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Can invading *Pinus* species facilitate congeneric invasion in a mountain grassland?

M. Florencia Spalazzi[a,*], Tomás Milani[a], Jason D. Hoeksema[b], Martin A. Nuñez[c,d], François P. Teste[a,e]

[a] Grupo de Estudios Ambientales – IMASL, Universidad Nacional de San Luis & CONICET, San Luis, Argentina
[b] Department of Biology, University of Mississippi, University, MS 38677, USA
[c] Department of Biology and Biochemistry, University of Houston, Houston, TX, USA
[d] Grupo Ecología de Invasiones, Instituto de Investigaciones en Biodiversidad y Medio Ambiente, CONICET – Universidad Nacional del Comahue, Bariloche, Argentina
[e] Swift Current Research and Development Centre, Agriculture and Agri-Food Canada. Government of Canada, Canada

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ABSTRACT

In the introduced range, the successful establishment of an invasive species may be influenced by positive plant-plant interactions. Pre-existing vegetation, particularly conspecific mature trees, can shape the availability and attributes of microsites, thus potentially creating favorable conditions for the establishment of conspecific seedlings through facilitation. Pines are widely introduced in the Southern Hemisphere primarily as forestry plantations; these pines can become invasive, causing detrimental effects on local ecosystems and economies. In the high-elevation grasslands of the *Sierras de Córdoba*, Central Argentina, pines have begun to invade the native grassland as a result of improper (or lack of) management of pine plantations. During early pine invasion in this semi-arid grassland, we aimed to quantify the influence of adult live pines and on congeneric pine seedling recruitment and survival. For this, 48,000 seeds of *Pinus elliottii and P. taeda* were sown in three consecutive field trials, under different tree status treatments: live pines, dead pines, and no pines (i.e., open grassland). Seed were sown with and without irrigation and seeded microsites were oriented to the north and south of the live and dead trees. We also considered the hillslope aspect where the sites were located. Our results show that pine seedling recruitment was 57 % higher under live pines compared to dead pines and no pines treatment, but only in the trials that were not irrigated. Microsites south of the live pine trees, more shaded from direct sunlight in the Southern hemisphere, presented 36 % more pine seed germination than those to the north. In terms of topography, hillslope aspects with lower solar incidence (wetter hillslopes) also showed higher pine seedling recruitment. Our results suggest that moisture availability is a dominant factor driving further invasion, and that adult pines may be facilitating the invasion process by creating moister microsites for germination and pine seedling establishment. Thus, the early removal of adult pines is important to consider in the management of pine invasions. They do not only serve as a source of propagules, but also positively affect the establishment of their congeneric seedlings.

1. Introduction

Introduced non-native trees must overcome environmental barriers that may limit successful establishment within their new range (Lonsdale, 1999; Carrillo-Gavilán et al., 2012). The relative importance of these barriers may vary throughout the tree's life cycle (Carrillo-Gavilán et al., 2012). Germination will depend on the conditions of the microsite where seeds fall, such as light availability and soil moisture (Fenner and Thompson, 2005). Seedling survival and growth will be affected by additional factors such as nutrient availability (Scholes and Archer, 1997), access to mutualistic organisms (Dickie et al., 2017), herbivory (Higgins et al., 2000), and facilitative effects (Gómez-Aparicio et al., 2004; Brooker et al., 2008).

Interactions between plants can play an important role in the structure of plant communities (Callaway and Walker, 1997). Plant interactions are either negative (e.g., competition) or positive (e.g.,

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* Corresponding author.
E-mail addresses: florenciaspalazzi@gmail.com (M.F. Spalazzi), milanitomas@gmail.com (T. Milani), hoeksema@olemiss.edu (J.D. Hoeksema), nunezm@gmail.com (M.A. Nuñez), francois.teste@agr.gc.ca (F.P. Teste).

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mutualism, facilitation) and can be subject to life cycle stages, as well as environmental conditions (Callaway and Walker, 1997; Castro et al., 2002; Schiffers and Tielbörger, 2006). Although in moderately to highly productive environments, where resources are relatively abundant, the community structure mostly results from negative interactions (Bertness and Callaway, 1994; Castro et al., 2002), in stressful environments such as those with marked drought and extreme temperatures, positive interactions tend to prevail (Callaway et al., 2002; He et al., 2013). In these arid environments, seedling recruitment and establishment will depend on the quantity and quality of favorable microsites (Bertness and Callaway, 1994; Legras et al., 2010). Occasionally, these microsites are created by pre-existing vegetation that serves as a 'nurse plant', for example, by maintaining suitable soil moisture conditions (Castro et al., 2002; Pueyo et al., 2016; Nuñez et al., 2009), providing water via hydraulic redistribution (Prieto et al., 2016), buffering seedlings from high temperatures (Callaway, 2007), or protecting them from herbivory (Gómez-Aparicio et al., 2004). These positive interactions are known as facilitation and can have a great influence on the invasion dynamics of plant species (Callaway, 1995; Brooker et al., 2008; Cavieres, 2021).

Specific mechanisms by which plant-plant facilitation occurs can be direct, when the presence of a species affects another species, or indirect, when they involve an intermediate species of the same or different kingdoms (Callaway, 2007). Many direct facilitation effects are related to the shading provided by trees, especially in limited water environments (Callaway, 2007). By dampening incoming radiation, tree shade allows for higher soil moisture and lower rates of evapotranspiration, improving the water status of the establishing plants (Castro et al., 2002; Simon et al., 2019). As for indirect facilitation effects, most studies within the *Pinus* genus involve interaction with ectomycorrhizal fungi (Nuñez et al., 2009; Dickie et al., 2017). The presence of conspecific trees influences seedling infection by ectomycorrhizal fungi and may positively affect their performance (Booth, 2004; McGuire, 2007; Teste and Simard, 2008; Liang et al., 2020), although the mechanism of such positive effects has proven difficult to confirm experimentally (Karst et al., 2023). Common mycorrhizal networks (CMNs) shared between plant individuals may be involved in some examples of plant-plant facilitation, but alleged CMN effects are just as often negative or neutral (e.g., Dehlin et al., 2008; Van Der Heijden and Horton, 2009; Brearley et al., 2016; Pec et al., 2020), and are commonly offset by direct effects of neighboring roots, which also range from positive to negative (e.g., Teste et al., 2009; Booth and Hoeksema, 2010).

In mountain ecosystems, a determining factor in microclimatic variation is the aspect, which is the direction a slope is facing (Ferraz et al., 2009). The differences between hillslope aspects are due to incoming solar radiation, which modifies the local water balance (Wang et al., 2011) and, therefore, affects vegetation dynamics (Badano et al., 2005). In the Southern Hemisphere, south-facing hillslopes receive less direct radiation than north-facing slopes. Due to the lower solar radiation, evapotranspiration rates on south-facing slopes are also lower, which favors higher soil moisture retention (Eisenlohr et al., 2013). Therefore, topography could be a relevant factor during the early establishment of invasive pine seedlings, driving variable germination and survival rates among different hillslope aspects.

In some regions of central Argentina mountains, introduced pines have succeeded in escaping from forest plantations. Between 1956 and 1990, more than 30,000 ha of *Pinus elliottii* Engelm. and *P. taeda* L. were planted through a government forestry program. After 1976, forestry activity decreased markedly and most of the plantations were abandoned (Izurieta et al., 1993). As a result of decades without forest management, these exotic pines have started invading the adjacent native grassland. A recent study in the area reported a singular invasion pattern characterized by low-density and widespread trees, leading to a stealthy, yet constant invasion process (Milani et al., 2020). Invasive pines are known to cause major ecological consequences (Richardson et al., 2014; Gioria et al., 2023) and economic losses (Diagne et al., 2021; Fernandez et al., 2023). Ecological impacts are often reported as increases in the intensity and severity of the fire regime (Cóbar-Carranza et al., 2014), loss of native biodiversity (Veldman et al., 2015), and unfavorable shifts in hydrological flow cycles (Jobbágy et al., 2013).

Understanding the major environmental constraints to invasive seedling recruitment is critical to developing targeted management strategies to prevent the spread of invasion in mountain grasslands, as well as identifying key stages on which to focus control efforts. As such, we aimed to better understand the influence of adult pine trees on the dynamics of early establishment of congeneric *Pinus spp.* seedlings in a mountain grassland ecosystem. Specifically, we quantified the effect of adult live pines, cardinal orientation with respect to live pines, and hillslope aspect orientation on seedling recruitment. Considering that our study area has a history of marked drought, and our study occurred during a drought period, we hypothesized that seedling recruitment is mainly restricted to sites with higher soil moisture, potentially due to the shading effect provided by conspecific trees and orientation relatively to the adult tree and/or the hillslope aspect. Consequently, we expected to find greater seedling recruitment under live adult pines than under dead pines or open grassland, when water limitation was not alleviated by irrigation. We also expected to find higher seedling recruitment on south-facing hillslopes and southern orientation relative to the focal trees, where, due to lower incoming solar radiation, the water balance favors soil moisture retention, generating more suitable conditions for seedling establishment.

2. Material and methods

2.1. Study site

The study was carried out in the *Sierras de Córdoba* (32°03'48.7"S 64°46'53.9"W), Córdoba province, central Argentina (Fig. 1a). The area is located among three mountain ranges running from north to south. Precipitation follows a monsoonal regime with an annual mean of 850 mm concentrated in the warm season (October to April). Above 1100 m elevation, mean annual temperature is 10 °C. The area is a large pre-Devonian metamorphic basement that appears as a mixture of homogeneous reliefs and rock outcrops (Bonalumi et al., 1999). Soils are typically shallow, sandy loam texture with an abundance of gravel (Farley et al., 2008; IDECOR). Characteristic vegetation physiognomy is mountain grasslands, co-dominated by species of *Stipa* and *Festuca* (Luti et al., 1979). Fire and grazing are the main disturbances that structure the native plant communities (Cabido et al., 1997; Cingolani et al., 2013; Argañaraz et al., 2015). Although grazing is known to be a driver of pine invasion by controlling grass competition (Boulant et al., 2008), the stocking rate in this system is relatively low and likely does not have a significant influence on pine establishment.

2.2. Study species

Pinus elliottii (Slash pine) and *P. taeda* (Loblolly pine) are both native to southeastern United States of America. They are extensively planted and grown around the world for forestry and ornamental purposes (Simberloff et al., 2010; Nuñez et al., 2017). Since these two pine species are phylogenetically close (Zeb et al., 2020) and ecologically similar (Proctor and Monroe, 2016), they often form mixed stands that lead to hybridization in the exotic range (Burns and Honkala, 1990). Within their native range, the climate is typically humid and warm (annual mean ~ 17°C) with average rainfall of 1270 mm mostly concentrated in summer (Burns and Honkala, 1990). In the Southern Hemisphere, pine seed fall peaks in April (Austral autumn) with large seed crops produced every three years (Bechara et al., 2013). Seeds are wind-dispersed, reaching maximum distances of about 91 m from parent trees for *P. taeda*, and 46 m for *P. elliottii* (Burns and Honkala, 1990). These two pine species are not serotinous, meaning that fire and high temperature are not needed for adequate seed dispersal from the pine cones of these two pine species. However, both species exhibit traits that confer fire

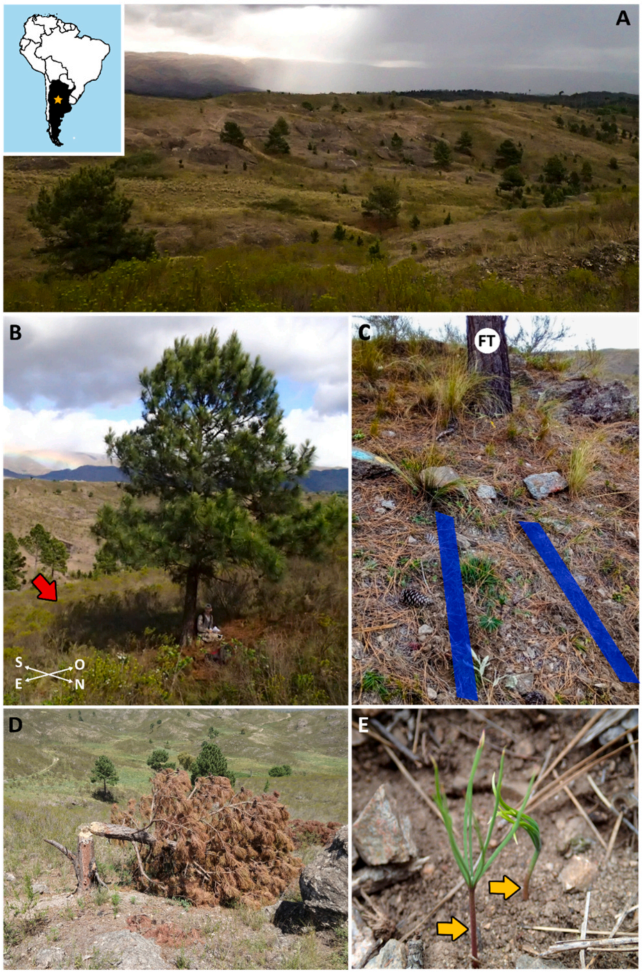

Fig. 1. **A.** Mountain grassland with invasive *P. elliottii* and *P. taeda* in *Sierras de Córdoba*, central Argentina (yellow star). **B.** Photo of 'live pines' treatment with the crown projected on the surface in south direction (red arrow). **C.** *Pinus elliottii* individual from "live pine" treatment with transects highlighted by solid blue lines (taken in May 2021); FT: focal tree. **D.** *Pinus elliottii* individual from "dead pine" treatment. **E.** *Pinus elliottii* seedlings growing under a live pine (yellow arrows; taken in November 2019).

tolerance, such as lower needle density and thicker bark when they reach adulthood (Pile et al., 2017). Pine plantation in the area contains both species (Dorado et al., 1997), with *P. elliottii* being the most abundant (Simberloff et al., 2010; Giorgis and Tecco, 2014).

2.3. Pine selection and treatments

Invading pine trees were selected within an area with low invasion density and similar topographic conditions (Fig. S1 – Supplementary material 1). All selected pines (hereafter, focal trees) were sexually mature, with a diameter at breast height ≥ 25 cm and spatially separated from each other across the landscape by at least four times the focal tree height (Table S1 – Supplementary material 1). This spatial separation criterion was used to consider the focal trees as independent units so that the likelihood of root or extraradical ectomycorrhizal fungi overlap between neighboring focal trees would be extremely low. Each focal tree (30 in total) was randomly assigned to one of three treatments to assess adult pine influence: (a) tree left intact (i.e., live pine) (Fig. 1b), (b) felled trees (i.e., dead pine) (Fig. 1d) and (c) felled trees after sowing seed (i.e., interrupted (see below)); the field trials also had a fourth treatment (d) no pines (i.e., open grassland), which involved sowing seed around native grassland vegetation without any nearby invading pine trees (same spatial separation criterion as above). For the "dead pine" treatment, trees were mechanically killed eight months before seeding (February 2019) to allow the site to recover from bark removal disturbance. In the "interrupted" treatment, it was planned to kill the trees between 4 and 12 weeks after seedling emergence. However, seedling mortality was higher and earlier than expected, so we were not able to apply this treatment. As such, we kept three treatments, the "interrupted" treatment was considered as "live pines" with an n = 20, while "dead pines" and "no pines" had n = 10 each. It is worth noting that the "interrupted" treatment, as originally proposed, failed consecutively in all experiments. Therefore, the doubling of replicates with "live pines" is common to all three field trials (see below).

The treatments were originally designed to test the role of mutualistic ectomycorrhizal interactions in pine seedling establishment. Due to high seedling mortality on the first attempt, we replanted pine seed twice (see Section 2.4), but similar mortality patterns prevented us from quantifying fungal facilitation. However, this experimental design provided insights into more general facilitation effects and the abiotic factors likely responsible for subsequent pine invasion in this system (Milani et al., 2022).

2.4. Seeds sown and seedling survival surveys

Field Trial 1. Between 9 and 10 of October 2019 (Austral spring), a total of 5400 *P. taeda* and *P. elliottii* seeds were sown in the field. Sowing consisted of burying 10 cold-stratified pine seeds (see Table S2 – Supplementary material 1) in microplots of 10 cm diameter and 0.5 cm depth haphazardly located around each focal tree (Fig. 2a), or in the "no pines" treatment, arranged in a row. In the case of "live pines" and "dead pines", treatments microplots were established at two distances: four at the drip line of the focal tree and four at twice the distance between the

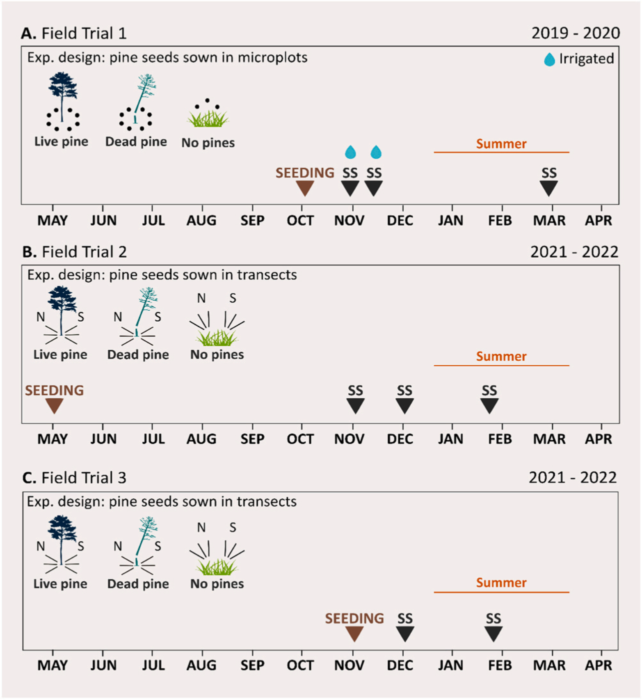

Fig. 2. Diagram of experimental design, seeding, and data collection. **A.** Field Trial 1 was carried out in 2019 – 2020, and pine seeds were sown in October 2019 and irrigated. Surveys of surviving seedlings (SS) were conducted at 25, 39, and 146 days post-seeding; **B.** Field Trial 2 was carried out in 2021 – 2022, and pine seeds were sown in May 2021. Surveys of surviving seedlings (SS) were conducted at 183, 224, and 268 days post-seeding; **C.** Field Trial 3 was carried out in 2021 – 2022, and pine seeds were sown in November 2021. Surveys of surviving seedlings at 41 and 85 days post-seeding.

drip line and the focal tree's trunk. In the case of the "no pines" treatment, the microplots were established at a distance of 2 m from each other. Each focal tree had eight microplots per planted pine species, and there were three microplots per planted pine species in each replicate of the "no pines" treatment. Ten replicates per treatment were established resulting in 510 microplots. For the "dead pine" treatment, we made sure to choose microsites in such a way that the dead crown of the felled trees would not shade or interfere in any way with seed germination or seedling establishment processes. Seeds were irrigated with 100 cm^3 of distilled water at three dates (at day of sowing, as well as 25 and 39 days after sowing). The aim of this irrigation was to help seedling germination and early establishment at the beginning of Field Trial 1, but not to ensure water availability over the entire trial period.

Field Trial 2. This second field trial was conducted only with *P. elliottii,* since it is the most abundant species, and since we did not detect any differences in survival between the two pine species in Field Trial 1 (data not shown). As such, between 7 and 10 of May 2021 (Austral autumn), a total of 24,000 *P. elliottii* seeds were sown using the same focal trees and treatments as in Field Trial 1 ("live pines" with n = 20, "dead pines" with n = 10, "no pines" with n = 10.). Seeds were not cold-stratified in the laboratory since natural *in situ* cold stratification could occur in the field due to the time of sowing in this trial compared to Field Trial 1 and 3. Sowing was carried out in six 2 m transects per focal tree (Figs. 1c and 2b), in order to place a greater number of seeds. Because the intensity of solar radiation varies with cardinal orientation (Fig. 1b), the transects were strategically oriented around each focal tree. In the Southern Hemisphere, shade is cast to the south, so seedlings growing south of the focal tree receive more shade per day than seedlings growing north. To control the shading effect, we oriented three transects in a southerly direction and three in a northerly direction, spaced at least 1.5 m apart from each other (Fig. 2b). In the no pines treatment (i.e., no focal tree) the transects were oriented in the same way, three to the north and three to the south. Each of the six transects were planted with 100 seeds and again had 10 replicate focal trees in the "dead pines" and "no pines" treatments and 20 replicate focal trees in "live pines", giving a total of 240 transects. In this second field trial, seeds were not irrigated.

Field Trial 3. Between November 4 and 8, 2021 (Austral spring) another set of 24,000 *P. elliottii* cold-stratified seeds were sown in six 2 m transects and again with the same focal trees and treatments as Field Trial 1 and 2. We oriented the six transects per replicate as in Field Trial 2 (three to the north and three to the south – Fig. 2c), and each transect was sown with 100 seeds. As in the previous trial, we had 10 replicate focal trees in "dead pines" and "no pines" treatment and 20 replicate focal trees in "live pines", giving a total of 240 transects (Fig. 2c).

2.5. Climatic and soil moisture data

To better understand the climatic context in which the experiments took place, potential evapotranspiration (PET) and rainfall data were collected from nearby weather stations (*Cuenca El Durazno, San Miguel de los Ríos* and *Río Santa Rosa;* https://new.omixom.com). The stations collected daily data that we averaged to obtain the monthly means (Fig. 3). Monthly temperature was also calculated but showed no relevant patterns (Fig. S2; Table S3 – Supplementary material 1). In February 2024, volumetric soil water content data were collected with the aid of a handheld TDR sensor (Theta Probe; Delta-T Devices, Cambridge, UK) at three random points north and south of the live trees, dead trees, and in the no pines treatment (the exact same field sites mentioned above). In total, 120 points were collected. In addition, we took a soil sample north and south of 10 live pines, five dead pines, and five in the no pines treatment, for a total of 40 soil samples. The samples were taken to the laboratory to process and calculate gravimetric soil water content (Fig. S3; Table S6 – Supplementary material 1).

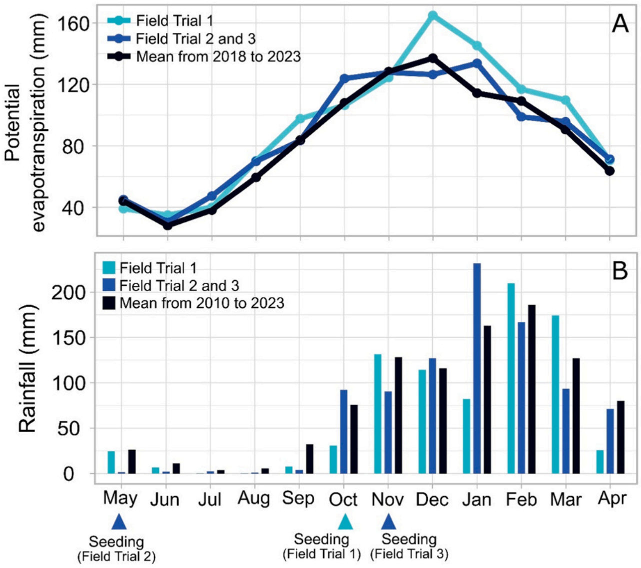

Fig. 3. Climate data for study periods. **A.** Monthly potential evapotranspiration for 2019 – 2020 (Field Trial 1); for 2021 – 2022 (Field Trials 2 and 3); and mean evapotranspiration from 2018 to 2023; details in Table S4 – Supplementary Material 1. **B.** Monthly rainfall for 2019 – 2020 (Field Trial 1); for 2021 – 2022 (Field Trial 2 and 3); and mean monthly rainfall from 2010 to 2023; details in Table S5 – Supplementary Material 1.

2.6. Data analysis

To understand the dynamics of seedling recruitment (Fig. 1e), we analyzed differences in the proportion of microsites with emerging seedlings. The proportion of microsites with seedlings arises from the ratio between the number of microsites that had at least one emergent seedling over the total number of microsites per treatment. Sown microplots and sown transects will be referred to as "microsites" hereafter. Differences were analyzed by fitting a Generalized Linear Mixed Model (GLMM, Zuur et al., 2009) with a binomial error distribution for each Field Trial. We considered tree treatment (live pines, dead pines, and no pines) as a fixed factor, focal tree as a random factor, and hillslope aspect index as a fixed covariate. Hillslope aspect index was calculated as cos (θ – 315°), where θ is the aspect of the site in degrees (Morales et al., 2015), and takes values between 1 and −1. Thus, sites facing NW (i.e., drier conditions for the southern hemisphere) take values of 1 and sites facing SE (i.e., wetter conditions in the southern hemisphere) take values of −1.

Subsequently, we analyzed the differences in the proportion of microsites with seedlings considering the orientation of the microsite (north and south) with respect to the focal tree. In this analysis, only data from Field Trial 2 and 3 were considered since they were the only ones that contained the variable "microsite orientation". For this purpose, we used GLMM with a binomial error distribution considering tree treatment, microsite orientation (north and south), and seeding date (May and November, Fig. 2b and c) as fixed factors, focal tree as a random factor, and hillslope aspect as a fixed covariate. During the seedling survival surveys, we recorded the distance to the focal tree trunk to analyze the influence of this variable (Fig. S2. Supplementary Material 2). Climate data (precipitation, temperature and evapotranspiration) as well as soil moisture data were not included in the statistical analyses. Model selection was based on the Akaike's information criterion (AIC; (Akaike, 1998). Significance of explanatory variables was determined by an analysis of deviance using a Chi squared test for unbalanced data (Type III Sums of Squares, (Fox and Weisberg, 2019) and pairwise post hoc multiple comparison using Tukey's HSD test. Data were analyzed using the R statistical computing and graphics software v. 4. 3. 0 (R (Core Team, 2023). We used package *lme4* (Bates et al., 2015) to fit GLMMs and used the *Dharma* package (Hartig, 2020) to test overdispersion in model residuals. Deviance Analysis test was carried out with *car* package (Fox and Weisberg, 2019), and graphing with *ggplot2* package (Wickham, 2016).

3. Results

The number of seedlings was substantially higher under live pines than near dead pines or with no pines (Fig. 4a; Table S1 – Supplementary material 2) for all field trials at every survey date. The same trend was observed with proportion of surviving seedlings (Fig. 4b), where the highest proportion of live seedlings (calculated as the number of seedlings over the number of seeds sown) was found under live trees.

In Field Trial 2 and 3, with absence of irrigation, a greater proportion of microsites with seedlings was found under live pine trees compared to either dead pines or no pines treatments (Fig. 5). But in Field Trial 1, where microsites were irrigated, there was no difference in the proportion of microsites with seedlings among the three treatments (Fig. 5, Table S2 – Supplementary material 2).

Regarding microsite orientation, the proportion of microsites with seedlings was higher in the south-facing sides of the live pine treatment only in Field Trial 2 (Fig. 6, Table S3 – Supplementary material 2) and not in Field Trial 3. Overall, the hillslope aspect index also showed a significant effect on seedling recruitment (Fig. S1 – Supplementary material 2). Wetter hillslopes (values close to −1) had slightly higher seedling recruitment than drier hillslopes (values close to 1). This pattern was similar across all irrigation and tree treatments (Fig. 7).

4. Discussion

Our results show that after three consecutive trials, seedling recruitment was higher under congeneric live trees than under dead trees or in the open grassland, an effect that was not observed when water limitation was reduced by irrigation. The same trend was seen in microsites south of the live trees (i.e., more hours of shade per day) and on south-facing slopes (i.e., wetter slopes). In combination, our results suggest that the establishment of invasive seedlings is influenced by potentially favorable soil moisture conditions and that adult pines could facilitate this process by creating water-favorable microsites for germination and early seedling development.

4.1. Seedling recruitment

At the microsite scale, in the absence of irrigation, live pines had a higher number of seedlings and a higher proportion of microsites with seedlings than the other treatments. This result indicates that live pines play a fundamental role in the early stages of the life cycle of invasive seedlings. The fact that these differences were not evident in Field Trial 1 (i.e., the irrigated trial) suggests that water is an important factor that operates in this ecosystem and that live pines may be creating microsites where water balance for seedlings is less severe. To further explore this idea, we observed that there was a greater proportion of microsites with seedlings when they were oriented to the south of the living pine trees. South microsites in the Southern Hemisphere receive more shade per day and therefore less direct solar radiation, possibly allowing lower water demand than the northern microsites (Castro et al., 2002). Ultimately, this series of findings validates our hypothesis that mature pine trees partly mitigate the harsh arid conditions inherent in this ecosystem and create favorable micro environments for the germination and recruitment of invasive seedlings. Hillslope aspect also showed a strong effect on pine seedling recruitment. Hillslopes with lower solar incidence (south-facing slopes) showed a higher proportion of microsites with seedlings, supporting the idea that southern hillslopes tend to be more suitable regarding soil moisture for germination. This finding is consistent with the current spatial invasion pattern at the site, indicating a preference for locating more established pines on south-facing slopes as observed in our satellite images.

Interestingly, the proportion of microsites with seedlings to the north of the live trees was highly variable. This variation could be due to sporadic instances of fungal mediated facilitation by adult pine trees,

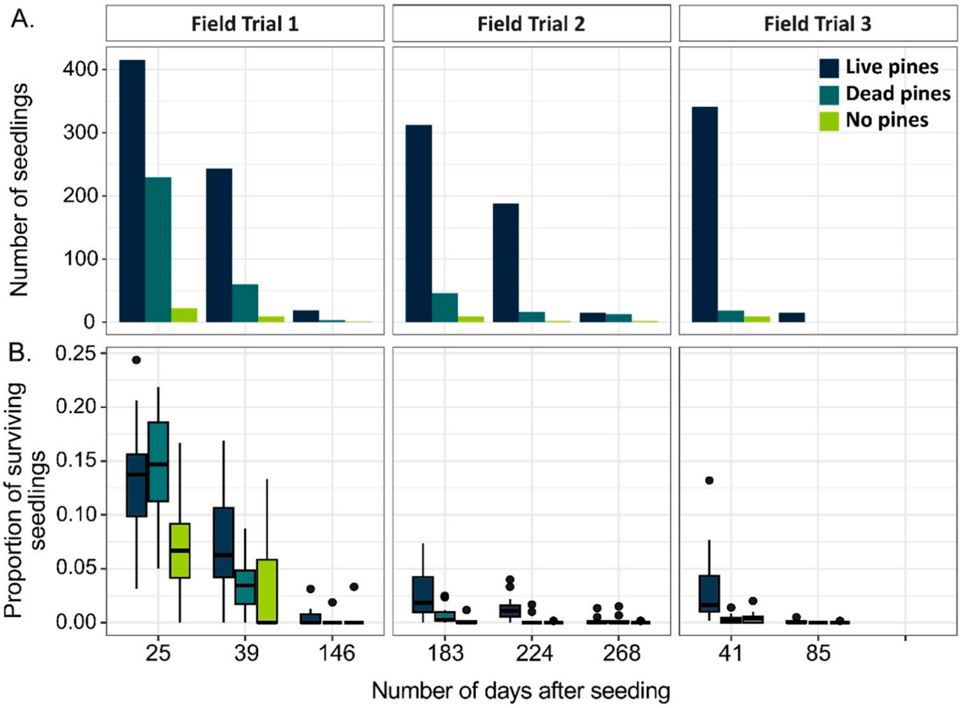

Fig. 4. *Pinus elliottii* seedling recruitment under live pines, dead pines, and no pines treatment for Field Trial 1, 2 and 3. The seeding dates for each Field Trial are detailed in the diagram in Fig. 1. **A.** Total number of observed emerged seedlings across all the treatments at every survey post seeding. **B.** Surviving seedlings calculated as the proportion between living seedlings and number of sown seeds.

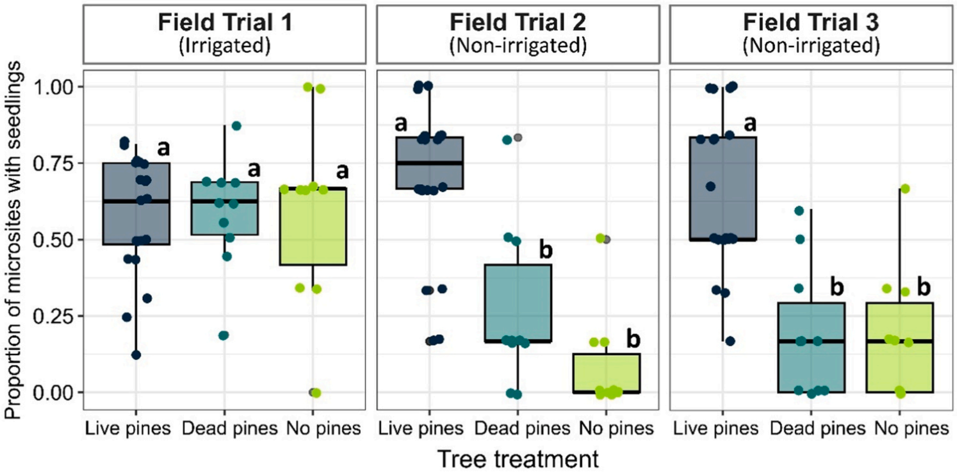

Fig. 5. Proportion of microsites with seedlings under live pines, dead pines, and no pines treatments for Field Trial 1, 2 and 3. Boxplots are shown with different letters to indicate significant differences between treatments (Field Trial 1: $P = 0.91$; $\chi^2 = 0.17$; Field Trial 2: $P < 0.001$; $\chi^2 = 34.13$; Field Trial 3: $P < 0.001$; $\chi^2 = 30.81$).

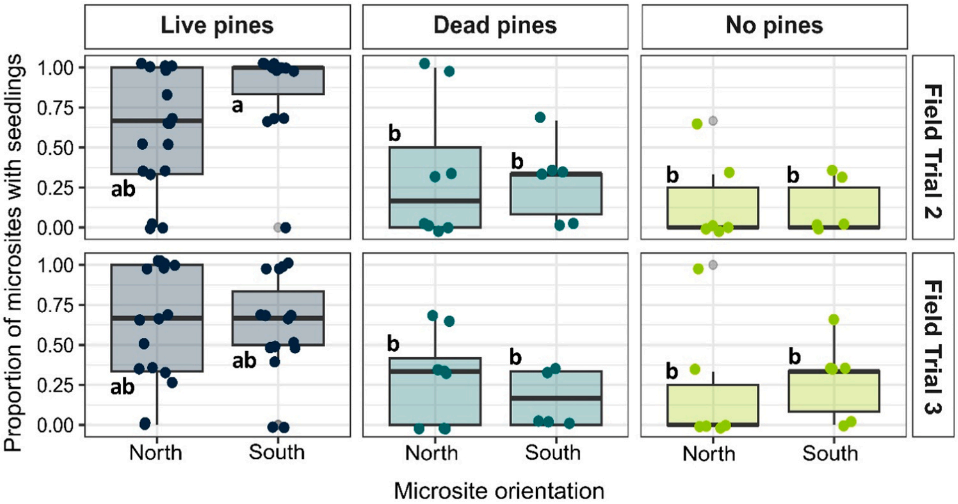

Fig. 6. Seedling recruitment under tree treatments considering microsite orientation (transects) for Field Trials 2 and 3. Boxplots are shown with different letters to indicate significant differences between treatments ($P < 0.05$; $\chi^2 = 6.26$).

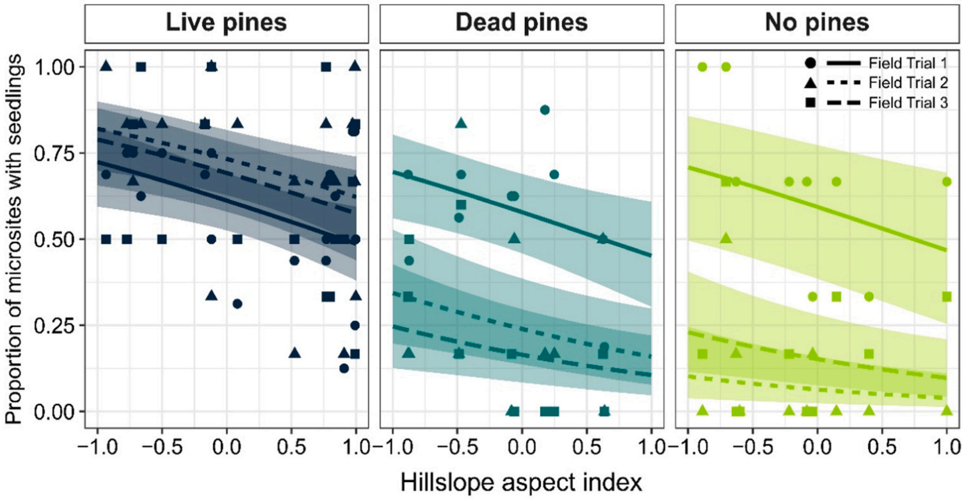

Fig. 7. Microsites with seedlings considering hillslope aspect index as covariate for tree treatment in Field Trial 1 (circles and solid lines; $P < 0.05$; $\chi^2 = 4.88$), Field Trial 2 (triangles and dotted lines; $P < 0.05$; $\chi^2 = 3.87$), and Field Trial 3 (squares and dashed lines; $P = 0.07$; $\chi^2 = 3.21$).

which benefit their congeneric seedlings during stressful conditions. The roots of mature trees may act as a source of ectomycorrhizal fungal mycelium that can colonize the roots of seedlings and improve their performance in the early stages of life, or may reduce water limitation through hydraulic lift and redistribution through common mycorrhizal networks (Egerton-Warburton et al., 2007; Teste et al., 2009; Booth and Hoeksema, 2010). Nevertheless, both soil moisture retention due to shading of adult trees and the role of ectomycorrhizal fungal mycelium in hydraulic redistribution are mechanisms that need to be further addressed with field experiments that can simultaneously test the occurrence of these phenomena (Karst et al., 2023).

4.2. *Plant-plant interactions*

The balance between negative (competition) to positive (facilitation) plant-plant interactions is expected to change over time during development or succession (Callaway and Walker, 1997; Bullock, 2009; Langdon et al., 2019). Several studies in arid environments have demonstrated facilitative effects on establishing vegetation during germination and early establishment that then shift to negative interactions (i.e., competition) later in the life cycle (Miriti, 2006; Valiente-Banuet and Verdú, 2008; Bullock, 2009). *Pinus elliottii* is considered a shade-intolerant species, so it naturally regenerates in small openings (Bullock, 2009). After germination, the availability of light or even water and nutrients could modify the balance between facilitation and competition of adult pines with their congeneric seedlings and this might be the cause of the high mortality of seedlings. This means that the results presented here do not necessarily indicate that the positive effects observed will persist; more data on long-term interactions between nurse plants and conspecific seedlings are needed.

New invading pine seedlings in our study site also interact with the species of the native grassland, yet the biotic resistance of this ecosystem to woody invasions is thought to be weak (Rundel et al., 2014). In grassland systems, above- and belowground competition is also a fundamental interaction determining successful woody invasion (Richardson and Bond, 1991). Coomes and Grubb (Coomes and Grubb, 2000) suggest that the response of seedlings to root competition will depend on which resources are in scarce supply, and on the inherent responsiveness of each species. When it comes to water, grasses often exert strong competition over pine seedlings (Richardson and Bond, 1991). Others have found that within its native range, competition with the understory significantly reduces the growth and survival of *P. elliottii*. (Burns and Honkala, 1990; Dickens et al., 2004). This observation is relevant since in all of our experiments, the no pines treatment (i.e., sites in open grassland) had the lowest seedling recruitment and survival, except in Field Trial 1 when they were irrigated. This pattern may be due to biotic resistance exerted by the native plant community that limits seedling establishment.

4.3. *Management and implications*

This particular pine invasion is in its early stages (Milani et al., 2020), and thus at an ideal stage for control measures to be effective (Nuñez et al., 2017). Although incipient, the evidence suggests that this invasion is accelerating with periods of more establishment, possibly related to variables such as precipitation and grazing pressure (Boulant et al., 2008; Nuñez and Paritsis, 2018). However, underlying processes operating at smaller spatiotemporal scales, such as the availability of favorable microsites generated by conspecific adults, might be the basis for a slow but steady establishment. Although *Pinus* establishment seems to require the facilitating effect of shelter trees in this semi-arid system, it may be crucial to analyze inter-annual variation in long-term recruitment to perceive the importance of water resources on population dynamics.

Isolated reproductive pines are an important source of propagules, but also play an important role in mediating seedling establishment. Prioritizing the removal of these individuals would not only reduce the number of viable seeds dispersing further in the ecosystem, but would also neutralize their facilitating effects, potentially slowing down the invasion. Our results also suggest that south-facing slopes are more prone to recruit invasive seedlings. It would therefore be key to focus control efforts in these specific areas in order to prevent further invasion and benefit grassland conservation.

5. Conclusion

Our study reveals that invasive pine seedling establishment is limited by moisture availability in this high-elevation grassland of central Argentina. We also observed that adult pines appear to facilitate the invasion process by creating favorable microsites in terms of moisture for germination and early seedling development. However, the interactions that continue to occur between older seedlings and saplings and the adult pines are unclear and deserve further research in this system. Management could leverage these negative outcomes by focusing on removal of adult pines, especially on south-facing slopes with modest legacy effects left by pines. Continuing to study the interactions that occur between invading pines and, their co-invading ectomycorrhizal fungi, as well as the biotic resistance of native grasslands, will help in the design of better control strategies for pine invasions at such sites. Finally, our results indicate that legacy effects once trees are removed are far less important than the facilitation that occurs under living trees. This is important since restoration programs need to understand the trajectories after intervention (Torres et al., 2023), and the dead pine treatment did not show high germination and survival rates due to liberation of resources.

CRediT authorship contribution statement

Martin A. Nuñez: Writing – review & editing, Validation, Funding acquisition, Conceptualization. **Jason D. Hoeksema:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **Tomas Milani:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Maria Florencia Spalazzi:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **François P. Teste:** Writing – review & editing, Validation, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.foreco.2024.122254.

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