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RESEARCH ARTICLE

Symbiotic interactions above treeline of long-lived pines: Mycorrhizal advantage of limber pine (*Pinus flexilis*) over Great Basin bristlecone pine (*Pinus longaeva*) at the seedling stage

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Abstract

1. In response to contemporary changes in climate, many tree species are shifting upslope to find favorable habitat. In the case of obligate ectomycorrhizal species, seedling growth above upper treeline depends on fungal spore availability. In the mountain ranges of the Great Basin, a recent shift in tree species stratification has been recorded, with limber pine (LP, *Pinus flexilis*) leapfrogging above the ancient bristlecone pine (BCP, *Pinus longaeva*) forest and establishing above current treeline.
2. We compared the ability of LP and BCP to interact with soil spore banks collected at different microhabitats (next to dead trees, young live trees or in a treeless control) above current treeline in the White Mountains of California.
3. We found an ectomycorrhizal fungal spore bank community composed of 15 species that was dominated by an undescribed and a hitherto unsequenced species of *Geopora* and *Rhizopogon*, respectively. This represents a much richer community than was found previously in this system. While both LP and BCP were able to establish ectomycorrhiza, LP was twice as likely to do so, and when comparing only seedlings that were colonized, its root system was colonized to a three-fold greater extent. BCP seedlings grown on soils collected under young live trees were much more likely to be colonized compared to soils from the other two microhabitats.
4. *Synthesis*. These differences in ectomycorrhizal receptivity might help to explain why LP is currently establishing at higher rates above the BCP treeline. Furthermore, it is possible that LP saplings above treeline can provide ectomycorrhizal facilitation for BCP seedlings, enabling the subsequent shift of BCP above treeline.

KEYWORDS

dispersal, dormancy, ectomycorrhiza, facilitation, *Pinus flexilis*, *Pinus longaeva*, plant–soil (below-ground) interactions, spore-bank

1 | INTRODUCTION

The role of environmental changes in shaping the evolution and the ecology of organisms has long been recognized. In light of environmental change, organisms either adapt, move to a new location where the environment still suits their needs, or die (Kubisch, Degen, Hovestadt, & Poethke, 2013). Because, adaptation is unlikely under rapid environmental change (Corlett & Westcott, 2013),

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migration is the more likely solution in such cases. Tracking environmental changes is expected to be even harder for long-lived, sessile organisms, where dispersal is only intergenerational.

Subalpine trees are a premier example of organisms that must disperse up or down mountain slopes to track environmental changes (Harsch, Hulme, McGlone, & Duncan, 2009). This need is expected to increase due to the rapid rate of predicted changes in the global climate (Bell, Bradford, & Lauenroth, 2014). The efficiency of such tracking is expected to depend on the rate of climatic change (affecting the dispersal distance that needs to be covered), the dispersal ability of the seeds (determining the dispersal distance that can be covered; Dullinger, Dirnböck, & Grabherr, 2004, but see Engler et al., 2009), time to reproductive maturity (Coutts, Klinken, Yokomizo, & Buckley, 2011), and the likelihood of seedling establishment and survival (Hampe, 2011). A tree with limited dispersal capacity and low rate of seedling establishment is expected to grapple with tracking a rapidly changing environment.

The role of seed dispersal has been emphasized as an important determinant of treeline movement (Dullinger et al., 2004; McLachlan, Clark, & Manos, 2005; Pearson, 2006). However, environments above treeline (alpine) are often harsh and can greatly limit seedling establishment post dispersal due to abiotic stresses (Maher & Germino, 2006). For tree species that rely on obligate root symbiosis to establish, the challenge might be much greater (Collier & Bidartondo, 2009; Hayward, Horton, Pauchard, & Nunez, 2015). Such obligatory mutualisms require essentially two dispersal events to achieve establishment: one for the plant, and one for mutualist. This requirement should reduce the chances and rates of successful establishment.

Mycorrhizal associations are one of the most studied belowground symbiotic interactions, with their effects on plant growth, survival and distribution receiving increasing attention (Smith & Read, 2008). Mycorrhizae may also play a major role in plant succession and invasion (Hayward et al., 2015; Horton, Bruns, & Parker, 1999), with the availability of specific mycorrhizal fungi often limiting seedling establishment of obligatory mycorrhizal plants (e.g. Ashkannejhad & Horton, 2006; Horton, Ashkannejhad, & Hazard, 2002; Policelli, Bruns, Vilgalys, & Nuñez, 2019). When dispersing beyond forest edges, seedlings of obligatory ectomycorrhizal (hereafter: **EM**) trees must associate with EM fungal spores within a relatively limited timeframe. Under high water availability, pine seedlings have been shown to survive for one year without colonization, but they lack significant growth (Collier & Bidartondo, 2009). In harsher subalpine setting we doubt that uncolonized pine seedlings would have the luxury of prolonged survival. The chance of both seed and spore arriving at the same spot at the same time is low and is expected to reduce the chances of seedling establishment (Bruns et al., 2009; Terwilliger & Pastor, 1999). This limitation can be overcome if EM fungal spores are able to survive in the soil for long periods of time, as this would increase the chances of a successful overlap in time and space (Nguyen, Hynson, & Bruns, 2012).

The spores of some ectomycorrhizal fungal species are extremely hardy and can produce a long-lived spore bank (Bruns et al., 2009; Nguyen et al., 2012) that can provide a solution to the seed-spore synchronization challenge. This should be especially important for tree species that lack a dormant soil seed bank (Daskalakou & Thanos, 1996; Ziffer-Berger, Weisberg, Cablk, Moshe, & Osem, 2017). Such spore banks have been shown to be important in pines, which are obligately EM, when they expand into habitats lacking ectomycorrhizal hosts (Hayward et al., 2015; Livne-Luzon et al., 2016; Peay, Schubert, Nguyen, & Bruns, 2012; Smith, Steidinger, Bruns, & Peay, 2018). While similar conditions can be expected to occur above the treeline of obligately EM tree species, the extreme conditions at treeline are expected to reduce the number of spores and seeds produced while increasing their inter-seasonal variability. Under such conditions, lack of EM fungal spores could inflict a significant restriction on seedling establishment and climate tracking of high elevation forests.

Great Basin Bristlecone pine (BCP, *Pinus longaeva* Bailey) and limber pine (LP, *Pinus flexilis* James) are important components of the high elevation forests of the dry mountain ranges of the southwestern United States. Both species have great longevity with LP living up to 1,700 years (Schulman, 1954) and BCP, the longest living tree species on earth, living near to 5,000 years (Currey, 1965; Lanner & Connor, 2001; Schulman, 1958). The harsh conditions of these high elevation forests result in extremely rare and variable establishment events (Barber, 2013; Emlen, 1970). Recruitment events are so rare that hundreds and even thousands of years (Barber, 2013) can pass between successful establishment within large areas, as can be seen from tree ring records (Millar, Charlet, Delany, King, & Westfall, 2019).

In many parts of their joint distribution range, BCP inhabits the upper most forest belt (including treeline) and overlaps at lower subalpine areas with LP (Lanner, 1984). However, a few recent studies have shown that this historically observed stratification might be changing in recent decades (Millar, Westfall, Delany, Flint, & Flint, 2015; Smithers, 2017; Smithers, North, Millar, & Latimer, 2018). Specifically, LP has been found to leapfrog above the main treeline that is composed almost entirely of mature BCP trees. In many cases this establishment is taking place in proximity to ancient, relic BCP groves, composed entirely of dead trees (Millar et al., 2015). This differential establishment of LP seedlings above the BCP treeline highlights the lack of BCP seedling establishment above the treeline and raises concerns regarding this very slow growing tree's ability to track climate change up the mountain slope. At the same time this pattern raises the question of how LP seedlings established in areas long barren of live pines where EM inoculum would not be expected to persist.

Being obligate EM trees, the presence or absence of EM spore banks is expected to affect the ability of both species to advance up these dry mountain slopes. A prior study had shown that spore banks associated with BCP in this area were patchy, sparse, contained few species, and were limited almost exclusively to groves of living trees (Bidartondo, Baar, & Bruns, 2001). Thus we expected to find a similar pattern in the current study. We hypothesized that the ancient groves of dead BCP trees might retain viable EM spore banks if spores can live for hundreds or thousands of years, and this could explain establishment of pines in or near these relic groves of dead trees (Millar et al., 2019). We further

hypothesized that young trees established above treeline would create an inoculum hotspot. To test these hypotheses we sampled soils from sites near treeline that were either in dead groves, next to young trees or distant from any trees. We used pine seedling bioassays to test for the presence of living spores. We used both LP and BCP to assay soils in order to test whether they responded differently to the available inoculum. Our expectations were that we would find the most inoculum next to young live trees and that inoculum would be more common in the dead groves than away from them.

2 | MATERIALS AND METHODS

2.1 | Study area

The White Mountains are a semi-arid range lying east of the Sierra Nevada along the California-Nevada border at the western margin of the Great Basin. LP and BCP compose the subalpine forest zone, which starts at about 2,900 m. Mature LP forests extend from ~2900–3350 m. BCP occurs in monotypic as well as mixed stands with LP, and mature trees extend to higher elevations than LP, ~3,500 m. The climate of the White Mountains is cold and dry (Powell and Klieforth in Hall, 1991). The scant precipitation falls largely in the winter and varies with elevation, ranging from 142 mm/year at the Bishop Airport in Owens Valley (1,250 m) to 455 mm/year at the White Mountain Research Center's Barcroft Station (3,800 m). Summer precipitation falls sporadically during convective monsoon storms. Temperature is also strongly dependent on elevation, with mean annual temperature ranging from 13.3°C at Bishop (1,260 m) to −1.7°C at the Barcroft Station (3,800 m, Western Regional Climate Center, accessed 2017).

2.2 | Soil collection

On July 2017 soil was collected at 5 different sites in the White Mountains (Table S1 for site coordinates and elevations, Figure 1). All sites had mature dead BCP trees but lacked mature live trees. In each site we sampled next to mature dead trees or in adjacent areas distant from either live or dead trees (treeless-control). Special care was taken so that selected dead trees were at least 50 m from live trees (either mature or seedling) and that treeless-control sites were at least that distance from both live and dead trees. The distances of every sampling point to both live and dead trees was measured with a laser distance meter (Nikon Aculon). Distances were taken to the closest live and dead tree in four different quadrates (90 degrees each. 4 × 2 = 8 measurements). Distance to living treeline was calculated using Google-earth (the distance to the closest edge of the closest living grove that had more than 10 living trees).

In each treeless-control sampling location we collected soil from 8 different points along a circle of 1 m radius. At dead tree sampling locations, soil was collected in 8 different spots around the trunk. At each collection point soil was sampled from the surface to a depth of approximately 10 cm. The 8 samples collected in each location were bulked and coarse sieved (5.6 mm) prior to being sealed in a plastic Ziploc bags. All tools and hands were cleaned and sterilized with ethanol wipes between samples. Overall we had 54 soil samples (27 dead trees and 27 treeless-controls). However, since one bag was not labeled correctly we were left with 53 samples.

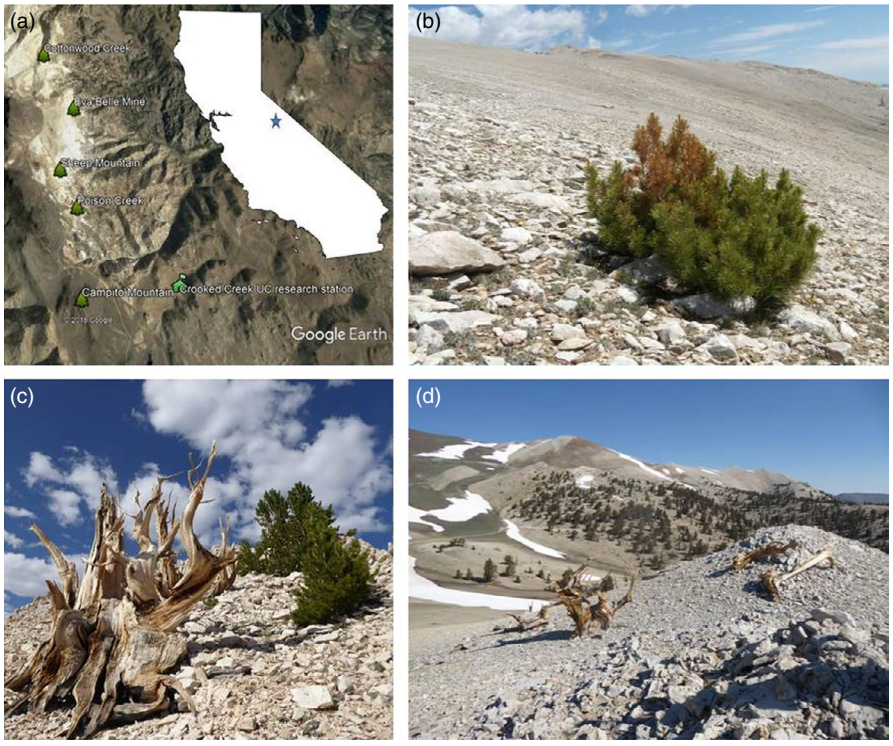

FIGURE 1 A map of the sampling sites (a). Limber pine (LP) establishing above tree line at the Eva Belle site. The dead needles are common in young (~20 years old) saplings and are the result of winter freeze in years when snow cover is light (b). Recent establishment of young LP sapling (25–35 years old) in the vicinity of an ancient bristlecone pine (BCP) snag (c). BCP treeline as seen from the Poison Creek site, dead trees can be seen in the front (d) [Colour figure can be viewed at wileyonlinelibrary.com]

In addition soils were collected next to 5 young pine seedlings (3 BCPs and 2 LPs). Soils were collected in a similar manner as mentioned above. All 5 young trees were sampled at the Eva Belle Mine site.

In the lab, all soils (dead trees, treeless-controls and live young trees) were sieved with a fine sieve (2 mm). The soil was then kept in a cold room (4°) for 3 months until the beginning of the bioassay in October 2017.

2.3 | Dead tree dating

To estimate the time since death of trees used as soil sampling points, we collected increment cores from a subset of the stems in four of the five stands (Poison Creek was excluded). In the laboratory, air-dried increment cores were processed using standard dendrochronological techniques (Cook & Kairiukstis, 1990; Holmes, Adams, & Fritts, 1986). The annual ring sequence of each sample was measured to 0.001-mm accuracy using a Velmex measuring system interfaced with MEASURE J2X measurement software (Consulting Voortech, 2005). The program COFECHA was used to assess measurement quality and perform cross-dating correlation analyses (Grissino-Mayer, 2001; Holmes, 1983). A 10–15-yr cubic smoothing spline (50% frequency cutoff) was employed in COFECHA to maximize series intercorrelation and minimize the number of generated error flags (Krusic & Cook, 2013). Samples having ambiguous cross-dating features or correlations less than 0.2 were excluded from analysis. Dating was achieved first by developing a site chronology with reference to local trees, then confirming with regional chronologies we have built and long chronologies from the White Mountains (ITRDB accessed May 2018). Additional evidence to support the identity of the deadwood as BCP was the inability to cross-date the sample series with local and regional LP standard chronologies.

2.4 | Bioassay

Each soil sample was used to fill 20 cone-tainers (50-ml,Super 'Stubby' Cell Cone-tainer; Stuewe & Sons Inc., hereafter: pots). A few soil samples were not large enough to fill 20 pots resulting in an average of 18.6 (±3.8) pots per soil sample. Polyester Fiber fill (Joannes Fabrics) was placed in the bottom of every pot to prevent soil washout. A 2 cm layer of sand was added on top of the fiber followed by the field soils that were then covered by a thin layer of sand to minimize between seedling contaminations. The pots of each soil sample were divided into two. Half of the pots were sown with BCP and the other half were sown with LP. Nine sterile-soil controls of each pine species were planted to monitor for EM contamination. All the control plants were found to lack ectomycorrhiza at the end of the bioassay. Two seeds were sown in each cone-tainer to account for lack of germination. All seeds were provided by the USDA Forest Service, Institute of Forest Genetics, Placerville, California from their seed archive. After approximately a month, seedlings were thinned down to one seedling per pot. All pots were placed in a greenhouse in the Berkeley campus under natural daylight. Seedlings were watered every two days.

Seedlings were harvested after 8 months of growth. Each seedling was thoroughly washed over a fine sieve and the clean root system of each seedling was scanned under a dissecting scope. Root tips covered with a fungal mantel, or that were swollen and lacked root hairs were classified as potentially mycorrhizal. Samples of all such root tips that differed in color or general shape from a single seedling were placed individually into Xtract and Amp buffer (Sigma), and heat extracted following manufacturer's instructions. The ITS region was amplified with ITS 1f & 4 primers (Gardes & Bruns, 1993; White, Bruns, Lee, & Taylor, 1990), and amplicons were Sanger sequenced (UC Berkeley DNA sequencing facility). Colonization was estimated for each seedling using one of 5 ranks (not colonized, 0%–25%, 25%–50%, 50%–75%, 75%–100% of the root system colonized). All tools were sterilized between plants.

2.5 | Tree building and OTU annotation

A total of 407 sequences were obtained from individual EM root tips. These were blasted against the UNITE database (Koljalg et al., 2005). Sequences were clustered into 55 different OTUs when using a 97% similarity threshold using Usearch. Seventeen of the OTUs were found to be none ectomycorrhizal. The sequences of the remaining 38 OTUs were grouped by genus (*Rhizopogon*, *Geopora* & *Suillus*). All sequences for *Rhizopogon* and *Geopora* from this study were combined with selected sequences deposited in NCBI (Flores-Rentería, Lau, Lamit, & Gehring, 2014; Guevara-Guerrero, Stielow, Tamm, Cázares-Gonzalez, & Göker, 2012; Southworth & Frank, 2011; Tamm, Põldmaa, & Kullman, 2010) and aligned separately for each genus. Neighbor joining trees were produced (Geneious software) to test for possible redundancies due to differences in sequence quality and length. Based on the trees, we consolidated the 38 OTUs into 15.

2.6 | Statistical analysis

Because ~9 pots per tree species had soil originating from the same soil sample, we could not treat them as independent replications. Therefore, we analyzed the colonization rates per soil sample rather than per seedling. In the genetic analysis we recorded the presence absence of fungal species in all 9 seedlings together rather than per individual seedling.

Since each soil sample was used for both LP and BCP, we used repeated measures ANOVA in order to test for the effect of tree species (within), soil type (between) and the interaction between them on the percent of seedlings colonized. A chi square test of independence was used separately for LP and BCP in order to test for a correlation between soil type and the presence of either *Geopora* or *Rhizopogon*. Pearson correlation as well as a quadratic regression was used to quantify the relationship between the colonization of the two tree species.

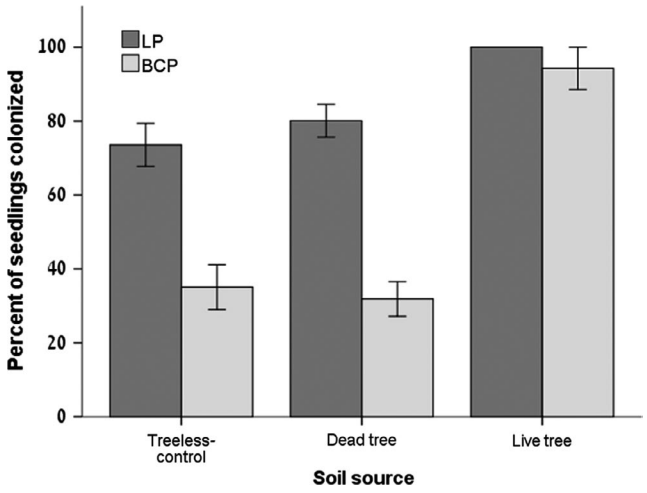

FIGURE 2 The effect of soil source on the percent of seedlings colonized. Error bars represent ± *SE*. The bar lacking error bars represents 18 plants, all of which were colonized. BCP (bristlecone pine); LP (limber pine)

3 | RESULTS

3.1 | Percent colonization and its relationship to sample type

Nearly 100% of pine seedlings of both species were colonized when grown in soil collected from beneath live, young trees (Figure 2). This represented a significant increase, relative to seedlings grown in soil collected from beneath ancient, dead trees or from areas lacking trees (soil type effect; $F_{2,55}$ = 7.421, p = .001). When comparing all soil types together, the two pine species exhibited different receptivity to colonization. An average of 78.89% (±26.09) LP were colonized compared to 38.69% (±31.65) for BCP (pine species effect; $F_{1,55}$ = 53.433, p < .001). However, the significant interaction suggests that the advantage of LP did not exist on soils collected next to live trees (species × soil interaction $F_{2,55}$ = 6.69, p = .003 Figure 2). A similar trend was found when looking at the percent of the root system colonized, although the interaction wasn't significant (see Figure S1).

Although the two tree species differed in their colonization frequencies, colonization values of the two pine species obtained from each soil sample were linearly correlated (r_p = .597, n = 58, p < .001; Figure 3a). However, the colonization of LP saturated more often, and the variance was better explained by quadratic function (r = .675, $F_{2,55}$ = 23.078, p < .001; Figure 3a).

Distance from the soil sample location to the closest live tree, dead tree, or living treeline did not significantly correlate with the percent seedlings colonized, or percent of the root system colonized for either species.

3.2 | Species richness

We found a total of 15 EM fungal species from 3 different genera: 9 species of *Rhizopogon*, 5 species of *Geopora* (inclusive of *Picoa*) and

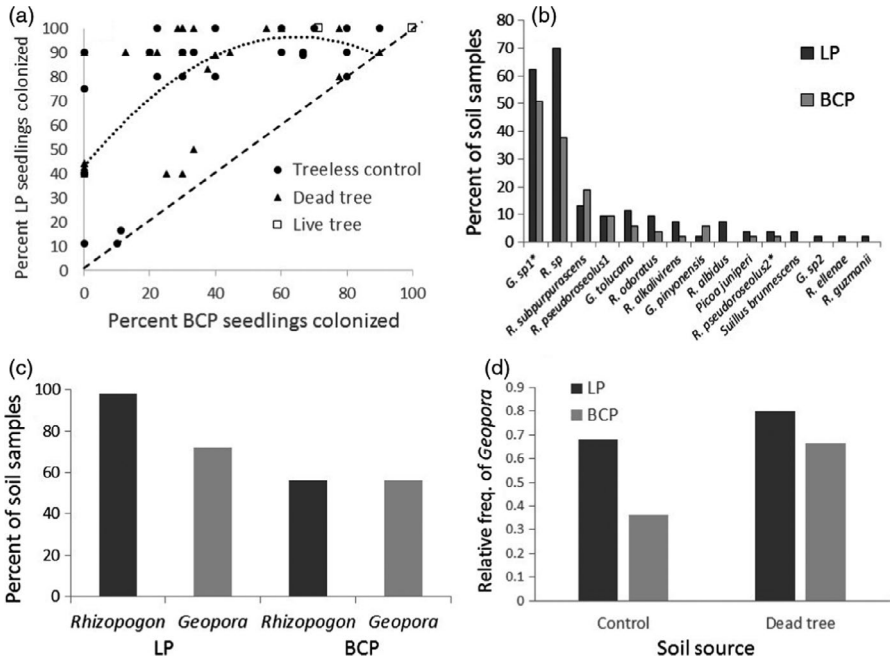

FIGURE 3 The correlation between colonization rates of the two pine species, displayed by treatment. Points above the dashed reference line represent soil samples in which the percent of LP (limber pine) seedlings colonized was greater than that of BCP (bristlecone pine). Four out of the five samples taken under live young trees resulted in 100% colonization for both pine species. The black line represents the best fit quadratic function (a). The percent of soil samples at which each fungal species was identified on the roots of either LP or BCP. *Rhizopogon* (R), *Geopora* (G). Species marked with an asterisk were also found in the White mountains by Bidartondo et al. (2001) (b). The percent of the soil samples in which at least one *Rhizopogon* or *Geopora* species colonized the roots of LP or BCP (c). The fraction of soil samples where a *Geopora* species were found, presented by soil source for either LP or BCP. Soils collected next to live trees were not analyzed due to small sample size (d)

1 species of *Suillus*. Of these 15 species, all were found at least once on the roots of LP and 10 were found at least once on the roots of BCP. The ranked frequency on the two pine species was very similar, and only species that were rarely found on LP were absent on BCP (Figure 3b; Figure S2 presents the same data, divided into the 3 different soil sources). The two most common species were two unidentified species of *Rhizopogon* and *Geopora*.

Rhizopogon species were found in 98% of the soil samples assayed with LP seedlings. *Geopora* species were found in 72% of the same samples. Since genetic analysis was done per soil sample, these values are per soil sample and not per seedling. This means that in 98% of the soil samples at least one LP seedling interacted with a *Rhizopogon* species. The respective values for BCP were 56% for both *Rhizopogon* and *Geopora* (Figure 3c).

3.3 | Age of legacy (dead) pines

As is common for dendrochronology work in the White Mountains, appearance of dead trees gives little clue as to how long they have been dead. Due to ring erosion, the outer rings dated are not a tree's exact death date, but represent maximum dates. Of 28 dated cores from four of the soil sampling sites, the average outer ring date was 1,150 BCE (Table S2), which corresponds to (maximum of) 3,167 ± 1,268 years since death in 2017 CE. The ranges of outer ring dates were large, but no tree had an outer ring younger than 457 CE (1,560 years since death) and there were not large differences in age range among the sites.

3.4 | Legacy effect of the dead trees

We found a marginally significant effect of dead trees on the presence of *Geopora* on the roots of BCP (χ^2_1 = 4.224, p = .04) but not on the roots of LP (χ^2_1 = 0.860, p = .354; Figure 3d), although the trend was in the same direction. No such effects were found for *Rhizopogon* in either BCP (χ^2_1 = 0.113, p = .736) or LP (χ^2_1 = 1.161, p = .281). Soils collected next to live trees were not analyzed due to small sample size.

4 | DISCUSSION

In response to contemporary climate change, the treelines of many tree species are moving up slope (Harsch et al., 2009). In the case of obligate EM trees, spore availability above the current live forest might be expected to limit seedling establishment and, when absent or reduced, severely hamper environmental tracking. However, we found that the EM spore banks were present at all sample points whether live trees were present, legacy dead trees remained, or no trees-stems were present. The spore banks were dominated by two species, *Rhizopogon* sp. and *Geopora* sp., but contained 15 different EM fungal species. While both LP and BCP were able to establish ectomycorrhizae on all soils sampled from these environments, LP did so twice as often and its root colonization percentage was three-fold greater. These differences in ectomycorrhizal receptivity, coupled with the dispersal differences of the two species (Smithers et al., 2018), might explain why LP is currently establishing at higher rates above the BCP treeline.

4.1 | Colonization differences between LP and BCP

The two pine species studied, interact with the same EM fungal species. However, LP does so at a higher rate (Figures 2 and 3a). This difference is expected to be greater under field conditions. The conditions experienced by seedlings in our bioassay are by far more luxurious than those experienced in the field, in that bioassay seedlings receive ample water, a longer growing season, benign climate and no competition. These differences are bound to create a positive bias in survival and colonization and result in larger seedlings and larger root systems that effectively explore more soil. For these reasons we assume that colonization levels in the field are actually much lower (Dove & Hart, 2017). Reduced colonization levels might be especially detrimental for BCP, which even under nursery conditions of our bioassay was often not colonized at all (Figure 2). This potential bias highlights the importance of understanding colonization dynamics in the field, a challenging task when studying a species that can have 100 year establishment gaps (Barber, 2013).

A recent study described the establishment of LP over the BCP treeline, suggesting it might be the result of superior dispersal abilities (Millar et al., 2015). In light of their findings it was hypothesized that LP might be a long-lived early succession species that is later outcompeted or outlived by BCP (Smithers et al., 2018). Our results suggest that LP has the potential to also be a mycorrhizal facilitator. Due to its greater ability to associate with EM inoculum and its dispersal abilities, LP is more likely to initially establish above the treeline. Once established, LP would create an EM hotspot locally rich in hyphae, increasing the probability of BCP seedlings to establish in its vicinity. In addition, our sample from beneath young 10–25 years old LP and BCP trees shows that they also serve as a source for newly produced spore inoculum (Figure 2) or capture wind-dispersed spores. Thus the EM hot-spot created by a successful tree establishment may disperse some distance away from the tree. The fact that mature LP are rare near the tree line raises the possibility that it does not currently reach maturity at these high altitudes, possibly dying back due to extreme climate episodes or being outcompeted by the more stress tolerate BCP. However, it is also possible that climate changes have altered the physical niche and the competitive balance between the two pine species and will change the forest structure for many years to come.

4.2 | Compatibility with previous studies

An earlier study conducted at slightly lower elevations (3,216 m ± 71.4 compared to 3,488 m ± 44.82 in the present study) in the same general area of the White Mountains, bioassayed soils from forest and sagebrush (*Artemisia tridentata*, Nutt) scrub using BCP and found a less diverse and more spatially restricted EM spore bank (Bidartondo et al., 2001). Only four species of fungi were found in bioassays from the forest soils and only one of these was found

outside of the pine forest. The latter was a *Rhizopogon* species that was common in the earlier study but occurred infrequently in our current study where we refer to it as *Rhizopogon pseudoroseolus* sp2 (Figure 3b). *Geopora* sp1, the most abundant species in the current study, was also the most abundant species in the forest soils sampled by Bidartondo et al. (2001), where it was not found outside the forest and was identified only to family (Pyronemataceae) due the unpopulated ITS database of the time.

These earlier results differ from our current study, in which we found 10 different species of EM fungi from bioassays with BCP, when using soils collected above treeline in areas lacking sagebrush. The contradicting results regarding the occurrence of a significant spore bank outside the forest between the two studies might originate from the differences in the distances to live trees, the history of the sites or other biotic or abiotic differences between the studied sites. Specifically, the average distance of 328 m from the closest living pine tree (for sagebrush sites) in the previous study was greater than the 88 m average for the sampling locations in the current study. Furthermore, while the current study was conducted in sites where clear evidence of ancient groves exists, the sagebrush sites of the previous study show no evidence that trees ever existed. In an environment where wood decay can take as long as 5,000 years, this is a clear sign of historical differences between the sites.

4.3 | Species specific dynamics of the soil spore bank

Regardless of the discrepancies between the two studies, it seems clear that as in other water limited systems (Gehring, Mueller, Haskins, Rubow, & Whitham, 2014; Gehring, Sthultz, Flores-Rentería, Whipple, & Whitham, 2017; Gordon & Gehring, 2011; Livne-Luzon et al., 2017, Patterson, Flores-Rentería, Whipple, Whitham, & Gehring, 2019) *Geopora* is an important pine-symbionts in the White Mountains. However, the frequent occurrence of *Geopora* outside of the forest (72% and 56% of soil samples with LP and BCP respectively) is somewhat surprising. For example, in a dry Mediterranean system were *Geopora* dominated the soil spore bank of the pine (*P. halepensis*) forest, a nearby abandoned field was nearly devoid of *Geopora* and was instead dominated by *Suillus* (Livne-Luzon et al., 2016) which is known to be a prolific aerial disperser (Hayward et al., 2015; Peay et al., 2012).

The occurrence of *Geopora* spores outside of the forest in our current study shows that effective dispersal is taking place. *Geopora* species are known to vary in their morphology and location within the soil (Flores-Rentería et al., 2014; Guevara-Guerrero et al., 2012; Southworth & Frank, 2011; Tamm et al., 2010), variability which probably also affects their dispersal ability. The fact that *Geopora* was found in a study that used fly ash dump soil lacking any plants, shows some level of dispersal ability (Hrynkiewicz, Baum, Niedojadło, & Dahm, 2009).

Long-term legacy effects might provide an alternative to current dispersal in explaining the occurrence of *Geopora* at high concentrations outside of the current forest. The fact that *BCP* was more likely to be colonized with *Geopora* next to dead trees compared to treeless-control soils, raises the possibility that *Geopora* spore concentration might be relicts of spore banks established when the ancient trees were living. However, the average time since death of the trees next to which we sampled (based on coring) is estimated to be 3,167 ± 1,268 years (Table S2). Perhaps spores of *Geopora* could remain viable for such a long period of time, but it is also possible that episodical establishment of pine saplings (which do not reach maturity and do not leave behind recognizable wood) above the treeline maintains this *Geopora* spore bank. Such unsuccessful establishment periods, which can occur on the scale of decades to centuries (Millar et al., 2019, 2015) might be able to sustain the *Geopora* spore bank. Another possibility is that the vicinity of dead trees provides a preferred habitat for small mammals that disperse the spores of *Geopora* or that the trees create a physical trap for airborne spores. That being suggested, we find the statistical significance of this finding (p = .04) to be marginal and think that more specific research is needed in order to draw more certain conclusions regarding possible extreme long spore dormancy in this dry environment.

5 | CONCLUSIONS

The occurrence of ectomycorrhizal spore banks has been shown to be crucial for the establishment of pines outside of the forest limits, both within natural (Smith et al., 2018) as well as introduced (Hayward et al., 2015; Policelli et al., 2019) habitats. Mycorrhizal facilitation has been suggested to occur when one plant increases the mycorrhizal colonization of other plants (Dickie, Koide, & Steiner, 2002; Kennedy, Izzo, & Bruns, 2003; Kennedy, Smith, Horton, & Molina, 2012; Richard, Selosse, & Gardes, 2009). Our findings suggest that such mycorrhizal facilitation might be taking place above treeline, where both young trees and dead ancient trees are possibly increasing the colonization potential of young BCP seedlings.

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AUTHORS' CONTRIBUTIONS

H.S. T.D.B. and C.I.M. conceived the ideas and designed methodology; all authors collected the data; H.S. T.D.B. and B.E.B. analyzed the data; H.S. and T.D.B. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

All 407 sequences were submitted to NCBI Genbank (Accession numbers: MK841646–MK842051)

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