hardham, 1989), although spatial variation in its ploidy status and genetic (or epigenetic) structure is currently unknown. Within this context, local extinction events at westerly populations pose a threat to the overall genetic diversity of the species, weakening both its status and the tools for its conservation and restoration.

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## AUTHOR CONTRIBUTIONS

JS and JK designed and conducted the research. JK analyzed the data and wrote the manuscript. JS and DS guided the analyses and edited the manuscript.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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