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# A test of the Janzen-Connell hypothesis in a species-rich Mediterranean woodland

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**Abstract.** The Janzen-Connell (JC) hypothesis predicts that conspecific negative density dependence contributes to the maintenance of plant diversity by lowering the recruitment of locally abundant plant species. The JC hypothesis is a widely evoked explanation for the high species diversity in tropical forests, but remains poorly tested in other species-rich systems such as Mediterranean woodlands. As such, we tested if the JC operates and the role of soil-borne oomycetes in a species-rich Mediterranean woodland of Western Australia, where post-fire recruitment can lead to high seedling densities, using the common *Banksia attenuata* as a case study. We attempted to decipher the effects of oomycete pathogens and distance from conspecific trees on intraspecific seedling survival and growth. Contrary to the JC hypothesis, we found little evidence of negative density dependence, but our results suggest positive density dependence survival under conspecific trees. Oomycete-driven mortality in seedlings was also found regardless of the type of tree. Our results suggest that short-term seedling recruitment patterns in this species-rich, fire-prone ecosystem do not follow the JC hypothesis. Future studies should explore whether negative conspecific density and distance dependence could play a greater role in later stages of the post-fire recovery process.

**Key words:** coexistence and diversity; fungal pathogen; Kwongan vegetation; Mediterranean; non-mycorrhizal *Banksia attenuata;* seedling recruitment and survival.

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## INTRODUCTION

The Janzen-Connell (JC) hypothesis (Janzen 1970, Connell 1971) is a leading theory seeking to explain the high plant diversity of tropical rainforests (Wright 2002, Terborgh 2012). This hypothesis states that seedling recruitment will be higher away from conspecific mature trees (negative distance dependence) and at lower conspecific seed or seedling densities (negative density dependence). Negative distance and density dependence are widespread in lowland tropical rainforests (Harms et al. 2000, Comita et al. 2010, Metz et al. 2010) and in temperate forests (Deniau et al. 2018, Seiwa et al. 2019, Yao et al. 2020). However, it has been far less studied in other vegetation types such as species-rich fire-prone Mediterranean woodlands. For example, a recent meta-analysis of the effects of the JC hypothesis on seedling survival did not present any data for Mediterranean ecosystems (Comita et al. 2014). To our knowledge, the JC hypothesis remains untested in Mediterranean woodlands that harbor diverse and abundant non-mycorrhizal plant species, which may suffer great mortality early in life to soil-borne

pathogens due to lower defense against pathogens (Laliberté et al. 2015). In addition, seedling recruitment in these ecosystems typically occurs after a fire, which can lead to high seedling densities post-fire (Lamont et al. 1993), potentially leading to strong JC effects.

Southwestern Australia is one of the world's 25 global biodiversity hotspots (Myers et al. 2000, Hopper and Gioia 2004) and hosts some of the most species-rich shrublands and woodlands in the world (Lamont et al. 1977, 1984). In species-rich *Banksia* woodlands, a sparse canopy of *Banksia* tree species (e.g., *Banksia attenuata*) occurs over a species-rich understory composed of woody shrubs and perennial herbs. Cones of most *Banksia* species in Western Australia are serotinous (Lamont et al. 1984); that is, their seeds are stored in closed cones and are mostly released only after a fire. Most seeds and dead leaves fall directly beneath the mature plant, leading to litter patches with high seed densities among otherwise bare sand with low seed densities (Lamont et al. 1993); however, long-distance seed dispersal by birds does also occur particularly relevant after fire (Cowling et al. 1987, He et al. 2009). Despite higher nutrient availability in litter patches, seedling survival and growth is much lower than in bare sand, and this phenomenon has been attributed to intraspecific competition for water (Lamont et al. 1993). Lamont et al. (1993) had shown evidence for density-dependent mortality attributed to competition for water in this fire-prone Mediterranean ecosystem. A potential role for soil-borne pathogens (i.e., oomycetes), as another mechanisms, however, has not been explored (Laliberté et al. 2015).

In tropical rainforests, oomycete pathogens (e.g., *Phytophthora*, *Pythium*) appear to be important causal agents of negative distance- and density-dependent seedling mortality (Bell et al. 2006, Bagchi et al. 2010, 2014). Native and endemic *Phytophthora* species that have co-evolved with the flora have been isolated from species-rich woodlands and shrublands from southwestern Australia, including *Banksia* woodlands (Rea et al. 2011). These *Phytophthora* species rarely kill mature plants but could affect juveniles and generate JC effects, particularly for Proteaceae species such as *Banksia*, which are known to have high sensitivity to these pathogens (Rea et al. 2011, Shaw 2020). The high sensitivity of *Banksia* species to oomycetes might be linked to their non-mycorrhizal status given that mycorrhizal fungi can protect roots against pathogens (Pozo and Azcón-Aguilar 2007, Laliberté et al. 2015). However, the potential role of oomycetes in maintaining plant species diversity in species-rich southwestern Australian woodlands and shrublands remains overlooked.

Studying the distance- and density-dependent aspects of the JC hypothesis, as well as identifying the underlying biotic agents behind negative density dependence, is important to understand the mechanisms controlling local plant diversity in species-rich ecosystems Mediterranean woodlands. Few experiments have simultaneously considered both the distance- and density-dependent aspects of the JC hypothesis (Terborgh 2012). In addition, causal agents underlying JC effects are often unidentified, although the importance of soil-borne pathogens is being increasingly recognized (Bell et al. 2006, Bagchi et al. 2010, 2014, Laliberté et al. 2015). Here, we report the results of a field experiment, where we manipulated seed density of *Banksia* in a recently burnt species-rich *Banksia* woodland, and where seeds were sown either close to conspecific mature trees or not, and either in the presence or absence of a fungicide that targets oomycetes. The post-fire nature of our experiment ensured its ecological relevance since most seedling recruitment occurs after a fire in these ecosystems. This design allowed us to determine (1) distance- and density-dependent effects on seedling mortality, (2) the functional form of negative density dependence, and (3) whether oomycetes cause negative density dependence in this widespread non-mycorrhizal tree species. Our specific hypotheses were that: (1) Density-dependent seedling mortality would be stronger close to conspecific mature trees, and that (2) suppression of oomycetes would reduce such density-dependent mortality.

## Methods

### *Study area and site description*

We established a seedling survival and recruitment tracking field study at the University of Western Australia's (UWA) Shenton Park Field Station (31.96°S, 115.79°E). The field station hosts

a *Banksia* woodland dominated by *Banksia attenuata*, *Eucalyptus gomphocephala*, *Banksia menziesii*, *Eucalyptus marginata*, *Allocasuarina fraseriana*, and *Xanthorrhoea preissii* and is located in the Kwongan vegetation type on the Swan Coastal Plain within the Southwest Australian Floristic Region (*sensu* Hopper and Gioia 2004). The field station is part of the Spearwood coastal dune system (Wyrwoll and King 1984). The climate is Mediterranean with a mean annual temperature of 19.2°C and receives approximately 700 mm of rain per year, ~80% of which falls in winter (Australia Bureau of Meteorology; http://www.bom.gov.au/climate/data/). Fire is the main natural disturbance with an estimated mean fire interval of 5 yr based on a study of fire history at a nearby natural reserve (Fisher et al. 2009). In January 2013, 18 months prior to our experiment was established, a small fire burnt approximately half of the bushland where the seed plots were positioned afterward; this fire did not kill the focal trees used in this experiment (Appendix S1: Fig. S1). We conducted the field experiment post-fire to increase the ecological relevance of our study, given that most seedling recruitment occurs after fire (Lamont et al. 1993).

### *Field experiment*

In July 2014, 84 0.3 m × 0.3 m *Banksia attenuata* seed plots were placed close to *Banksia attenuata* and *Eucalyptus marginata* adult trees at the UWA Shenton Park Field Station (Appendix S1: Fig. S1). These two tree species are referred to hereafter by their generic names. We chose *Eucalyptus* as the focal tree species because it is widespread and common, and also representative of widely occurring ectomycorrhizal nutrient-acquisition strategy. Around each focal tree, 14 seed plots were randomly positioned at distances between 0.5 m and 2.5 m from the base of the focal tree trunk. The seed plots were never <0.5 m apart from each other. Furthermore, each focal tree was at least 25 m away from any other focal tree. Around each focal tree, we sowed a total of 2166 *Banksia* seed at seven different densities: 1 to 7, 7 to 14, 14 to 21, 21 to 28, 28 to 35, 35 to 42, and 42 to 48 seeds per plot (i.e., 11 to 77; 78 to 155; 156 to 233; 234 to 311; 312 to 388; 389 to 466; and 467 to 533 seed $m^{-2}$). In these woodlands, sunlight conditions under older trees are relatively uniform, mainly due to the open canopy of mature trees such as *Eucalyptus* and *Banksia* used here. Therefore, potential effects of shade on seedlings are very minimal.

We selected a maximum seed density ($d_{max}$) of 48 seeds per 0.3 m × 0.3 m plot, corresponding to 533 seeds $m^{-2}$. In a field survey near the town of Eneabba (~275 km north of Perth), the mean seed density of *Banksia* in litter patches was estimated at 41.7 seeds $m^{-2}$, with a standard deviation of 15.5 (Lamont et al. 1993). Therefore, our maximum seed density is about five times greater than the value of the quantile function for this normal distribution, corresponding to a 99.99% probability. While such high seed density is unlikely to be observed in the field, we wanted to evaluate negative density dependence across a wider range of seed densities. Our approach is similar that of Bagchi et al. (2010), who selected a maximum seed density corresponding to four times the maximum seedling density recorded in the field for their species. Seven seedling densities were selected using a uniform series. Within each block/treatment combination, the $i$th seed number per 30 cm × 30 cm plot was selected as a uniform random draw (rounded to the next larger integer) from the interval $\{d_{max}(i-1)/7+1, d_{max}(i)/7\}$.

### *Fungicide treatment*

We applied the oomyceticide Apron XL 350 ES (active constituent: 350 g/L metalaxyl-m; Syngenta Australia Pty Ltd, Macquarie Park, New South Wales, Australia) as a liquid spray directly to the soil of the small plots at a rate of 0.25 g metalaxyl-m·$m^{-2}$·$week^{-1}$, following earlier studies (e.g., Bagchi et al. 2010, Gripenberg et al. 2014). Apron was used to kill oomycetes such as *Pythium* and *Phytophtora*. Control plots were sprayed with an equivalent volume of sterilized water. However, for the first month after sowing the seed, we maintained moisture levels on the plots to avoid seed desiccation and germination failure. This entailed a few extra watering and fungicide applications per week. Metalaxyl-m is considered to have little negative impacts on arbuscular mycorrhizal (AM) fungi (e.g., Seymour et al. 1994), although some studies have observed negative impacts on AM fungi (Sukarno et al. 1993, 1996). In our case, the target plant species, *Banksia*, is non-mycorrhizal (Teste et al. 2017) and thus would not benefit from AM fungi.

Immediately after *Banksia* seed were sown, we applied the fungicide treatment on 15 different dates (18 July 2014; 25 July 2014; 1 August 2014; 7 August 2014; 15 August 2014; 22 August 2014; 29 August 2014; 4 September 2014; 19 September 2014; 2 October 2014; 17 October 2014; 14 November 2014; 12 December 2014; 14 January 2015; and 16 February 2015) spanning nine months.

### *Seedling recruitment dynamics*

We followed seedling recruitment through time at the same date the fungicide was applied (Appendix S1: Fig. S2). At each survey date (Appendix S1: Fig. S2), total number of germinating seeds, and then live seedlings, were scored at each 30 × 30 cm plots until the end of the experiment. Due to a large storm on 25 August 2014, we could not record all seedling counts close the conspecific trees on that date. Between 16 February and 19 February 2015, the surviving seedlings found within each plot were completely harvested (with roots and shoots), oven-dried at 70°C during 48 h, and then weighed to quantify their final dry mass.

### *Data analyses*

All graphical and statistical analyses were conducted in R (R Core Team 2020). First, we used a binomial generalized linear mixed model (GLMM) to test for the effects of type of focal tree (conspecific or heterospecific), fungicide treatment (with or without), and seed density, and the interactions among these factors on seedling survival (i.e., number of live seedlings per seed sown per plot) at the end of the field experiment; replicated blocks were set as a random factor in these analyses. We then determined the functional form of density dependence graphically by plotting percent survival over seed density using the ggplot2 package (Wickham 2016). Total (i.e., sum) and mean dry weight of remaining live seedling(s) per seed plot at the end of the experiment were calculated and then analyzed with a LMM using seed density (number of seed $m^{-2}$) as prior weight (using weights function) in the fitting process in the statistical model. Assumptions for the validity of the statistical models were assessed graphically in R with residual plots and with overdispersion tests in the case of the GLMMs. Finally, we generated 95% confidence for the means of statistically significant fixed effects with the effects package (Fox and Weisberg 2019). Means among treatment levels were compared with Tukey's HSD tests with the multcomp package (Hothorn et al. 2008) or type III Wald chi-square tests (Fox and Weisberg 2019).

## Results

### *Three- and two-way interactions between seed density, fungicide, and focal tree type*

Both of our specific hypotheses required a test of the three-way interaction between seed density, fungicide treatment, and type of focal tree, but also tests of the two-way interactions between these treatments on seedling survival. First, we found no statistically significant three-way nor two-way interactions between type of focal tree, fungicide treatment, and seed density on seedling survival (Appendix S1: Table S1). Second, we did not find any statistically significant two-way interactions between these three treatments from either the global model fit or model fitted without the three-way interaction (Appendix S1: Table S1**)**. These results were corroborated with an alternative generalized linear model, with quasibinomial error distribution, analysis of seedling survival based on total seed sown in the field (Appendix S1: Table S3).

### *Density dependence, effects of focal tree, and oomycetes on seedling survival and biomass*

Overall, we did not find evidence for negative density dependence; in fact, in some cases such as under conspecific trees there was evidence for positive density dependence (Fig. 1). Based on graphical assessment, seedling recruitment temporal dynamics followed bell-shaped curves under both conspecific or heterospecific trees in which peak seedling numbers arose ~70 d after seed were sown in the field (Appendix S1: Fig. S2). Under conspecific trees, the fungicide-treated plots tended to have greater numbers of live seedlings at the end of the experiment, ~231 d after seed were sown (Appendix S1: Fig. S2a).

Seedlings growing close to conspecific trees had higher survival than those growing near heterospecific trees (Appendix S1: Table S1; Fig. 2a). We also found overall (i.e., regardless of focal

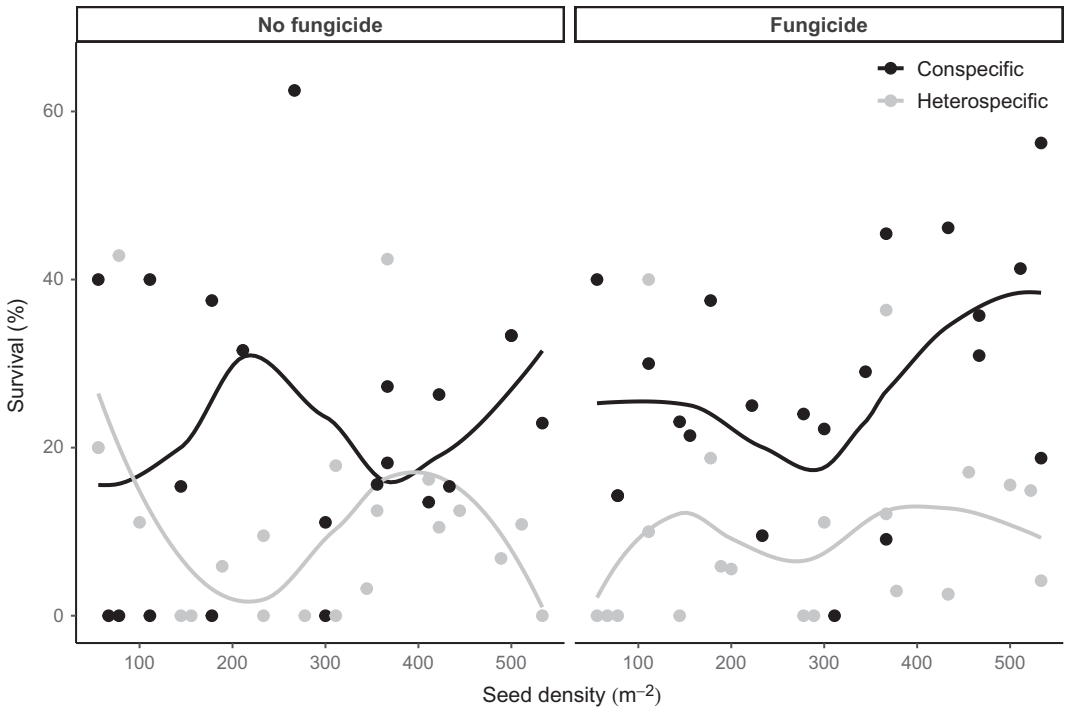



Fig. 1. Relationship between sown seed density and seedling survival of *Banksia attenuata* at the end of the experiment. Shown are the raw data points and the resulting form of density dependence in *B. attenuata* seedlings grown under conspecific or heterospecific (*Eucalyptus marginata*) trees with or without the fungicide treatment. We did not detect any significant three-way or two-way interactions between seed density, focal tree type, and the fungicide treatment (Appendix S1: Table S1).

tree type), a marginally higher survival of *Banksia* seedlings when fungicide was applied to the plots (Appendix S1: Table S1; Fig. 2b). No significant interactions between focal tree type and the fungicide treatment were found for the survival response (Appendix S1: Table S1).

At the end of the experiment, there was greater total biomass of surviving *Banksia* seedlings produced per plot when growing close to conspecific trees compared to heterospecific trees, but only at high seed density (>388 seed $m^{-2}$; Appendix S1: Table S2; Fig. 2c). However, when all seed densities were included in the statistical model, the interaction between focal tree type and fungicide was marginally non-significant ($F = 3.35$, $P$-value = 0.067; Appendix S1: Table S2). When mean seedling biomass per plot was analyzed, there was only a marginally non-significant interaction between focal tree type and the fungicide treatment ($F = 3.25$, $P$-value = 0.071; Appendix S1: Table S2).

## Discussion

### *Density-dependent effects in non-mycorrhizal trees*

In a diverse Mediterranean woodland, we found poor evidence for negative density dependence for a common, widespread non-mycorrhizal tree species. In fact, under conspecific parent trees, our results suggest that there is positive density dependence. Such positive density dependence has been observed in certain plant species (e.g., *Olea europaea* and *Tamarix africana*) in the context of ecological restoration of disturbed Mediterranean sites (Ibáñez and Rodríguez 2020). However, negative density dependence effects are prevalent in species-rich tropical (Harms et al. 2000, Bagchi et al. 2014, Du et al. 2017) and temperate forests (Comita et al. 2014). In our study, *B. attenuata* seedling survival increased with seed density, with marginally lower survival under

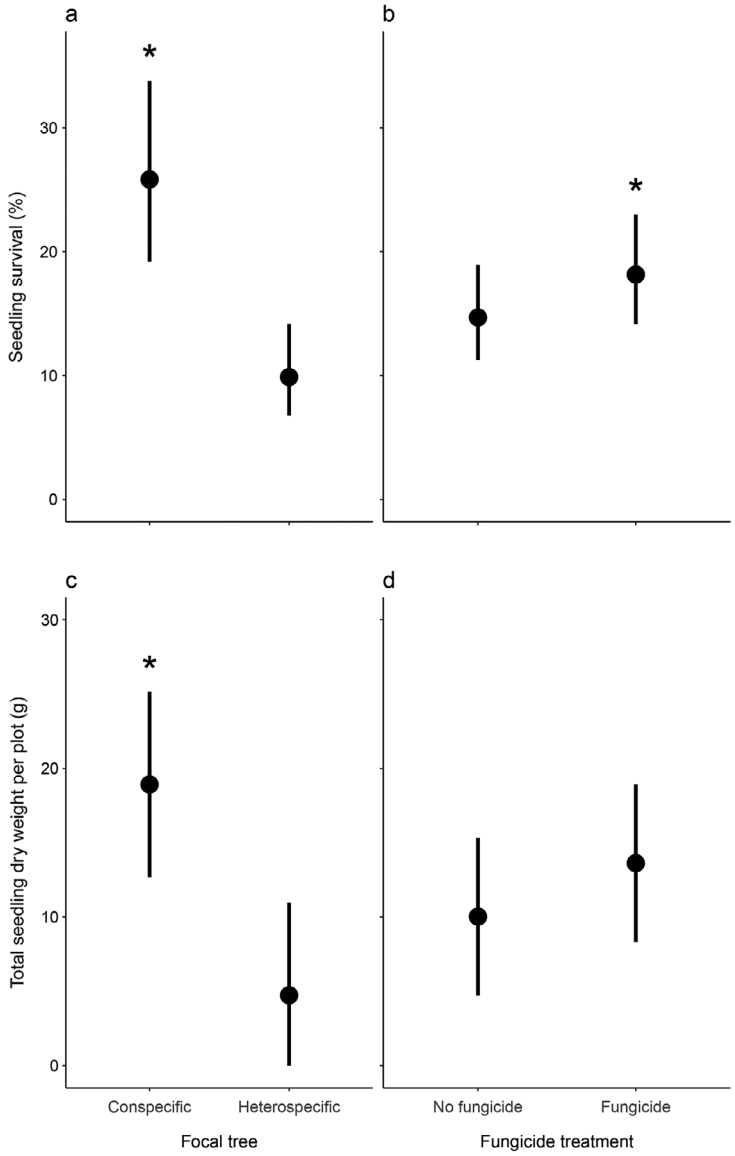



Fig. 2. *Banksia attenuata* seedling survival (a) close to conspecific and heterospecific (*Eucalyptus marginata*) trees and (b) without or with fungicide at the end of the experiment. Values in (a) and (b) are means with 95% confidence intervals derived from a generalized linear mixed effect model (GLMM), and asterisks (*) identify statistically significant differences between means following a type III Wald chi-square test the GLMM model (see Appendix S1: Table S1, S3 for statistical output). We did not detect an interaction between focal tree type and the fungicide treatment ($\chi^2 = 2.19$, $P = 0.138$; Appendix S1: Table S3). Lower panels show *B. attenuata* seedling (c) total dry weight (at the end of the experiment) close to conspecific and heterospecific trees and (d) without or with fungicide at high seed density (>35 seeds per plot, i.e., >388 seed $m^{-2}$). This significant trend was not found at other seed densities (see Appendix S1: Table S2 for statistical output). Overall, the relationship between seedling dry weight per plot and seed density showed a typical intraspecific competition effect on seedling biomass (Appendix S1: Fig. S4). Values in (c) and (d) are means with 95% confidence intervals from a linear mixed effect model (LMM), and asterisks (*) identify statistically significantly different means following a Student's *t*-test (*t*-test = 4.46, df = 13.3, *P*-value <0.001).

heterospecific *Eucalyptus* trees compared to conspecific trees. This trend is contrary to the distance-dependent predictions of the Janzen-Connell hypothesis. In animal-dispersed tree species, where a considerable amount of seed can disperse far away from the parent trees and under high seed densities, positive conspecific density effects have been observed (Sugiyama et al. 2018). They concluded that both directed dispersal and escape hypotheses were operating, and regardless of high densities of conspecific seedlings, favorable conditions under bat feeding roosts and lack of negative density-dependent effects were evolutionary advantageous. Although long-distance seed dispersal can occur in *Banksia attenuata* (He et al. 2009), it is likely more common that seed fall close to conspecific parent trees shortly after fire (Lamont et al. 1993). As such, the lack of evidence for negative density dependence and the higher seedling survival closer to conspecific than heterospecific mature trees that we observed suggest that the Janzen-Connell hypothesis does not play a major role in the maintenance of *Banksia* tree diversity in these Mediterranean woodlands, at least not directly after a fire which is when most seedling recruitment takes place. As suggested by Ibáñez and Rodríguez (2020), we also think that the relatively short time frame of the field experiment was a caveat. As such, longer studies are needed to provide more robust conclusions on density dependence in Mediterranean systems.

### *Distance-dependent effects and role of oomycetes after fire*

Instead of conspecific negative distance dependence, as we hypothesized, we observed conspecific positive distance dependence on *Banksia* seedling survival. We also found that soil-borne oomycetes played a role in reducing *Banksia* seedling survival. The magnitude of the effect was small and was noticed regardless of the distance from conspecific trees. The small magnitude of the oomycete effect may have resulted from low densities of oomycetes in the soil due to a recent fire disturbance. Indeed, much of the native vegetation at the field station had recently been burned one year prior to the start of our field bioassay, since fire in these woodlands is a common disturbance (Keith et al. 2014) and negatively affects soil microbes (Holden et al. 2016), including fungal pathogens (Hart et al. 2005, Dooley and Treseder 2012, Holden and Treseder 2013), due to high soil surface temperatures (Raison et al. 1986). We suggest that shortly after fire, *Banksia* seedlings have a short-lived (i.e., first year after fire) advantage growing under parent trees, since soils under burnt parent trees would likely have lower densities of conspecific (i.e., *Banksia*-specific) soil-borne pathogens (D'Ascoli et al. 2005, Goberna et al. 2012, Holden et al. 2013, Muñoz-Rojas et al. 2016). Therefore, we surmise that time since fire is a potentially important factor that explains the selective pressure to maintain high seedling survival close to conspecific parent trees shortly after fire in Mediterranean woodlands.

### *Factors that dampen the Janzen-Connell effects*

Overall, we did not find consistent patterns predicted by the Janzen-Connell hypothesis, since survival of *Banksia* seedlings was significantly greater under conspecific trees compared to heterospecific trees. Large-seed species are possibly less affected by Janzen-Connell effects, since they may experience less initial seedling mortality (Lebrija-Trejos et al. 2016, Seiwa et al. 2019) and less negative distance-dependent mortality (Marchand et al. 2020). As also suggested by theoretical modeling, common plant species, such as *Banksia*, should be the least strongly affected by Janzen-Connell effects and possibly make plant communities less stable if only conspecific negative density dependence operates (Stump and Comita 2018).

In temperate forests, the Janzen-Connell pattern appears offset by ectomycorrhizal fungal mutualists in *Quercus* tree species (Deniau et al. 2018, Calderon-Sanou et al. 2019) and by arbuscular mycorrhizal fungi in temperate and tropical tree species (Liang et al. 2015, Bachelot et al. 2018, Wulantuya et al. 2020). Ectomycorrhizal host tree species may show positive conspecific density effects on seedlings, due to high abundance of specialist mycorrhizal fungal mutualists close to adult plants, which may also provide root protection against soil-borne pathogens (Laliberté et al. 2015). Since the focal species *Banksia attenuata* used in our experiment is non-mycorrhizal, such positive effects of mycorrhizal fungi should not operate. Instead, we propose that: (1) Micro-environmental conditions are

more favorable under parent *Banksia* trees due to specialized decomposer fungi similar to what has been shown with large-seed *Quercus* species (Deniau et al. 2018) and comparatively greater phosphorus accumulation in litter (Qiu et al. 2012); or (2) nutrient mobilization-based facilitation (Wright et al. 2017, Lambers et al. 2018) to some degree offsets Janzen-Connell effects; or (3) conspecific mother trees possibly host beneficial soil bacteria that induced root growth (Malajczuk and Bowen 1974) or suppress soil pathogens responsible for negative density dependence (Mendes et al. 2011, Liu et al. 2016). More studies are needed in plant communities hosting dominant or widespread non-mycorrhizal species to test some of these particular hypotheses.

We provide evidence that oomycete-seedling mortality may be less important shortly after fire, yet gain relevance as time since fire increases (Holden et al. 2013). This is indirectly supported by a large plant–soil feedback study using non-mycorrhizal *Banksia attenuata* and *menziesii* species; where biotic negative plant–soil feedback was found from field sites that were not as recently burned (Teste et al. 2017). Spatial and temporal variation in virulence of soil-borne pathogens due to environmental conditions or time-dependent accumulation of pathogens (Liu et al. 2015) could explain some of the conflicting results about the importance of the Janzen-Connell effects (Liu and He 2019).

Recent studies have found negative plant–soil feedback in non-mycorrhizal plants such as *Banksia* species (Teste et al. 2017, Shaw 2020), which theoretically should reduce the establishment of conspecific plants under mature *Banksia* trees, such that *Banksia* seedling establishment would be favored away from conspecific trees (Janzen 1970, Connell 1971). However, other studies provide evidence that phylogenetically related neighboring plants can significantly alter Janzen-Connell patterns through their effects on the community of host-specific soil-borne pathogens (Cheng and Yu 2020). Indeed, abundant heterospecific neighboring plants can regulate fungal pathogen community composition, thus "diluting" the relative proportion of conspecific pathogens and reducing infection of seedlings, and ultimately improving survival rates of conspecific seedlings (Cheng and Yu 2020). Such neighbor effects that ultimately reduce the negative effects of conspecific soil-borne pathogens on seedlings are potentially relevant in these diverse Mediterranean woodlands, since it is common to find several plant species growing near or under parent trees. Furthermore, since these neighbor effects are not directly tested in common plant–soil feedback experiments conducted in glasshouses, they could partly explain the inconsistency between this study that of others with *Banksia* (Teste et al. 2017, Shaw 2020). These neighbor effects on the magnitude of oomycete-driven seedling mortality require further testing in species-rich ecosystems.

### *Favorable seedling growth under conspecific trees*

Surviving *Banksia* seedlings had higher total biomass under conspecific trees compared to heterospecific trees, although effect that was significant only at a high initial seed density (>388 seed $m^{-2}$). At these high seed densities, analysis of mean biomass per plot was marginally (but non-significantly) greater under conspecific trees and with the fungicide treatment. Growth of individual seedlings was not followed, as such we cannot determine with accuracy the relative contribution of other factors. However, overall we found evidence that conspecific trees reduced intraspecific competition, possibly because of relatively nutrient-rich *Banksia* litter, that is, richer than *Eucalyptus* litter (Qiu et al. 2012), or by nutrient mobilization facilitation (Lambers et al. 2018, Teste et al. 2020).

Identifying specific mechanisms explaining the greater seedling survival and total biomass under conspecific trees remains a challenge (Germany et al. 2019, Liu and He 2019, Ke and Wan 2020). In our study, we suggest that non-mycorrhizal *Banksia* seedlings: (1) benefited from more nutrient-rich litter under conspecific trees compared to heterospecific trees (particularly relevant shortly after fire); or (2) experienced some nutrient-based facilitation under mature conspecific *Banksia* trees; or (3) were more severely affected by specific soil-borne pathogens associated with the heterospecific *Eucalyptus* trees; or (4) were more negatively affected by generalist soil fungal pathogens found away from conspecific trees and capable of reducing seed viability and germination, similarly to effects seen in other species-rich forests (Sarmiento et al. 2017); or (5) a combination of (1) to (4). There is a need for

more field studies to test these hypotheses simultaneously using more comprehensive experimental approaches (Ke and Wan 2020).

## Conclusion

In this study, we found greater seedling survival under conspecific trees compared to under heterospecific trees, contrary to our hypothesis and that predicted by the Janzen-Connell hypothesis. A modest oomycete-driven mortality in *Banksia* seedlings was also found, and we propose possible mechanisms involving other soil biota such as beneficial bacteria and organic matter fungal decomposers. The strength of negative density dependence has been shown to increase with resource availability (LaManna et al. 2016), as such it is possible that the weak evidence for negative density dependence effects in *Banksia* may reflect the very low nutrient availability in the soils of this ecosystem (Turner and Laliberté 2015) and the comparatively more nutrient-enriched litter, particularly richer in phosphorus, found under conspecific *Banksia* trees compared to the heterospecific *Eucalyptus* (Qiu et al. 2012). The role of soil-borne pathogens in driving conspecific negative distance and density-dependent seedling mortality has been highlighted in several field studies (Comita et al. 2014), yet, if both pathogens and mutualists, that affect niche differences, are tested (i.e., controlled for), it is expected that a more general theory on coexistence will result (Bever et al. 2010, Bachelot et al. 2015, Ke and Wan 2020). We expand on this concept and suggest that greater generality and robustness will be gained if plant coexistence theory and future field experiments can include all major soil biota groups (i.e., pathogens, mutualists, decomposers). Seedling recruitment patterns in these diverse Mediterranean ecosystems do not appear to follow predictions from the Janzen-Connell hypothesis, at least not shortly after fire and for the common species studied here. We propose that a more complex and context-dependent hypothesis incorporating time since fire, role of facilitation by mature conspecifics, and key plant traits (e.g., nutrient acquisition strategies; Teste et al. 2017, seed nutrient reserves) may prove to better predict the interacting drivers responsible for the maintenance of diverse plant communities in Mediterranean ecosystems.

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## Data Availability Statement

Data are available from Zenodo: https://doi.org/10.5281/zenodo.5567037

## Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3821/full