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Salt Life: Salinity Drives Ectomycorrhizal Community Structure in the Endangered Pine Rocklands

Elena Karlsen-Ayala[1,2,3] | Michelle A. Jusino[4,5] | Matthew E. Smith[2] | Romina Gazis[1,2]

[1]Tropical Research and Education Center, University of Florida, Homestead, Florida, USA | [2]Department of Plant Pathology, University of Florida, Gainesville, Florida, USA | [3]USDA Forest Service, Northern Research Station, Hamden, Connecticut, USA | [4]USDA Forest Service, Northern Research Station, Center for Forest Mycology Research, Madison, Wisconsin, USA | [5]Department of Botany, University of Wisconsin-Madison, Madison, Wisconsin, USA

Correspondence: Elena Karlsen-Ayala (elena.karlsen-ayala@usda.gov; ekarlsenayala@ufl.edu)

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ABSTRACT

Pinus densa, an endemic and keystone tree in Florida's endangered pine rocklands ecosystem, faces increasing threats from sea level rise and salt intrusion. Ectomycorrhizal (ECM) fungi are critical for pine recruitment and survival, yet their diversity and response to salinity in this ecosystem have been unstudied. We used metabarcoding to survey the naturally occurring ECM fungi on the roots of mature *Pinus densa* at eight field sites with varying elevations, soil salinities, habitat patch sizes and distances from the ocean, followed by a manipulative greenhouse experiment to assess potential impacts of rising salinity, with four salinity levels on *P. densa* seedlings in soils that spanned a salinity gradient to evaluate survival and shifts in ECM communities. Results show that salinity stress threatens both *P. densa* and its ECM symbionts, with ECM fungal richness positively correlated with elevation and negatively correlated with salinity. Habitat patch size, distance from the ocean and soil pH showed no significant effect on richness, and pH was less predictive of community structure. In seedlings, higher salinity was associated with greater mortality and shifts in ECM community composition favouring *Rhizopogon* species and Pezizales taxa. These findings underscore the susceptibility of ECM fungi to increased salinity, which may disrupt mutualisms critical for coastal resilience. Understanding how salinity affects mutualistic fungi can inform predictions on the vulnerability of other coastal ecosystems to climate change and sea level rise.

1 | Introduction

Sea level rise and subsequent salt intrusion into low-lying areas are major threats to coastal ecosystems facing climate change (Nicholls and Cazenave 2010). Rising sea levels, when coupled with rising temperatures, drought and increased storm surge, threaten biological communities, especially those that are rare or endemic. Increases in salinity can arise either as 'pulse disturbances' during specific events (e.g., salt intrusion due to storm surge from hurricanes) or by 'ramp disturbances' over time (e.g., salt intrusion due to sea level rise; Hisano et al. 2018; Lake 2013; Ross et al. 2009). Increased salinity causes detrimental osmotic stress to salt-sensitive plants, resulting in reduced plant growth, loss in ecosystem productivity and biodiversity (Kirwan et al. 2007; McCarthy et al. 2022; Parida and Das 2005). Examples of the negative effects of increased storm surge and sea level rise include white mangrove trees (*Conocarpus erectus*) invading buttonwood forests in the Everglades (Florida,

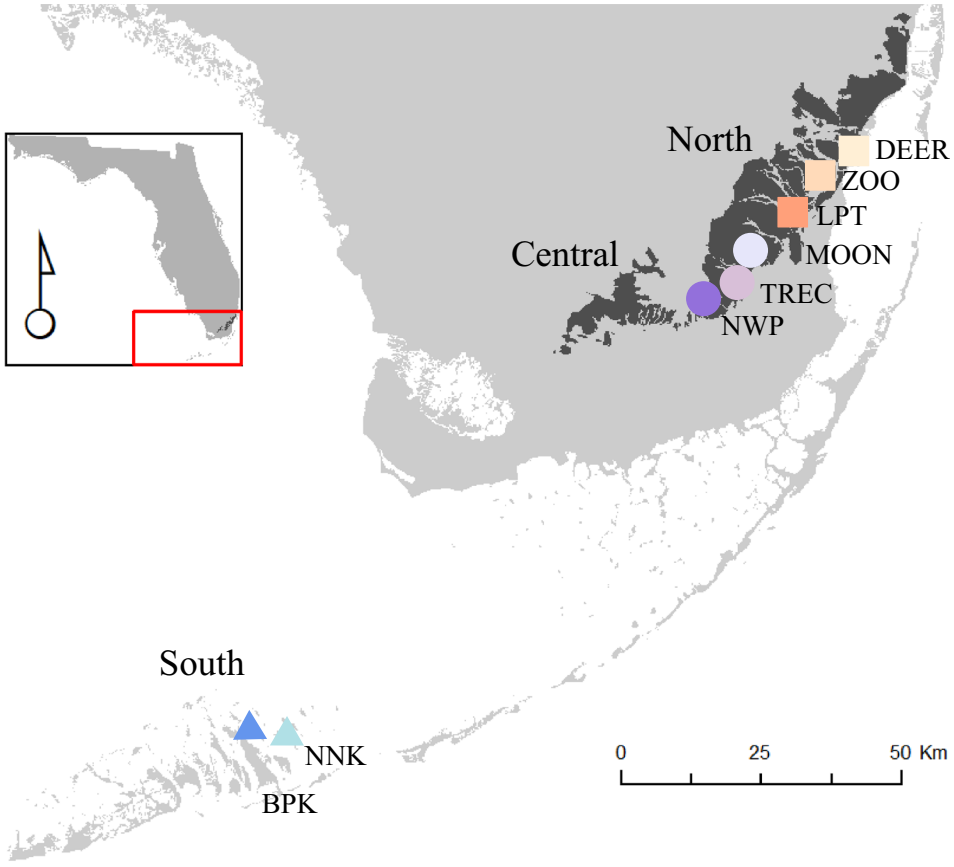

FIGURE 1 | Map of the eight field sites where ectomycorrhizal (ECM) fungi were sampled on the roots of mature *Pinus densa*. Historical range of the pine rocklands habitat is outlined in dark grey, land is outlined in light grey, and water is shown in white. Northern sites (Deering Estate—DEER, Miami Zoo—ZOO, Larry & Penny Thompson—LPT) are designated with orange squares, central sites are designated with purple circles (Private rocklands—MOON, Tropical Research and Education Center—TREC and Navy Wells Preserve—NWP), and southern sites are designated with blue triangles (No Name Key—NNK, Big Pine Key—BPK). At each of the eight sites, we sampled ectomycorrhizal pine roots and associated soils from 10 mature pine trees ($n = 80$ trees). Sampling of ECM fungi on mature pines was used to identify three sites that differed in baseline salinity to obtain initial soils for the manipulative salinity experiment.

USA) (Saha et al. 2011), salt-tolerant marshes encroaching on pine forests in the Florida Keys (Ross et al. 1994), and population declines of the Florida Key tree cactus (*Pilosocerus robinii*) (Goodman et al. 2012; Maschinski et al. 2009). Recently, the federally endangered salt-intolerant population of Key Largo tree cactus (*Pilosocerus millspaughii*) was reported as the first known local species extirpation in the USA linked to sea level rise (Possley et al. 2024). Other examples of coastal forest losses due to salt intrusion in North America include pine and cedar forests across coastal mid-Atlantic states (Schieder and Kirwan 2019) and pine or deciduous forests that have transitioned into marshes in North Carolina (Ury et al. 2021). This type of forest loss results in 'ghost forests', or standing dead trees on land that have transitioned to marshes due to salt intrusion (Schieder and Kirwan 2019).

The Florida pine rocklands, a critically endangered ecosystem of pine-dominated upland coral 'islands' located in south Florida and the Florida Keys (Snyder et al. 1990), are under threat of becoming a 'ghost ecosystem'. The pine rocklands are unique because of their tropical location, patchy distribution, karst geology and their many endemic plant taxa (Possley et al. 2008; Snyder et al. 1990; Trotta et al. 2018). This patchy, fire-dependent ecosystem relies on *Pinus densa* as a keystone species to fuel low-intensity fires critical for maintaining the open savanna-like landscape (Snyder et al. 1990; Williams et al. 2007). Less than 2% of the original ecosystem remains outside of Everglades National Park, and all patches are 2–7 m above sea level (Figure 1; Snyder et al. 1990). Mature pine density and pine seedling recruitment in this ecosystem have been reduced due to hurricanes, increased soil salinity and decreased freshwater availability (Ross et al. 1994; Sah et al. 2010). Increased hurricane activity and sea-level rise are projected to amplify storm-induced mortality of mature pines through wind damage, salt toxicity, and the creation of anoxic conditions due to waterlogging (Ross et al. 2020). The decline of pines and pine recruitment also threatens the persistence of other endemic organisms in this ecosystem, including 16 federally protected plants and 11 animals (Jones and Koptur 2017).

While the negative effects of increased salinity on coastal vegetation have been well documented, less is known about how increased salinity impacts the functional diversity of microbial communities. Functional diversity, a product of the ecological functions that different organisms provide, is critical for maintaining ecosystem health and resilience (Tilman 2001). Many

coastal forest trees are dependent on ectomycorrhizal (ECM) fungal associations because they cannot establish or survive without this mutualistic relationship in which the fungi facilitate nutrient absorption in exchange for photosynthetic sugars (Smith and Read 2008). Besides their critical importance in nutrient and water uptake, mycorrhizal fungi can ameliorate the impact of abiotic stressors such as drought, heavy metals and salinity (Bandou et al. 2006; Bingham and Simard 2012; Gordon and Gehring 2011; Souza et al. 2012). Ectomycorrhizal symbioses have evolved independently >70 times and are functionally diverse, offering a variety of benefits to their host plants (Lebreton et al. 2021; Miyauchi et al. 2020; Tedersoo and Smith 2013). Different lineages of ECM fungi vary in their tolerance to stressors, host colonisation strategy, saprotrophic capability, and extramatrical mycelium production (Bazzicalupo et al. 2020; Ishida et al. 2008; Jörgensen et al. 2023; Lindahl and Tunlid 2015) and it is believed that ECM lineage diversity and species diversity are therefore intrinsically linked to function (Looney et al. 2018).

There is experimental evidence that salt-tolerant ECM fungi may improve their host plant's tolerance of salt stress, but the mechanisms are not fully understood and are likely species-dependent (Bandou et al. 2006; Bois et al. 2006; Langenfeld-Heyser et al. 2007). There are several potential mechanisms through which ECM fungi may help improve salt tolerance in their host. For example, the ECM mycelium can form a physical barrier covering the host roots and sequester salt molecules. Additionally, ECM symbiosis improves host nutrition, which may mitigate negative impacts of high salinity (Guerrero-Galán et al. 2019). Some groups of fungi have also been found to adapt to salinity stress. Branco et al. (2015) compared the genomes of two populations of *Suillus brevipes* that were genetically isolated and diverged under contrasting soil salinity concentrations. This study found that genetic loci related to salt tolerance were under selection, suggesting that salinity is a critical environmental stressor that can drive local adaptations of ECM fungi populations (Branco et al. 2015).

Despite the ecological importance of *P. densa* and its obligate ECM associations, fungal communities in the pine rocklands remain poorly studied and are only characterised on seedlings grown *ex situ* in pine rockland soil (Karlsen-Ayala et al. 2022, 2023). Although salinity-induced declines of *P. densa* populations are anticipated to increase, the impact on ECM communities is largely unknown. Most salinity studies have focused on individual plant–fungus combinations under greenhouse conditions (Bandou et al. 2006; Bois et al. 2006; Kernaghan et al. 2002) and only a few have looked at the effects of increasing salinity on entire ECM fungal communities (Zwiazek et al. 2019).

In this study, we examined the effects of increasing salinity on ECM fungi associated with south Florida slash pines (*Pinus densa*) by employing two data sets. First, we used DNA metabarcoding to document the ECM fungi communities on roots and in soils beneath mature, naturally occurring *Pinus densa* at eight sites representing a natural salinity gradient across the pine rocklands. Second, we conducted a manipulative salinity experiment to test the effects of increased salinity on ECM communities in pine seedlings grown in soils from three pine rockland sites with different initial soil salinities. We hypothesised that sensitivity to salinity would vary across ECM lineages and that increased salinity would therefore negatively impact overall ECM diversity in both the mature pine community and the greenhouse salinity experiment. We also hypothesized that soil salinity and pH would be the most important explanatory variables shaping the ECM fungal communities in the pine rocklands. Our overarching goal was to survey mature pines to identify the most vulnerable locations, then use a manipulative experiment to preview how imminent sea level rise will affect the pine–ECM symbiosis. By identifying sites with high ECM diversity and identifying fungi that confer salt tolerance, we are providing baseline data to identify and prioritise key sites for conservation efforts, and we are providing a critical tool to be used in future restoration efforts.

2 | Materials and Methods

2.1 | Ectomycorrhizal Fungi Associated With Mature Pines

Mature *Pinus densa* trees were sampled from sites that varied in elevation, distance from the ocean, habitat patch size and soil salinity across eight sites in three regions in Florida, USA: Miami (northern region), Homestead (central region) and the Keys (southern region; Table S1). Pine roots were collected from eight sites: three in the north [Deering Preserve (DEER), Larry and Penny Thompson (LPT), Zoo Miami (ZOO)], three in the central [a private rocklands site (MOON), Tropical Research and Education Center (TREC) and Navy Wells Preserve (NWP)] and two in the south [No Name Key (NNK) and Big Pine Key (BPK)]. At each site, 10 randomly selected pines 30–50 m apart were sampled (Figure 1).

Due to the presence of limestone soils, soil cores could not be used to collect roots. Instead, the topsoil and vegetation were removed using a hand rake, and ECM pine roots and adhering soil were collected with a cleaned hand trowel and placed in a Ziploc bag and transported to the laboratory on ice. From each tree, a bag was filled with ~950 cm^3 of roots and adhering soil collected 0.5 m from the base of the tree in four cardinal directions. We weighed the roots to ensure approximately equal weights between samples prior to removing ectomycorrhizal root tips.

Ectomycorrhizal fungi were sampled from soil and roots (10 trees × 8 sites = 80 soil samples and 80 root samples). Prior to washing, the roots were shaken in the original sample bag to separate adhering rhizosphere soil from ECM roots. The adhering rhizosphere soil from each tree was homogenised, and 250 mg of soil per tree was transferred to 60 μL of Qiagen PowerSoil DNA buffer (Qiagen, Germantown, MD, USA) and stored at 4°C until processed. Additional soil was air dried for soil pH and soil electrical conductivity (EC) measurements. Pine roots were processed separately. Roots were washed in deionised (DI) water, and healthy, ECM-colonised roots were sorted into morphotypes using a dissecting microscope (Agerer 1995). Up to 10 root tips from each morphotype were blotted dry on a kimwipe and pooled (approximately 50–100 root tips per sample) in one tube containing DNA-sterile cell lysis solution (CLS; Lindner and Banik 2009) which contains cetyltrimethylammonium bromide (CTAB). We aimed to include representatives of

all morphotypes that we observed in each sample. Samples were stored at −20°C until processed.

2.2 | Manipulative Salinity Experiment on Seedlings

Soils from 3 of the 8 mature pine sites were sampled as the initial soils prior to salinity treatments (Figure 1): Larry and Penny Thompson Park (LPT), Navy Wells Preserve (NWP) and Big Pine Key (BPK). At each site, soil was collected from beneath 10 randomly selected mature pines that were 10–30 m apart. The soil was sieved once through a 2.5 mm sieve and homogenised as described above. To determine the pool of fungi in the initial soil prior to the salinity treatments, 250 mg of fresh soil was taken from each tree (10 trees × 3 sites, $n = 30$ soils) and preserved for DNA extraction (see below). Subsamples of the initial soils were also air-dried and sent to the UF-IFAS Analytical Services Laboratory (Gainesville, FL, USA) and analysed for Calcium (Ca^{2+}), soil electrical conductivity (EC), Magnesium (Mg^{2+}), Ammonium (NH_4N), Nitrate (NO_3N), Sodium (Na^{+}), Phosphorus (P) and pH (Table S2). The remaining soil was used to plant seedlings for the manipulative salinity experiment.

Pinus densa seeds were obtained from Andrews Nursery (Chiefland, FL, USA). Seeds were surface sterilised with 30% H_2O_2 and a drop of Tween 20 for 10 min and then rinsed and soaked overnight with autoclaved water. Seeds were cold-stratified for 10 days at 4°C before being planted. A calcinated clay product, Turface (Turface Athletics MVP, Buffalo Grove, IL, USA), was autoclaved twice and then mixed in a 2:1 field soil:Turface ratio and placed in 50 mL cone-tainers (Stuewe & Sons Inc., Tangent, OR, USA). Polyester fibre fill, Pol-fil (Fairfield Processing Corporation, Danbury, CT, USA) was added to the bottom of each cone-tainer to prevent soil leakage. Greenhouse negative control seedlings ($n = 20$) were used to verify that there was no ECM inoculum contamination in the greenhouse environment. These were planted in a mixed 3:1:1 ratio of sterilised topsoil:sand:Turface (Turface Athletics, Buffalo Grove, IL, USA). For all treatments, three seeds were planted per cone-tainer and grown for 1 month, after which only the most vigorous seedling was left, and the two remaining seedlings were removed. Throughout the entire experiment (a 1-month establishment period followed by 6 months of growth), seedlings were grown inside a greenhouse at the Tropical Research and Education Center (Homestead, FL, USA) at ambient light and temperature (average 35°C), watered three times per week, and fertilised once a week with 10 mL of 1:10 Hoagland's solution (Sigma Aldrich, St. Louis, MO, USA).

We selected realistic, sublethal salinity levels that mimic natural differences observed along existing salinity and elevation gradients (Table S2) and that were comparable to previous experiments targeting pine rockland plants (Goodman et al. 2012; Zwiazek et al. 2019). Salinity concentrations were chosen to accurately reflect how gradual salinity increases might occur in this ecosystem (i.e., salt spray from storms, drier conditions, gradual sea level rise, saltwater intrusion) and ensured that there was an appropriate attenuation period before starting the experiment to reduce osmotic stress. Salinity treatments were made by mixing DI water with Instant Ocean Sea Salt (Pentair Aquatic Ecosystems Inc., Apopka, FL, USA). The four salinity treatments were: control (deionised water, 0.006 mS/cm), low (25 mM, 4.38 mS/cm), medium (50 mM, 8.72 mS/cm) and high (75 mM, 12.36 mS/cm). Based on soil availability, each salinity × site treatment consisted of 3–5 replicate seedlings (mean 4.79 ± 0.52 seedlings). To prevent plant osmotic shock, all salinity treatment seedlings were initially watered at the low salinity level (25 mM) and then salinity was gradually increased (i.e., 2 weeks after the start of the experiment the medium and high seedlings were increased to 50 mM salt and then after two additional weeks the high salinity treatment was increased to 75 mM). The total number of seedlings (herein called 'treatment seedlings'), including greenhouse controls, was 595 (Figure S1).

Treatment seedlings were harvested by removing the roots and shoots from the soil. Soil from five seedling replicates in each treatment was pooled and air-dried for post-experimental pH and EC measurements (10 initial soils × 3 sites × 4 salinity treatments = 120 soil samples). Soils were mixed in a 1:2 soil:water ratio, and both pH and EC were measured using a Fisher Scientific accumet AB200 (Fisher Scientific, Waltham, MA, USA).

Ectomycorrhizal root tips from seedlings were harvested as described for the mature pines and individually placed into sterile 1.5 mL tubes with CLS buffer. ECM root tips were then frozen at −20°C, heated for 2 h at 65°C and crushed with a sterile micropestle. Then 100 μL of CLS buffer was removed from each replicate seedling and combined by soil × salinity × site treatment prior to DNA extraction (10 initial soils × 3 sites × 4 salinity treatments = 120 treatment seedling's ECM root tips). We pooled DNA for each initial soil × salinity × site treatment (120 samples, one for each set of 5 replicate seedlings) rather than processing each seedling sample separately (595 samples, one for each individual seedling) because a preliminary experiment determined that Illumina sequencing from pooled samples were similar to results from sequencing the ECM fungi from individual seedlings and then combining them bioinformatically ($p = 0.74$, Figure S2).

2.3 | Molecular Methods

DNA was extracted from soil samples using the Qiagen PowerSoil DNA kit following the manufacturer's instructions. Fungal DNA from root tips was extracted via a modified glass milk extraction protocol (Jusino et al. 2014). Fungal DNA from soils and mature pine roots was amplified using ITS1F (Gardes and Bruns 1993) and ITS2 (White et al. 1990) modified with Illumina Nextera v2 adapters (Illumina, San Diego, CA, USA), following PCR conditions in (Karlsen-Ayala et al. 2023) with additional details in Table S3. DNA from the samples that did not initially amplify was used in subsequent PCR amplifications using 1:20, 1:50, 1:100 and 1:1000 dilutions. Amplicons were dual indexed with indices from the Illumina Nextera v2 kit (Illumina, San Diego, CA, USA) in an eight-cycle PCR reaction. Indexed products were cleaned with the Zymo Select-A-Size Clean up kit (Zymo Research, Irvine, CA, USA). For quality control, we used negative PCR

and extraction controls and two mock communities (positive controls), one biological mock (*Amanita* sp., *Entoloma* sp., *Lactarius* sp., *Sphaerosporella* sp., *Suillus decipiens*, *Delastria* sp. and *Tomentella* sp.) and SynMock (Palmer et al. 2018). DNA concentration and quality of cleaned products were measured using a Qubit 4.0 Fluorometer (ThermoFisher Scientific, Madison, WI, USA) with the HS-DS-DNA kit (Invitrogen, Carlsbad, CA, USA). Indexed libraries were then equilibrated and pooled. Two libraries were sequenced on the Illumina MiSeq (Illumina, San Diego, CA, USA) using 2 × 300 paired-end v3 chemistry at the Interdisciplinary Center for Biotechnology Research (ICBR) at the University of Florida (Gainesville, FL, USA).

2.4 | Bioinformatics

Both Illumina libraries were concatenated and processed with zero mismatches in the indices using the AMPtk pipeline (version 1.5.3, https://amptk.readthedocs.io/en/latest/; Palmer et al. 2018). ITS1 barcode reads were merged using VSEARCH (version 2.18.0; Rognes et al. 2016) before removing the primers. Reads < 125 bp were discarded, reads > 300 bp were trimmed, and reads 125–300 bp were padded with Ns before clustering. To generate operational taxonomic units (OTUs), sequences were filtered based on quality and denoised using the UNOISE3 algorithm (Edgar 2010) with expected errors less than 0.5, and the resulting ASVs were clustered into OTUs at 97% similarity. Singletons were removed. Both of our mock communities were used to parameterise our bioinformatics. SynMock was used to determine the rate of index bleed using the filter module in AMPtk (Palmer et al. 2018). Taxonomic assignment of OTUs was conducted using the hybrid taxonomy algorithm in AMPtk, and all non-fungal reads were removed. FUNGuild (v1.1) was used to assign guilds (Nguyen et al. 2016). FUNGuild assigns 'possible', 'probable' and 'highly probable' rankings to each guild assignment. Because we focused on ECM fungi, we manually curated FUNGuild assignments of 'ectomycorrhizal fungi' with rankings of 'possible' and 'probable'. We performed NCBI BLAST searches and verified whether OTUs were ECM or non-ECM based on the ECM lineages identified by Tedersoo and Smith (2013).

2.5 | Statistical Analyses

All analyses were conducted in R (v3.4.2; Core R Team 2019). ECM community analyses were performed using the 'vegan' package (v2.5–6; Oksanen et al. 2015). To test if differences in the ECM community composition in roots of treatment seedlings were attributed to treatment type or site, we used a non-parametric permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) with the *adonis2* function, and tests for multivariate dispersion were performed with the *betadisper* function (Anderson 2006). NMDS and multivariate hypothesis tests were based on the βsim distance metric using 999 permutations from a presence/absence community matrix. These tests were also used on the presence/absence community matrix from the soil dataset to determine if the site had a significant effect on the ECM community. To assess the ECM communities associated with mature pines and treatment seedlings from the salinity manipulation experiment, we conducted a distance-based Constrained Correspondence Analysis (CCA) with βsim distance metric using the *capscale* function in *vegan*. To determine which environmental variables best explained the variation in ECM communities associated with mature pines and with treatment seedlings, we used a forward selection process using the *ordiR2step* function in *vegan* based on adjusted R^2 and *p*-values using: vif score < 10, variable significance $p < 0.01$, and 999 permutations (Blanchet et al. 2008). The *anova.cca* function in the *vegan* package was used for significance tests of the full model. The mature pines dataset included distance from the ocean, elevation, habitat patch size, soil pH, and soil EC as variables. Soil variables for the seedling manipulation experiment included Ca^{2+}, electrical conductivity (EC), distance from the ocean, Mg^{2+}, NH_4N, NO_3N, Na^{+}, P and pH as measured from the field soils, as well as the EC and pH measured at the end of the greenhouse experiment. Soil EC is a composite measure of all soil salts that was highly correlated with Ca^{+2}, Na^{+} and Mg^{+2}, so we used EC and excluded the individual salts from the model. Additionally, habitat patch size and elevation had high collinearity with EC and were excluded from the model.

For the mature pine dataset, we obtained data on elevation (en-gb.topographic-map.com), distance to the ocean (Google Maps measure tool) and pine rocklands habitat patch size at each site (data obtained from site managers). We examined correlations between OTU richness and the soil and site variables (soil EC, soil pH, elevation, distance to the ocean and habitat patch size) in R using the Spearman rank test with the function *cor.test*.

Diversity and richness measurements were performed on the presence/absence data set using the *alphaDiversity* function in the *otuSummary* package, and a one-way ANOVA was used to test for significant differences among treatments using the *aov* function. To test for significant differences in pine seedling mortality and differences in ECM richness and diversity due to salinity treatments, we used one-way ANOVAs and a post hoc Tukey test using the *aov* and *TukeyHSD* functions. All data were visually checked for normality via a Bartlett and Shapiro test in base R. We performed indicator species analyses using the *multipatt* function of the indicspecies package (Cáceres and Legendre 2009). We used the indval.g statistic and 999 permutations to determine potential indicator ECM fungi that might be preferentially associated with different salinity treatments or might be associated with the seedlings versus mature pines.

3 | Results

Overall, we generated a total of 20,027,277 denoised reads (excluding controls) for the mature pines and soils, initial soils and seedling root tip data set that grouped into 1760 fungal OTUs with an average read number of 48,966 per sample (range 6302–113,055 reads) and an average of 43 ECM OTUs per sample (range 1–233 OTUs). Additional information on read numbers can be found in Table S4. We recovered all members of our biological mock community and the synthetic

mock community. Species accumulation curves indicated that sequencing depth was sufficient since most sites reached a plateau (Figure S3).

3.1 | Ectomycorrhizal Fungi Associated With Mature Pines

For the mature pine trees, we amplified fungal DNA from ECM-colonised roots from 76 of 80 trees and 72 of 80 soils. Using a forward model selection with *ordiR2step*, the best fit model for ECM fungi composition in the soil and roots included elevation, distance to the ocean, habitat patch size, EC and pH (Table 1 and Figure 2). The southern sites (BPK, NNK) were positively associated with EC whereas the northern sites (DEER, ZOO and LPT) were negatively associated with EC and positively associated with elevation. The central sites (TREC, MOON and NWP) were positively associated with distance from the ocean and pH.

We also examined correlations between ECM fungi OTU richness and site variables. The average ECM OTU richness per region was as follows: northern DEER (91), ZOO (97), LPT (102), central MOON (57), TREC (96), NWP (78), southern NNK (41) and BPK (38). OTU richness was positively correlated with elevation ($p=0.024$, $R^2=0.78$) and negatively correlated with EC ($p=0.0075$, $R^2=-0.85$; Figure 3). It is notable, however, that elevation and EC are weakly but not significantly correlated ($p=0.07$, $R^2=-0.68$). On the contrary, OTU richness was not correlated with distance to the ocean, soil pH or habitat patch size. The BPK site is significantly larger than other pine rockland sites, so it was excluded from this analysis as an outlier (Figure 3). Yet even with this site included, the correlation between patch size and OTU richness was not significant (Figure S4). We ran additional correlation tests between these variables and found that distance to the ocean and elevation ($p=0.75$, $R^2=-0.13$) and EC and distance to the ocean ($p=0.45$, $R^2=-0.31$), and EC and pH ($p=0.84$, $R^2=-0.84$) were not correlated (Figure S4).

3.2 | Ectomycorrhizal Fungal Communities From Initial Soils Used in the Salinity Manipulation Experiment

We examined the ECM fungal communities in the initial soils prior to salinity treatments from three sites (BPK, NWP and LPT) used for the salinity manipulation experiment. Some samples could not be amplified via PCR or did not contain detectable ECM fungi. ECM fungi were detected in 3 of 10 samples from BPK, 7 of 10 samples from NWP and 10 of 10 samples from LPT. The ECM communities were also significantly different among sites (adonis $R^2=0.29$, *pseudo-F* = 3.6, $p=0.001$) with non-significant effects of dispersion (betadisper $p=0.10$).

TABLE 1 | Output parameters from our *ordiR2step* forward model selection for the constrained ordination and the corresponding Akaike Information Criterion (AIC), *F*-statistic, *p*-value and adjusted R^2 value. Top table refers to ectomycorrhizal (ECM) fungi detected on mature *Pinus densa* trees (see Figure 2A), the middle table refers to ECM fungi in soils prior to salinity experiment (see Figure 4), and the bottom table refers to ECM fungi detected on *Pinus densa* seedlings at the end of the salinity experiment (see Figure 5D).

Forward selected variables	AIC	*F*-statistic	*p*	R^2 adjusted
ECM fungi associated with mature pines				
Elevation	598.68	5.87	0.002	0.058
Elevation + distance from ocean	595.19	5.48	0.002	0.112
Elevation + distance from ocean + electrical conductivity (EC)	594.52	2.61	0.002	0.133
Elevation + distance from ocean + electrical conductivity (EC) + pH	593.91	2.54	0.002	0.155
ECM fungi in soils prior to salinity experiment				
Electrical conductivity (EC)	45.34	3.50	0.002	0.14
Electrical conductivity (EC) + distance from ocean	44.65	2.46	0.006	0.22
Electrical conductivity (EC) + distance from ocean + NO_3N	44.21	2.10	0.026	0.29
ECM fungi on seedlings after salinity experiment				
Electrical conductivity (EC)	400.16	5.85	0.002	0.082
Electrical conductivity (EC) + treatment	396.38	3.25	0.004	0.205

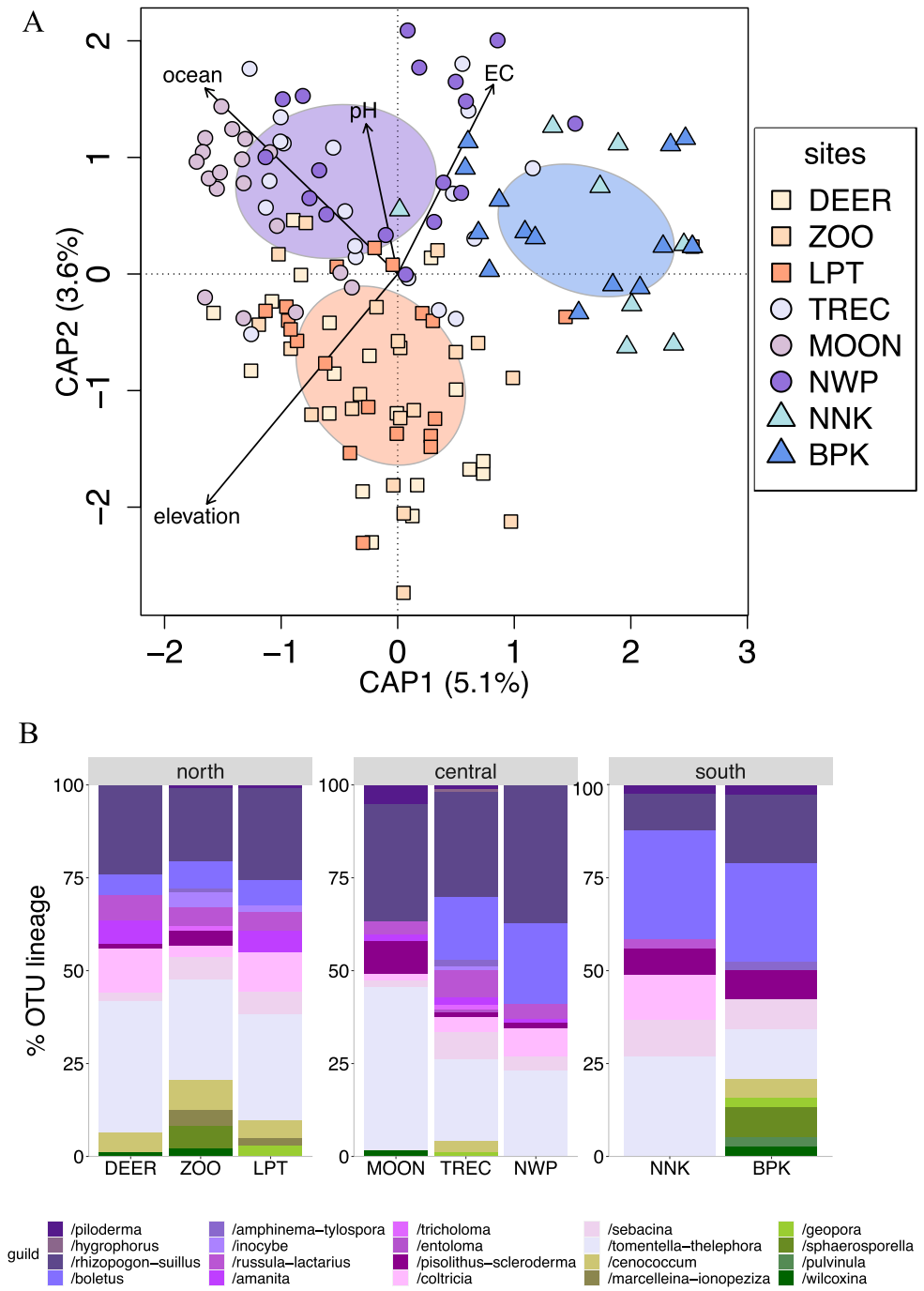

FIGURE 2 | (A) Constrained canonical ordination (CCA) of ectomycorrhizal fungi from healthy root tips and corresponding soils from mature *Pinus densa*. Arrows indicate variables that show significant impact on community composition; distance to the ocean, soil pH, soil electrical conductivity (EC) and elevation ($p < 0.05$). Northern sites are designated with orange squares (Deering Estate—DEER, Miami Zoo—ZOO, Larry & Penny Thompson—LPT), central sites are designated with purple circles (Private rocklands—MOON, Tropical Research and Education Center—TREC and Navy Wells Preserve—NWP), and southern sites are designated with blue triangles (No Name Key—NNK, Big Pine Key—BPK). (B) Relative frequency of all ectomycorrhizal fungal lineages recovered from mature pine roots.

Based on *ordiR2step* model selection, the best fit model included three variables that were the best predictors of ECM community composition: EC, distance to the ocean and NO_3N (Table 1 and Figure 4). Southern soils (BPK) were associated with higher EC and NO_3N values, and central sites were associated with greater distances from the ocean.

3.3 | Manipulative Salinity Experiment

A total of 542 of 575 treatment seedlings (and 18 of 20 greenhouse control seedlings) survived the manipulative salinity experiment. Soil EC and pH were measured after the experiment, and this post-experiment soil EC (EC-post) was

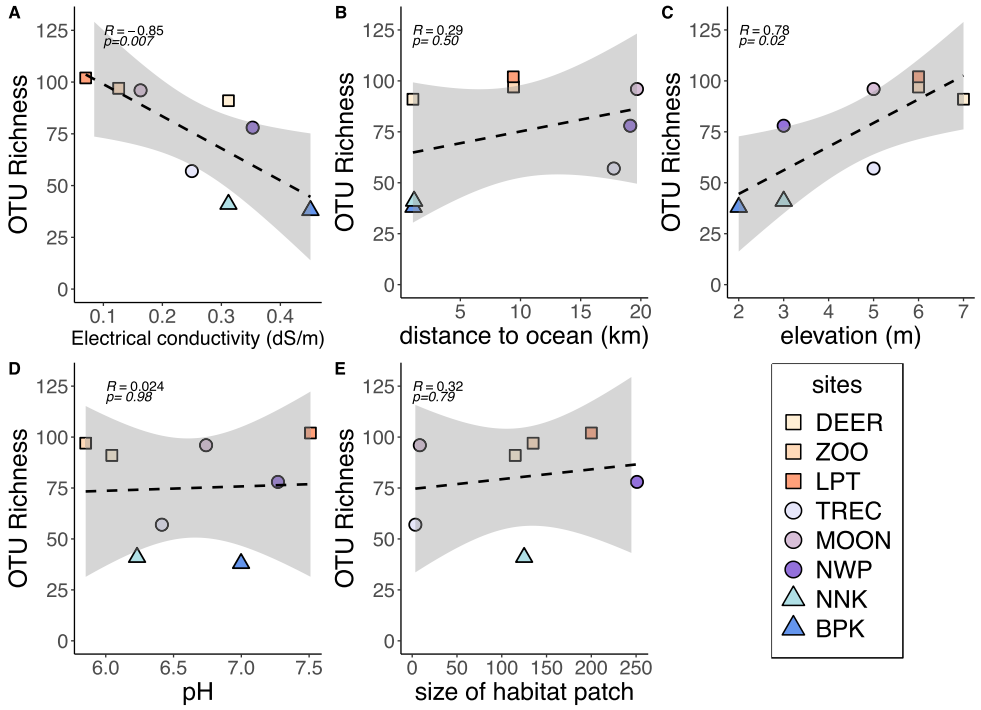

FIGURE 3 | Spearman correlations between average ectomycorrhizal operational taxonomic unit (OTU) richness in mature *Pinus densa* and measured variables along with the corresponding R^2 and p-values ($p < 0.05$): (A) soil electrical conductivity (dS/m), (B) distance to the ocean (km), (C) elevation (m), (D) soil pH and (E) average habitat patch size (in ha). Northern sites are designated with orange squares (Deering Estate—DEER, Miami Zoo—ZOO, Larry & Penny Thompson—LPT), central sites are designated with purple circles (Private rocklands—MOON, Tropical Research and Education Center—TREC, and Navy Wells Preserve—NWP), and southern sites are designated with blue triangles (No Name Key—NNK, Big Pine Key—BPK). All variables depicted are based on averages at each site. Big Pine Key (BPK) was removed from the analysis of average habitat patch size (E) because it is an outlier, but Figure S4a depicts a similar pattern for average habitat patch size even when BPK is included.

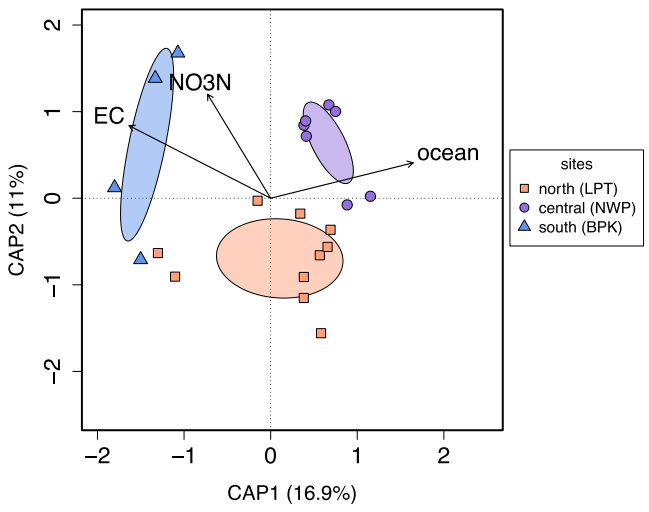

FIGURE 4 | Constrained canonical ordination (CCA) of initial soils used in the manipulative salinity experiment. Arrows indicate variables that show significant impact on ectomycorrhizal fungi community composition; soil electrical conductivity (EC), distance to the ocean and soil ammonium (NO_3N) ($p < 0.05$). The northern site (Larry & Penny Thompson—LPT) is designated with orange squares, the central site (Navy Wells Preserve—NWP) is designated with purple circles, and southern site (Big Pine Key—BPK) is designated with blue triangles.

significantly different among treatments ($F = 46.68$, $p < 0.001$, Figure 5A) whereas soil pH (pH-post) was not ($p = 0.16$; Figure 5B). Seedling mortality was also significantly different between treatments ($F = 4.70$, $p = 0.036$, Figure 5C); 27 seedlings died in the high salinity treatment, whereas only 5 died in the medium salinity treatment, and 1 died in the low salinity treatment (Figure 5C).

At the end of the experiment, we successfully amplified fungal DNA for 119 of 120 of the pooled seedling treatments and 25 of the 30 initial soils. These post-experiment roots and soils contained 55 ECM OTUs from 11 ECM lineages. Electrical conductivity measured at the end of the experiment (post-EC) and salinity treatment × post-EC (Figure 5D) were the best predictors of ECM fungal community composition (Table 1). Seedlings from the high salinity treatment were positively associated with soil EC, whereas seedlings from the control and low salinity treatment were negatively associated with soil EC. Our indicator species analysis identified one indicator, OTU136 Pyronemataceae that was positively associated with the high salt treatment (Table S5).

For most sites, ECM diversity on seedlings was not statistically different among treatments. Observed richness was lower in the highest salinity treatments for all sites, but only significantly

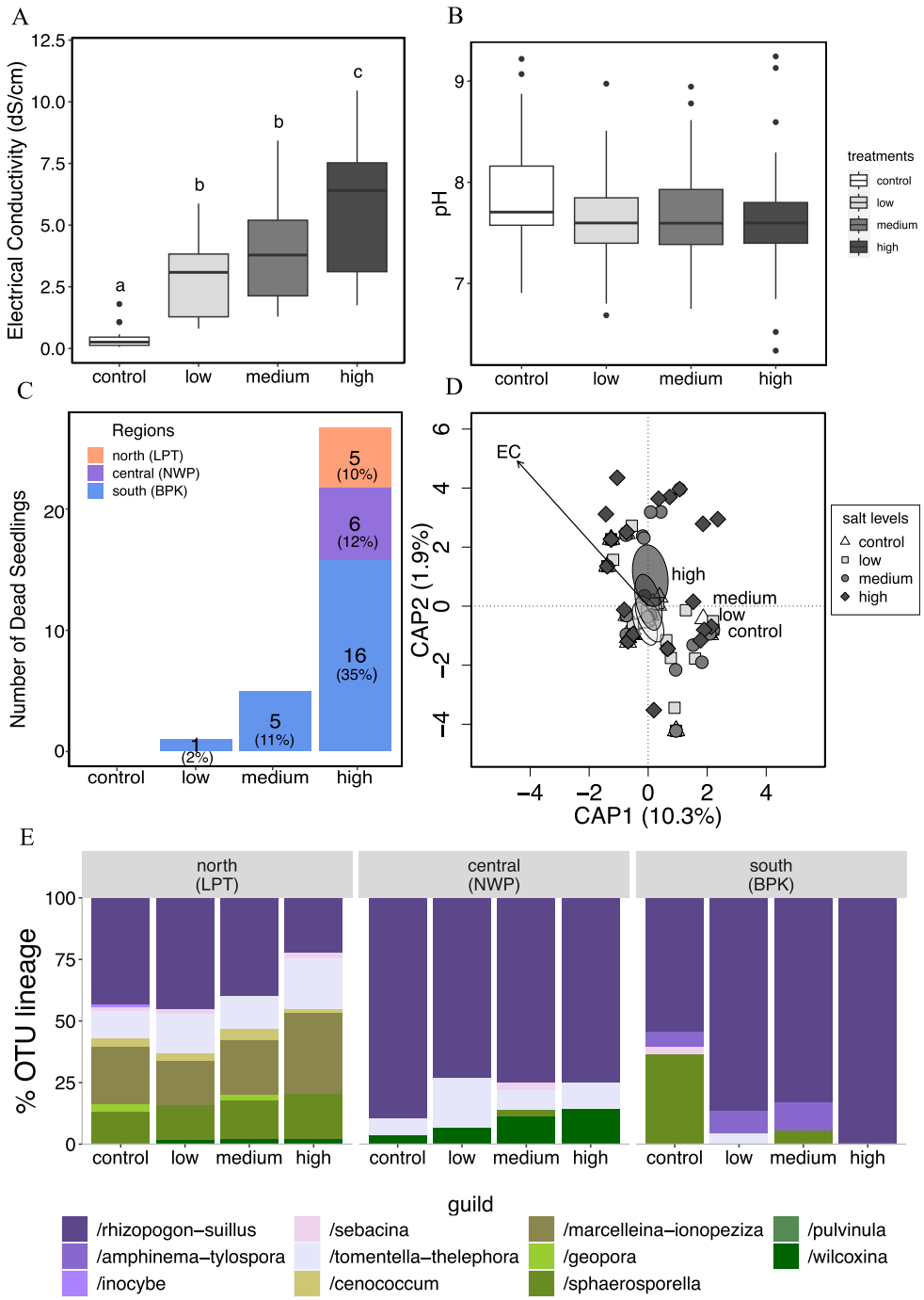

FIGURE 5 | Soil variables and ectomycorrhizal (ECM) fungi community composition on the roots of *Pinus densa* seedlings at the end of the manipulative salinity experiment. Boxplots showing average soil (A) post-experimental electrical conductivity (EC), (B) pH measured on treatment soils after the end of the experiment, (C) Number of dead seedlings at the end of the experiment per treatment and by site. Percent mortality for each treatment is listed below the number of dead seedlings on each bar. (D) Constrained canonical ordination (CCA) depicts the ectomycorrhizal fungi community on seedling roots by pooling the five seedling replicates from each treatment (salinity × site × tree). Arrows indicate significant environmental variables that influence the community composition of ECM fungi ($p < 0.05$). Colour coded points correspond to treatment type: White triangles (control), light grey squares (low salinity), medium grey circles (medium salinity) and dark grey diamonds (high salinity). Ellipses represent standard deviation around the centroid of each treatment. (E) Relative frequencies of all ECM fungal lineages from the northern (Larry & Penny Thompson—LPT), central (Navy Wells Preserve—NWP) and southern (Big Pine Key—BPK) sites. The ECM lineages present on seedlings are listed according to the nomenclature of Tedersoo and Smith (2013). Ascomycota ECM lineages in greenish colours, all other taxa are Basidiomycota.

lower for LPT (BPK: $p = 0.52$, NWP: $p = 0.49$, LPT: $p = 0.01$; Figure S5). Simpson diversity was only significantly lower at the highest salinity for NWP (BPK: $p = 0.65$, NWP: $p = 0.03$, LPT: $p = 0.09$). Shannon diversity was not significantly different for any site under any treatment (BPK: $p = 0.62$, NWP: $p = 0.10$, LPT: $p = 0.08$, Figure S5).

3.4 | Comparisons Between Mature Pines and Treatment Seedlings

Mature pines hosted a total of 92 ECM OTUs, while seedlings hosted 34 OTUs, with only 22 shared OTUs (Figure 6 and Table S6). We found 22 OTUs from the seedlings and 52 OTUs from the mature trees (total 74 OTUs) that were only present in one sample. Most notably, *Rhizopogon* was the most prevalent genus on the seedlings, with few *Suillus* species recovered, whereas *Suillus* was the most prevalent genus recovered on mature pines. Both these genera belong to the /rhizopogon-suillus lineage. The indicator species analysis identified 17 OTUs that were preferentially associated with mature trees and 6 OTUs that were preferentially associated with seedlings. This analysis also confirmed that *Rhizopogon* species were preferentially associated with seedlings, whereas *Suillus* species were preferentially associated with mature pines (Table S5).

The most prevalent taxa associated with mature pines aside from *Suillus* were *Cenococcum*, *Retiboletus* and Thelephoraceae. We recovered 20 ECM lineages from the roots of the mature pines, including OTUs in /amanita, /boletus, /coltricia, /entoloma, /hygrophorus, /piloderma, /pisolithus-scleroderma, /russula-lactarius and /tricholoma; most of these ECM lineages were not detected in seedlings (Figure 3B). The most prevalent genera associated with seedlings aside from *Rhizopogon* were *Sphaerosporella* and an undescribed genus in Pezizaceae; only 11 ECM lineages were found in treatment seedlings. Most of the lineages found in the seedlings were also found on mature pines, except for 13 OTUs belonging to the genera *Amphinema* and *Sphaerosporella*, as well as species in the Pezizaceae.

In the manipulative salinity experiment, the /rhizopogon-suillus lineage was the most prevalent across all sites and treatments. Higher ECM lineage diversity was found in the LPT soils (7 lineages) compared to BPK (5 lineages) and NWP (4 lineages). Seedlings grown in soil from two sites (LPT and NWP) were more heavily colonised by Ascomycota ECM lineages (e.g., /wilcoxina, /sphaerosporella) after the higher salinity treatments, but in soils from BPK the /rhizopogon-suillus lineage was the only lineage present at the highest salinity treatment (Figure 5E).

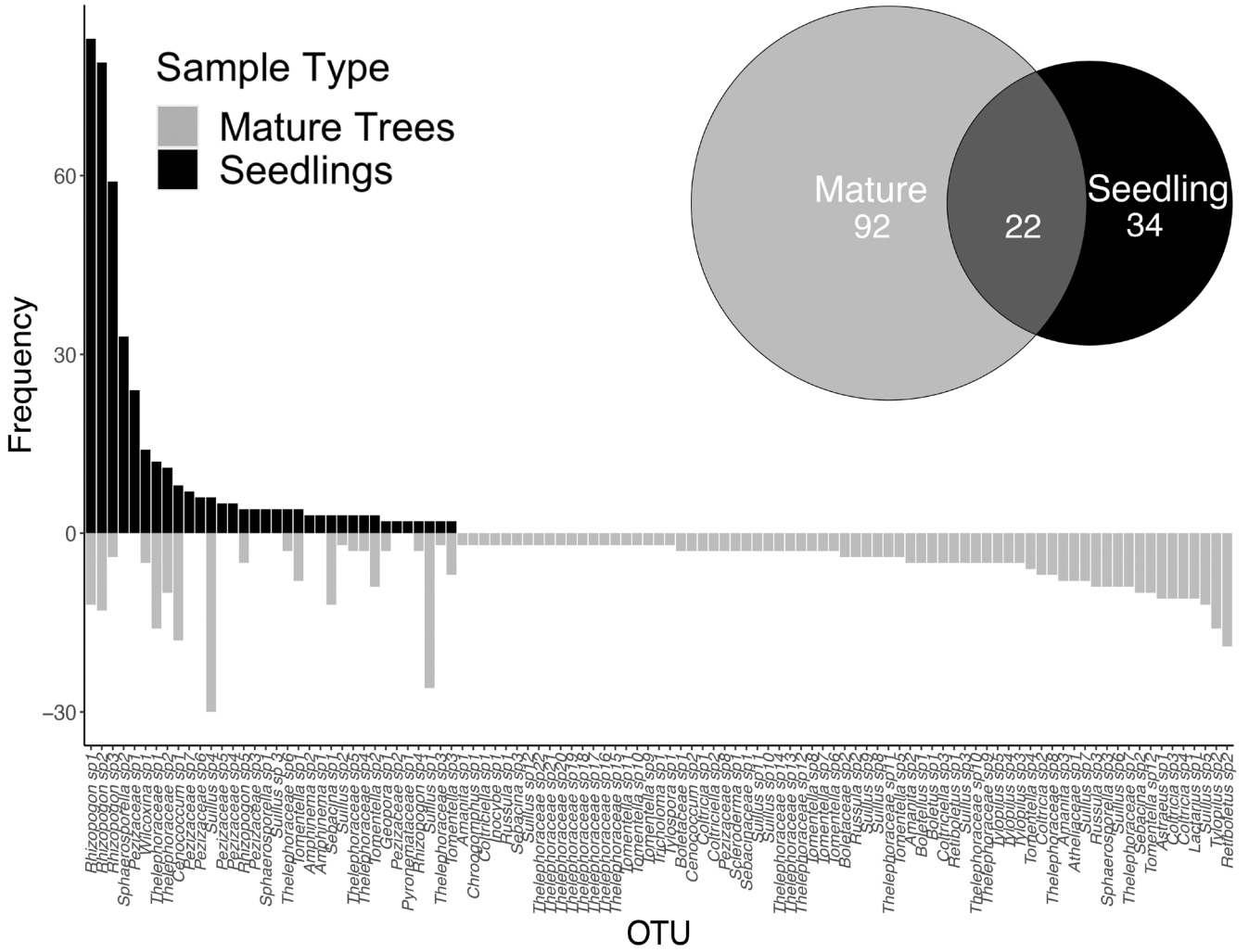

FIGURE 6 | Frequency of ectomycorrhizal (ECM) operational taxonomic units (OTUs) recovered from root tips of *Pinus densa* seedlings (black) and mature *Pinus densa* trees (grey). Only OTUs that were detected from two or more samples are shown (74 singleton OTUs found only in one sample are not shown). Venn diagram depicts the 92 total ECM OTUs detected on the roots of mature *Pinus densa*, the 34 OTUs detected on the roots of *Pinus densa* seedlings, and the 22 OTUs that were detected on the roots of both seedlings and mature trees.

4 | Discussion

This study is unique and provides insight into the ECM fungi on mature pine trees across a salinity gradient and also tests the community-level responses of ECM fungi to increased salinity in the same system. Both of our data sets showed that salinity was the most important variable in structuring ECM fungi, suggesting that increasing salinity is likely already impacting the fungi in the fragile rocklands ecosystem (Figures 2–5). We also determined that elevation and distance from the ocean are important related variables that impact ECM fungi in this system, even if they are not always directly correlated with salinity (Figure 2). The ecosystem is in a precarious position; all pine rocklands are 0.5–30 km from the ocean, less than 10 m above sea level, and subject to impacts from catastrophic hurricanes (Ross et al. 2009; Snyder et al. 1990). Given the fact that *Pinus densa* (Ross et al. 2009) and some pine-associated ECM fungi from other systems (Branco et al. 2022) are sensitive to increased salinity, we hypothesised that variation in soil salinity (soil electrical conductivity, EC) is a critical factor that impacts ECM fungal communities in the rocklands; not only in mature pines but also in seedlings (Figures 2 and 5 and Table 1). In ECM-colonised roots and the soil of mature pines, EC significantly influenced the structure of the ECM community composition and higher soil salinity was also correlated with lower ECM fungal richness (Figures 2 and 3). We found a similar pattern in the manipulative experiment whereby EC of the initial soils (prior to salt addition) and the post-experimental soils (measured after months of salinity inputs) were the most significant predictors of ECM community composition (Figures 4 and 5).

Salts are difficult to remediate from soils, which means that increased soil salinization is an irreversible process that creates new baselines for ecosystems (Ondrasek and Rengel 2021). Previous work demonstrated that there is a natural soil salinity gradient with elevated EC levels at the southernmost pine rocklands site (BPK) that are on average 5× higher than in the central site (NWP) and 12× higher than in the northernmost site (LPT) (Table S1; Karlsen-Ayala et al. 2023). This pattern was even more exaggerated for soil sodium (Na^+); Na^+ at the southern sites was up to 35× higher than northern sites (Table S2). These baseline soil measurements combined with the soil EC at the end of the manipulative experiment show that salt accumulation in the soil over time influences both the ECM communities associated with mature *P. densa* (Figure 2) and the ECM fungi that colonise pine seedlings from spores (Figure 5). Elevation, which was weakly but not significantly correlated with soil salinity ($p = 0.07$, $R^2 = -0.68$), was also an important predictor positively correlated with fungal richness on mature pines (Figure 3). Elevation has previously been shown in other studies to influence ECM communities, but typical elevation gradients in other studies span hundreds or even thousands of meters and are often correlated with differences in plant communities (Geml et al. 2017), soil pH (Truong et al. 2019), and other important environmental factors (Bahram et al. 2012; Gong et al. 2022). It is possible that other environmental factors which were not measured in this study, such as water retention, burn frequency or soil type, may also be correlated with elevation and therefore impact ECM richness. Regardless, it was surprising that elevation had such a profound impact on the ECM fungi communities even though the pine rocklands we studied contain only a single ECM host species and span a small elevation range of only 5 m (Figure 3). Previous studies have shown that elevation is positively correlated with *Pinus densa* seedling establishment and survival (Harley et al. 2015; Ross et al. 1994), and that elevational changes and depth of the water table drive plant community composition and diversity in pine rocklands (Schneider et al. 2024). Our data are the first to indicate that lower elevation sites also support different and less diverse ECM fungal communities, which suggests that elevational differences, even when they are only a few meters, are of critical importance within the pine rocklands (Figures 2 and 3).

Ectomycorrhizal fungal communities associated with mature pines were most influenced by one edaphic variable (soil EC) and two geographic variables (elevation, distance from the ocean) but these variables were not strongly correlated with each other (Figure S4). Based on data from previous studies (Geml et al. 2017; Glassman et al. 2017; Tedersoo et al. 2020), we expected that soil pH (which ranged 6.4–7.6) would strongly impact the ECM fungal communities in this system. Interestingly, soil pH was the least influential of the significant variables impacting ECM fungi in mature pines, and soil pH did not significantly influence the ECM fungi community or species diversity in seedlings during the manipulative experiment (Figure 5). We found similar patterns between our mature soils and initial soil communities, that distance from the ocean was strongly associated with central sites and soil EC was strongly associated with southern sites. Our study also found that nitrate (NO_3N) was a significant variable shaping the initial soil communities and was associated with southern sites; therefore, it is likely that similar patterns may apply to mature tree communities, although we currently lack the data to confirm this hypothesis. Based on island biogeography theory (Kirkby et al. 1968) and previous studies of 'islands' of *Pinus* in California (Peay et al. 2007, 2010), we also hypothesised that larger pine rocklands patches would host more diverse ECM fungi communities. However, we found no correlation between ECM fungi richness and habitat patch size, regardless of whether the large patch of habitat at BPK was considered or not (Figure 3 and Figure S3). We also found high collinearity between soil EC and habitat patch size for the initial soils that were used from three of our sites for planting our salinity experiment. There are many potential factors that might explain this observation, but it is possible that the heterogeneous, open, savannah-like pine rocklands may be functionally different from typical forest habitats when it comes to hosting ECM diversity. It is important to note that we could not easily account for management history for each of the rocklands patches, and it is possible that past disturbances, such as fires or hurricanes or other unmeasured factors, may also have influenced the ECM richness and community dynamics (Harris et al. 2021; Possley et al. 2008). Another important point to note is that the sites in the Keys are over 100 km from mainland pine rocklands habitat. So, even though patch size was not significantly correlated with ECM richness, it is possible that dispersal limitation could also be playing a role in the lower species richness in these more isolated southern sites.

Since forest regeneration requires seedling establishment, we wanted to experimentally test the responses of seedlings and their ECM fungi to increasing salinity. Our results show that higher salinity is strongly correlated with seedling mortality

and that even moderate increases in soil EC were detrimental to seedlings (Figure 5). Even though 27 out of 146 seedlings died in our high salinity treatment (and therefore the ECM fungi on these seedlings were also lost), we nonetheless documented a strong signal that soil salinity impacted ECM fungal communities, whereas soil pH did not (Figure 5D). Based on the results from control seedlings, the starting ECM communities and initial soil salinities also influence the downstream response to increased salinity. For the central (NWP) and northern (LPT) sites, increased salinity resulted in slight increases in the proportion of roots colonised by Ascomycota, including OTUs of /cenoccocum, /marcelleina-ionopeziza, /geopora, /sphaerosporella, /pulvinula and /wilcoxina (Figure 5E). Many of these Ascomycota lineages are thought to withstand stresses (such as high heat, drought, fire and salinity) better than some Basidiomycota because of traits such as large spores, thick cell walls and melanised tissues (Fernandez et al. 2023; Fujimura et al. 2005; Gordon and Gehring 2011; Tedersoo et al. 2006). Interestingly, we did not find this trend in the seedlings grown in the soils from BPK, the southernmost site with the highest salinity. Instead, our highest salinity treatments were completely dominated by the genus *Rhizopogon*. In fact, many seedlings grown at the highest salinity treatments with BPK soils contained only a single *Rhizopogon* OTU, which was identified by indicator species analysis as significantly associated with the high salt treatment (Tables S4 and S5). This reduction to only one or a few species from a single ECM lineage suggests the possibility that the other ECM fungi present in the soil had reached their upper salinity threshold and were no longer able to germinate normally and colonise ECM roots. Alternatively, it is possible that the added salinity tipped the competitive balance among ECM fungi and allowed *Rhizopogon*, which are highly competitive and known to readily colonise seedlings from spores (Kennedy et al. 2011; Moeller and Peay 2016), to dominate the system. Suilloid fungi are well documented as a dominant group of ECM fungi, as evidenced by their role in pine invasions in South America (Policelli et al. 2018) and their high prevalence after fires or in other high stress environments (Bidartondo et al. 2001; Glassman et al. 2016; Izzo et al. 2006). Our data suggest that suilloid fungi (particularly native *Rhizopogon* species that germinate readily from spores, are stress tolerant, and are highly competitive) may be a useful target group to employ for use in future restoration efforts (Policelli et al. 2018).

5 | Future Directions

Most studies have tested the tolerance of focal ECM fungi and/or plant species under increased salinity (i.e., Bandou et al. 2006; Wen et al. 2022) or have described the ECM community structure of mature trees in highly saline environments (i.e., Ishida et al. 2008; Thiem et al. 2018). Our experiment assessed seedling survival under four salinity levels and identified that fungi such as *Rhizopogon* were associated with the highest salt tolerance. This knowledge can be the basis for future in vitro screening for salt tolerance and genomic adaptation to high salt environments. Fungal isolates with the highest salt tolerance could be used for subsequent targeted seedling inoculation experiments to determine their potential applications in *P. densa* restoration efforts. Our data suggest that larger habitat patches at higher elevations might be better suited for *P. densa* restoration because high salt, low elevation sites present challenges. In the future, it will be critical to understand whether high salinity directly inhibits or kills ECM fungi, whether salinity mediates competition between ECM fungi that results in lower diversity on seedlings, or whether high salinity impacts carbon fixation by the host plants with subsequent downstream impacts on the ECM community (or perhaps some combination of these factors). Our study also established a baseline of the ECM communities associated with the roots of mature pines and highlights the need for long-term monitoring of these fungal communities. There are endangered ecosystems similar to the pine rocklands with another host pine species, *Pinus caribaea*, in Belize, the Bahamas and Turks and Caicos (Sanchez et al. 2019; Michelakis et al. 2016). Unfortunately, many of these tropical pine forests are understudied or completely unstudied (Corrales et al. 2018), and fundamental questions about ECM communities in these habitats remain unanswered. Here we provide the framework for future in situ studies that are needed to determine how salt intrusion impacts forest trees and fungal mutualists in other low elevation, tropical, coastal ecosystems.

Author Contributions

E.K.-A., M.A.J., M.E.S. and R.G. planned and designed the field sampling and the salinity experiment. E.K.-A. conducted all field, greenhouse, and lab work. E.K.-A. and M.A.J. analysed the data, M.E.S. and R.G. provided financial and lab support, and E.K.-A. and M.E.S. wrote the manuscript with input from M.A.J. and R.G.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The high throughput sequencing data are deposited in the NCBI Sequence Read Archive (SRA), seedlings PRJNA1142580 and mature pines PRJNA121482.

References

Agerer, R. 1995. "Anatomical Characters Identified Ectomycorrhizas: An Attempt Towards a Natural Classification." In *Mycorrhiza: Structure, Function, Molecular Biology and Biotechnology*, edited by K. Varma and B. Hock, 685–734. Springer.

Anderson, M. J. 2001. "A New Method for Non-Parametric Multivariate Analysis of Variance." *Austral Ecology* 26, no. 1: 32–41. https://doi.org/10.1046/j.1442-9993.2001.01070.x.

Anderson, M. J. 2006. "Distance-Based Tests for Homogeneity of Multivariate Dispersions." *Biometrics* 62, no. 1: 245–253. https://doi.org/10.1111/j.1541-0420.2005.00440.x.

Bahram, M., S. Põlme, U. Kõljalg, S. Zarre, and L. Tedersoo. 2012. "Regional and Local Patterns of Ectomycorrhizal Fungal Diversity and Community Structure Along an Altitudinal Gradient in the Hyrcanian Forests of Northern Iran." *New Phytologist* 193, no. 2: 465–473. https://doi.org/10.1111/j.1469-8137.2011.03927.x.

Bandou, E., F. Lebailly, F. Muller, et al. 2006. "The Ectomycorrhizal Fungus Scleroderma Bermudense Alleviates Salt Stress in Seagrape (*Coccoloba uvifera* L.) Seedlings." *Mycorrhiza* 16, no. 8: 559–565. https://doi.org/10.1007/s00572-006-0073-6.

Bazzicalupo, A. L., J. Ruytinx, Y. H. Ke, et al. 2020. "Fungal Heavy Metal Adaptation Through Single Nucleotide Polymorphisms and Copy-Number Variation." *Molecular Ecology* 29, no. 21: 4157–4169. https://doi.org/10.1111/mec.15618.

Bidartondo, M. I., J. Baar, and T. D. Bruns. 2001. "Low Ectomycorrhizal Inoculum Potential and Diversity From Soils in and Near Ancient Forests of Bristlecone Pine (*Pinus longaeva*)." *Canadian Journal of Botany* 79, no. 3: 293–299. https://doi.org/10.1139/cjb-79-3-293.

Bingham, M. A., and S. Simard. 2012. "Ectomycorrhizal Networks of *Pseudotsuga menziesii var. Glauca* Trees Facilitate Establishment of Conspecific Seedlings Under Drought." *Ecosystems* 15, no. 2: 188–199. https://doi.org/10.1007/s10021-011-9502-2.

Blanchet, F. G., P. Legendre, and D. Borcard. 2008. "Forward Selection of Explanatory Variables." *Ecology* 89, no. 9: 2623–2632. https://doi.org/10.1890/07-0986.1.

Bois, G., F. J. Bigras, A. Bertrand, Y. Piché, M. Y. P. Fung, and D. P. Khasa. 2006. "Ectomycorrhizal Fungi Affect the Physiological Responses of Picea Glauca and *Pinus banksiana* Seedlings Exposed to an NaCl Gradient." *Tree Physiology* 26, no. 9: 1185–1196. https://doi.org/10.1093/treephys/26.9.1185.

Branco, S., P. Gladieux, C. E. Ellison, et al. 2015. "Genetic Isolation Between Two Recently Diverged Populations of a Symbiotic Fungus." *Molecular Ecology* 24, no. 11: 2747–2758. https://doi.org/10.1111/mec.13132.

Branco, S., A. Schauster, H.-L. Liao, and J. Ruytinx. 2022. "Mechanisms of Stress Tolerance and Their Effects on the Ecology and Evolution of Mycorrhizal Fungi." *New Phytologist* 235, no. 6: 2158–2175. https://doi.org/10.1111/nph.18308.

Cáceres, M. D., and P. Legendre. 2009. "Associations Between Species and Groups of Sites: Indices and Statistical Inference." *Ecology* 90, no. 12: 3566–3574.

Corrales, A., T. W. Henkel, and M. E. Smith. 2018. "Ectomycorrhizal Associations in the Tropics—Biogeography, Diversity Patterns and Ecosystem Roles." *New Phytologist* 220, no. 4: 1076–1091. https://doi.org/10.1111/nph.15151.

Edgar, R. C. 2010. "Search and Clustering Orders of Magnitude Faster Than BLAST." *Bioinformatics* 26, no. 19: 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.

Fernandez, C. W., L. Mielke, A. Stefanski, et al. 2023. "Climate Change–Induced Stress Disrupts Ectomycorrhizal Interaction Networks at the Boreal–Temperate Ecotone." *Proceedings of the National Academy of Sciences* 120, no. 34: e2221619120. https://doi.org/10.1073/pnas.2221619120.

Fujimura, K. E., J. E. Smith, T. R. Horton, N. S. Weber, and J. W. Spatafora. 2005. "Pezizalean Mycorrhizas and Sporocarps in Ponderosa Pine (*Pinus ponderosa*) After Prescribed Fires in Eastern Oregon, USA." *Mycorrhiza* 15: 79–86. https://doi.org/10.1007/s00572-004-0303-8.

Gardes, M., and T. D. Bruns. 1993. "ITS Primers With Enhanced Specificity for Basidiomycetes-Application to the Identification of Mycorrhizae and Rusts." *Molecular Ecology* 2, no. 2: 113–118.

Geml, J., L. N. Morgado, T. A. Semenova-Nelsen, and M. Schilthuizen. 2017. "Changes in Richness and Community Composition of Ectomycorrhizal Fungi Among Altitudinal Vegetation Types on Mount Kinabalu in Borneo." *New Phytologist* 215, no. 1: 454–468. https://doi.org/10.1111/nph.14566.

Glassman, S. I., C. R. Levine, A. M. DiRocco, J. J. Battles, and T. D. Bruns. 2016. "Ectomycorrhizal Fungal Spore Bank Recovery After a Severe Forest Fire: Some Like It Hot." *ISME Journal* 10, no. 5: 1228–1239. https://doi.org/10.1038/ismej.2015.182.

Glassman, S. I., I. J. Wang, and T. D. Bruns. 2017. "Environmental Filtering by pH and Soil Nutrients Drives Community Assembly in Fungi at Fine Spatial Scales." *Molecular Ecology* 26, no. 24: 6960–6973. https://doi.org/10.1111/mec.14414.

Gong, S., B. Feng, S.-P. Jian, G. S. Wang, Z.-W. Ge, and Z. L. Yang. 2022. "Elevation Matters More Than Season in Shaping the Heterogeneity of Soil and Root Associated Ectomycorrhizal Fungal Community." *Microbiology Spectrum* 10, no. 1: e01950. https://doi.org/10.1128/spectrum.01950-21.

Goodman, J., J. Maschinski, P. Hughes, et al. 2012. "Differential Response to Soil Salinity in Endangered Key Tree Cactus: Implications for Survival in a Changing Climate." *PLoS One* 7, no. 3: e35228. https://doi.org/10.1371/journal.pone.0032528.

Gordon, G. J., and C. A. Gehring. 2011. "Molecular Characterization of Pezizalean Ectomycorrhizas Associated With Pinyon Pine During Drought." *Mycorrhiza* 21: 431–441. https://doi.org/10.1007/s00572-010-0349-8.

Guerrero-Galán, C., M. Calvo-Polanco, and S. D. Zimmermann. 2019. "Ectomycorrhizal Symbiosis Helps Plants to Challenge Salt Stress Conditions." *Mycorrhiza* 29, no. 4: 291–301. https://doi.org/10.1007/s00572-019-00894-2.

Harley, G. L., J. T. Maxwell, and G. T. Raber. 2015. "Elevation Promotes Long-Term Survival of *Pinus elliottii var. Densa*, a Foundation Species of the Endangered Pine Rockland Ecosystem in the Florida Keys." *Endangered Species Research* 29, no. 2: 117–130. https://doi.org/10.3354/esr00707.

Harris, B., A. Freidenreich, E. Betancourt, and K. Jayachandran. 2021. "Short-Term Vegetation Responses to the First Prescribed Burn in an Urban Pine Rockland Preserve." *Fire Ecology* 17, no. 1: 31. https://doi.org/10.1186/s42408-021-00116-1.

Hisano, M., E. B. Searle, and H. Y. H. Chen. 2018. "Biodiversity as a Solution to Mitigate Climate Change Impacts on the Functioning of Forest Ecosystems." *Biological Reviews* 93, no. 1: 439–456. https://doi.org/10.1111/brv.12351.

Ishida, T. A., K. Nara, M. Tanaka, A. Kinoshita, and T. Hogetsu. 2008. "Germination and Infectivity of Ectomycorrhizal Fungal Spores in Relation to Their Ecological Traits During Primary Succession." *New Phytologist* 180, no. 2: 491–500. https://doi.org/10.1111/j.1469-8137.2008.02572.x.

Izzo, A., M. Canright, and T. D. Bruns. 2006. "The Effects of Heat Treatments on Ectomycorrhizal Resistant Propagules and Their Ability to Colonize Bioassay Seedlings." *Mycological Research* 110, no. 2: 196–202. https://doi.org/10.1016/j.mycres.2005.08.010.

Jones, I. M., and S. Koptur. 2017. "Dead Land Walking: The Value of Continued Conservation Efforts in South Florida's Imperiled Pine Rocklands." *Biodiversity and Conservation* 26, no. 14: 3241–3253. https://doi.org/10.1007/s10531-017-1433-6.

Jörgensen, K., K. E. Clemmensen, H. Wallander, and B. D. Lindahl. 2023. "Do Ectomycorrhizal Exploration Types Reflect Mycelial Foraging Strategies?" *New Phytologist* 237, no. 2: 576–584. https://doi.org/10.1111/nph.18566.

Jusino, M. A., D. L. Lindner, J. K. Cianchetti, A. T. Grisé, N. J. Brazee, and J. R. Walters. 2014. "A Minimally Invasive Method for Sampling Nest and Roost Cavities for Fungi: A Novel Approach to Identify the

Fungi Associated With Cavity-Nesting Birds." *Acta Ornithologica* 49, no. 2: 233–242. https://doi.org/10.3161/173484714X687127.

Karlsen-Ayala, E., M. A. Jusino, R. Gazis, and M. E. Smith. 2023. "Habitat Matters: The Role of Spore Bank Fungi in Early Seedling Establishment of Florida Slash Pines." *Fungal Ecology* 62: 101210. https://doi.org/10.1016/j.funeco.2022.101210.

Karlsen-Ayala, E., M. E. Smith, B. C. Askey, and R. Gazis. 2022. "Native Ectomycorrhizal Fungi From the Endangered Pine Rocklands Are Superior Symbionts to Commercial Inoculum for Slash Pine Seedlings." *Mycorrhiza* 32, no. 5: 465–480. https://doi.org/10.1007/s00572-022-01092-3.

Kennedy, P. G., L. M. Higgins, R. H. Rogers, and M. G. Weber. 2011. "Colonization-Competition Tradeoffs as a Mechanism Driving Successional Dynamics in Ectomycorrhizal Fungal Communities." *PLoS One* 6, no. 9: 25126. https://doi.org/10.1371/journal.pone.0025126.

Kernaghan, G., B. Hambling, M. Fung, and D. Khasa. 2002. "In Vitro Selection Boreal Ectomycorrhizal Fungi for Use in Reclamation of Saline-Alkaline Habitats." *Restoration Ecology* 10, no. 1: 43–51. https://doi.org/10.1046/j.1526-100X.2002.10105.x.

Kirkby, M. J., R. H. MacArthur, and E. O. Wilson. 1968. "The Theory of Island Biogeography." *Geographical Journal* 134, no. 4: 592. https://doi.org/10.2307/1796430.

Kirwan, M. L., J. L. Kirwan, and C. A. Copenheaver. 2007. "Dynamics of an Estuarine Forest and Its Response to Rising Sea Level." *Journal of Coastal Research* 23, no. 2: 457–463. https://doi.org/10.2112/04-0211.1.

Lake, P. S. 2013. "Resistance, Resilience and Restoration." *Ecological Management and Restoration* 14, no. 1: 20–24. https://doi.org/10.1111/emr.12016.

Langenfeld-Heyser, R., J. Gao, T. Ducic, et al. 2007. "Paxillus Involutus Mycorrhiza Attenuate NaCl-Stress Responses in the Salt-Sensitive Hybrid Poplar *Populus* × *Canescens*." *Mycorrhiza* 17: 121–131. https://doi.org/10.1007/s00572-006-0084-3.

Lebreton, A., Q. Zeng, S. Miyauchi, A. Kohler, Y. C. Dai, and F. M. Martin. 2021. "Evolution of the Mode of Nutrition in Symbiotic and Saprotrophic Fungi in Forest Ecosystems." *Annual Review of Ecology, Evolution, and Systematics* 52, no. 1: 385–404. https://doi.org/10.1146/annurev-ecolsys-012021-114902.

Lindahl, B. D., and A. Tunlid. 2015. "Ectomycorrhizal Fungi—Potential Organic Matter Decomposers, Yet Not Saprotrophs." *New Phytologist* 205, no. 4: 1443–1447. https://doi.org/10.1111/nph.13201.

Lindner, D. L., and M. T. Banik. 2009. "Effects of Cloning and Root-Tip Size on Observations of Fungal ITS Sequences From *Picea glauca* Roots." *Mycologia* 101, no. 1: 157–165. https://doi.org/10.3852/08-034.

Looney, B. P., P. Meidl, M. J. Piatek, et al. 2018. "Russulaceae: A New Genomic Dataset to Study Ecosystem Function and Evolutionary Diversification of Ectomycorrhizal Fungi With Their Tree Associates." *New Phytologist* 218, no. 1: 54–65. https://doi.org/10.1111/nph.15001.

Maschinski, J., J. L. Goodman, and D. Powell. 2009. "Assessment of Population Status and Causes of Decline for *Pilosocereus robinii* i (Lem.) Byles & G.D. Rowley in the Florida Keys." Final Report to U.S. Fish and Wildlife Service, South Florida Ecological Services Office, Vero Beach, FL.

McCarthy, M. J., B. Dimmitt, S. DiGeronimo, and F. E. Muller-Karger. 2022. "Forest Loss Is Accelerating Along the US Gulf Coast." *Estuaries and Coasts* 45, no. 3: 913–919. https://doi.org/10.1007/s12237-021-01000-6.

Michelakis, D., N. Stuart, P. Furley, G. Lopez, V. Linares, and I. H. Woodhouse. 2016. "Woody Structure and Population Density of Pine (*Pinus caribaea* var. *Hondurensis*) (*Caribbean pine*) Dominated Lowland Tropical Savanna Woodlands Under Different Protection and Management Regimes." *Caribbean Journal of Science* 49, no. 1: 1–16. https://doi.org/10.18475/cjos.v49i1.a2.

Miyauchi, S., E. Kiss, A. Kuo, et al. 2020. "Large-Scale Genome Sequencing of Mycorrhizal Fungi Provides Insights Into the Early Evolution of Symbiotic Traits." *Nature Communications* 11, no. 1: 5125. https://doi.org/10.1038/s41467-020-18795-w.

Moeller, H. V., and K. G. Peay. 2016. "Competition-Function Tradeoffs in Ectomycorrhizal Fungi." *PeerJ* 2016, no. 7: e2270. https://doi.org/10.7717/peerj.2270.

Nguyen, N. H., Z. Song, S. T. Bates, et al. 2016. "FUNGuild: An Open Annotation Tool for Parsing Fungal Community Datasets by Ecological Guild." *Fungal Ecology* 20: 241–248. https://doi.org/10.1016/j.funeco.2015.06.006.

Nicholls, R. J., and A. Cazenave. 2010. "Sea-Level Rise and Its Impact on Coastal Zones." *Science* 328, no. 5985: 1517–1520. https://doi.org/10.1126/SCIENCE.1185782.

Oksanen, J., F. G. Blanchet, R. Kindt, et al. 2015. "OK-Package 'vegan.' Community Ecology Package, Version."

Ondrasek, G., and Z. Rengel. 2021. "Environmental Salinization Processes: Detection, Implications & Solutions." *Science of the Total Environment* 754: 142432.

Palmer, J. M., M. A. Jusino, M. T. Banik, and D. L. Lindner. 2018. "Non-Biological Synthetic Spike-In Controls and the AMPtk Software Pipeline Improve Mycobiome Data." *PeerJ* 6: e4925. https://doi.org/10.7717/peerj.4925.

Parida, A. K., and A. B. Das. 2005. "Salt Tolerance and Salinity Effects on Plants: A Review." *Ecotoxicology and Environmental Safety* 60, no. 3: 324–349. https://doi.org/10.1016/J.ECOENV.2004.06.010.

Peay, K. G., M. I. Bidartondo, and A. Elizabeth Arnold. 2010. "Not Every Fungus Is Everywhere: Scaling to the Biogeography of Fungal–Plant Interactions Across Roots, Shoots and Ecosystems." *New Phytologist* 185, no. 4: 878–882. https://doi.org/10.1111/j.1469-8137.2009.03158.x.

Peay, K. G., T. D. Bruns, P. G. Kennedy, S. E. Bergemann, and M. Garbelotto. 2007. "A Strong Species Area Relationship for Eukaryotic Soil Microbes: Island Size Matters for Ectomycorrhizal Fungi." *Ecology Letters* 10, no. 6: 470–480. https://doi.org/10.1111/j.1461-0248.2007.01035.x.

Policelli, N., T. D. Bruns, R. Vilgalys, and M. A. Nuñez. 2018. "Suilloid Fungi as Global Drivers of Pine Invasions." *New Phytologist* 222: 714–725. https://doi.org/10.1111/nph.15660.

Possley, J., J. J. Lange, A. R. Franck, et al. 2024. "First U.S. Vascular Plant Extirpation Linked to Sea Level Rise? *Pilosocereus Millspaughii* (Cactaceae) in the Florida Keys, USA." *Journal of the Botanical Research Institute of Texas* 18, no. 1: 211–223. https://doi.org/10.17348/jbrit.v18.i1.1350.

Possley, J., S. W. Woodmansee, and J. Maschinski. 2008. "Patterns of Plant Composition in Fragments of Globally Imperiled Pine Rockland Forest: Effects of Soil Type, Recent Fire Frequency, and Fragment Size." *Natural Areas Journal* 28, no. 4: 379–394.

R Core Team. 2019. *A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing.

Rognes, T., T. Flouri, B. Nichols, C. Quince, and F. Mahé. 2016. "VSEARCH: A Versatile Open Source Tool for Metagenomics." *PeerJ* 2016, no. 10: e2584. https://doi.org/10.7717/peerj.2584.

Ross, M. S., J. J. O'Brien, and L. da Silveira Lobo Sternberg. 1994. "Sea-Level Rise and the Reduction in Pine Forests in the Florida Keys." *Ecological Applications* 4, no. 1: 144–156. https://doi.org/10.2307/1942124.

Ross, M. S., J. J. O'Brien, R. G. Ford, K. Zhang, and A. Morkill. 2009. "Disturbance and the Rising Tide: The Challenge of Biodiversity Management on Low-Island Ecosystems." *Frontiers in Ecology and the Environment* 7, no. 9: 471–478. https://doi.org/10.1890/070221.

Ross, M. S., D. E. Ogurcak, S. Stoffella, J. P. Sah, J. Hernandez, and H. E. Willoughby. 2020. "Hurricanes, Storm Surge, and Pine Forest Decline

on a Low Limestone Island." *Estuaries and Coasts* 43, no. 5: 1045–1057. https://doi.org/10.1007/s12237-019-00624-z.

Sah, J. P., M. S. Ross, J. R. Snyder, and D. E. Ogurcak. 2010. "Tree Mortality Following Prescribed Fire and a Storm Surge Event in Slash Pine (*Pinus elliottii var. Densa*) Forests in the Florida Keys, USA." *International Journal of Forestry Research* 2010: 1–13. https://doi.org/10.1155/2010/204795.

Saha, A. K., S. Saha, J. Sadle, et al. 2011. "Sea Level Rise and South Florida Coastal Forests." *Climatic Change* 107, no. 1: 81–108. https://doi.org/10.1007/S10584-011-0082-0.

Sanchez, D. M., B. N. Manco, J. Blaise, M. Corcoran, and M. A. Hamilton. 2019. "Conserving and Restoring the Caicos Pine Forests: The First Decade." *Plant Diversity* 41, no. 2: 75–83. https://doi.org/10.1016/j.pld.2018.05.002.

Schieder, N. W., and M. L. Kirwan. 2019. "Sea-Level Driven Acceleration in Coastal Forest Retreat." *Geology* 47, no. 12: 1151–1155. https://doi.org/10.1130/G46607.1.

Schneider, O., R. M. Crandall, and B. Baiser. 2024. "The Large-Scale Restoration of Fire and Water Regimes in Everglades National Park Reveal Little Change in Plant Diversity Along an Elevational Gradient." *Restoration Ecology* 32: e14138.

Smith, S., and D. Read. 2008. *Mycorrhizal Symbiosis*. Academic Press. https://doi.org/10.1016/B978-0-12-370526-6.X5001-6.

Snyder, J. R., A. Herndon, and W. B. Robertsoon Jr. 1990. "South Florida Rocklands." *Ecosystems of Florida* 1: 230–277.

Souza, R. G., D. K. A. Silva, J. R. G. Oliveira, et al. 2012. "Use of Mycorrhizal Seedlings on Recovery of Mined Dunes in Northeastern Brazil." *Pedobiologia* 55, no. 6: 303–309. https://doi.org/10.1016/J.PEDOBI.2012.06.002.

Tedersoo, L., S. Anslan, M. Bahram, et al. 2020. "Regional-Scale In-Depth Analysis of Soil Fungal Diversity Reveals Strong pH and Plant Species Effects in Northern Europe." *Frontiers in Microbiology* 11: 1953. https://doi.org/10.3389/fmicb.2020.01953.

Tedersoo, L., K. Hansen, B. A. Perry, and R. Kjøller. 2006. "Molecular and Morphological Diversity of Pezizalean Ectomycorrhiza." *New Phytologist* 170, no. 3: 581–596. https://doi.org/10.1111/j.1469-8137.2006.01678.x.

Tedersoo, L., and M. E. Smith. 2013. "Lineages of Ectomycorrhizal Fungi Revisited: Foraging Strategies and Novel Lineages Revealed by Sequences From Belowground." *Fungal Biology Reviews* 27, no. 3–4: 83–99. https://doi.org/10.1016/j.fbr.2013.09.001.

Thiem, D., A. Piernik, and K. Hrynkiewicz. 2018. "Ectomycorrhizal and Endophytic Fungi Associated With *Alnus glutinosa* Growing in a Saline Area of Central Poland." *Symbiosis* 75, no. 1: 17–28. https://doi.org/10.1007/s13199-017-0512-5.

Tilman, D. 2001. *Functional Diversity BT—Encyclopedia of Biodiversity*. Encyclopedia of Biodiversity.

Trotta, L. B., B. Baiser, J. Possley, et al. 2018. "Community Phylogeny of the Globally Critically Imperiled Pine Rockland Ecosystem." *American Journal of Botany* 105, no. 10: 1735–1747. https://doi.org/10.1002/ajb2.1168.

Truong, C., L. A. Gabbarini, A. Corrales, et al. 2019. "Ectomycorrhizal Fungi and Soil Enzymes Exhibit Contrasting Patterns Along Elevation Gradients in Southern Patagonia." *New Phytologist* 222, no. 4: 1936–1950. https://doi.org/10.1111/nph.15714.

Ury, E. A., X. Yang, J. P. Wright, and E. S. Bernhardt. 2021. "Rapid Deforestation of a Coastal Landscape Driven by Sea-Level Rise and Extreme Events." *Ecological Applications* 31, no. 5: e02339. https://doi.org/10.1002/eap.2339.

Wen, Z., J. Xing, C. Liu, et al. 2022. "The Effects of Ectomycorrhizal Inoculation on Survival and Growth of *Pinus thunbergii* Seedlings Planted in Saline Soil." *Symbiosis* 86, no. 1: 71–80. https://doi.org/10.1007/S13199-021-00825-W.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. *Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics*. PCR Protocols. https://doi.org/10.1016/b978-0-12-372180-8.50042-1.

Williams, D. A., Y. Wang, M. Borchetta, and M. S. Gaines. 2007. "Genetic Diversity and Spatial Structure of a Keystone Species in Fragmented Pine Rockland Habitat." *Biological Conservation* 138, no. 1–2: 256–268. https://doi.org/10.1016/j.biocon.2007.04.024.

Zwiazek, J. J., M. A. Equiza, J. Karst, J. Senorans, M. Wartenbe, and M. Calvo-Polanco. 2019. "Role of Urban Ectomycorrhizal Fungi in Improving the Tolerance of Lodgepole Pine (*Pinus contorta*) Seedlings to Salt Stress." *Mycorrhiza* 29, no. 4: 303–312. https://doi.org/10.1007/s00572-019-00893-3.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.