A SINGLE RANGE-EXPANDING SPECIES RESHAPES ALPINE ECOSYSTEMS AND THEIR BELOWGROUND DIVERSITY

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“That land is a community is the basic concept of ecology, but that land is to be loved and respected is an extension of ethics” – Aldo Leopold (The Land Ethic, 1949)

ABSTRACT

A SINGLE RANGE-EXPANDING SPECIES RESHAPES ALPINE ECOSYSTEMS AND THEIR BELOWGROUND DIVERSITY

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As a result of rapid environmental change caused by humans, species around the globe are expanding their ranges and spreading into uncharted territories at an unprecedented rate. Range-expansion of foundation species, or any species that plays a disproportionately large role in structuring ecosystems, can have severe impacts on recipient communities and ecosystems. Such species can not only alter biological diversity and ecosystem function, they can also interfere with the fundamental processes underpinning community assembly. However, despite large implications for the integrity and functioning of the world's ecosystems, the ability of range expanders to alter community assembly processes is largely unknown. Here, we use an ongoing alpine invasion by a single foundation species (*Pinus contorta*) as a natural experiment to investigate the impacts of range-expansion on belowground community assembly and diversity. We report that abiotic selection exerts an increasingly strong influence as range expansion proceeds through the creation of micro-climatic islands with colder and wetter soils. Later stages of expansion were also associated with a decrease in the relative influence of dispersal and biotic interactions between fungi and their host plants. These changes, in turn, lead to an increase in the richness of fungal pathogens, and a decrease in the richness of symbionts, both locally and regionally. Taken together, these results suggest that range expanding species moving upslope have the potential to create novel ecosystems by rerouting the assembly of resident communities and reshaping biodiversity across scales. As climate change leads to poleward and upslope shifts in the distributions of plants and animals around the world, understanding the ecological

processes through which range-expanders reshape native biodiversity can improve our predictions of the threats and challenges facing impacted ecosystems and enable better, more informed, conservation decisions.

KEY WORDS

Range-expansion, species-invasion, biodiversity, community-assembly, novel-ecosystems

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INTRODUCTION

As a result of human-driven change, species around the planet are shifting their geographic ranges, expanding into new territories, and interacting with native taxa to form novel ecosystems (Hobbs *et al.* 2009; Boivin *et al.* 2016). The frequent introduction of exotic species has led to world-wide range-expansions for thousands of taxa (Hobbs 2000; Meyerson & Mooney 2007). The gradual warming of the earth's climate exacerbates this phenomenon, accelerating the geographic expansion of exotics in their introduced range (Dukes & Mooney 1999; Carlton 2000; Ward & Masters 2007; Hellmann *et al.* 2008; Colautti & Barrett 2013), as well as the spread of natives into new ranges (Parmesan *et al.* 1999; Hickling *et al.* 2006; Harsch *et al.* 2009). Climate change is creating new habitable conditions for lowland and equatorial species, resulting in upslope and poleward shifts in the geographic distributions of both natives and exotics (Chen *et al.* 2011; IPCC 2018; Warren *et al.* 2018; Freeman *et al.* 2020). These range-expanding taxa are interacting with resident native species, forming novel interactions, communities, and ecosystems with no historical analogues (le Roux & McGeoch 2008). Despite the rapid rate at which these novel communities and ecosystems are forming, and the widespread prevalence of this phenomenon, the consequences of range-expanding species remain poorly explored (Wallingford *et al.* 2020).

While all terrestrial taxa have the ability to spread and expand their range, some species have disproportionately large impacts on the construction and organization of novel ecosystems (Vitousek *et al.* 1987). Range-expanding *foundation species,* in particular, may play crucial roles in the structuring of novel communities (Ramus *et al.* 2017). A foundation species is any species that is common, makes up a considerable portion of ecosystem biomass, and influences the diversity of associated taxa through non-trophic interactions, resulting in their

ability to structure entire ecosystems through microhabitat creation and influence on ecosystem processes (Dayton 1972; Callaway 1995; Stachowicz 2001; Ellison *et al.* 2005). For example, due to its rapid growth rate, high tannin content, and leaves with low C:N ratio, the disappearance of American Chestnut from temperate forests fundamentally altered a variety of ecosystem processes including decomposition, nutrient cycling, woody plant composition, and productivity (Ellison *et al.* 2005). The disproportionately large structuring effects of foundation species can be broken down into biotic and abiotic components (Jones *et al.* 1994; Gutiérrez *et al.* 2011), and the range expansion of such species may accelerate the creation of novel ecosystems.

Range-expanding foundation species could alter the structure of biotic communities by interfering with the fundamental processes maintaining biodiversity, often referred to as community assembly processes. Communities are shaped by selection, dispersal and drift (Vellend & Agrawal 2010; Nemergut *et al.* 2013). Selection includes abiotic selection, which suggests that local conditions enable the persistence of only those species with matching adaptations (Keddy 1992) and biotic selection, which implies that the interactions between species either promote (e.g. facilitation, mutualism) or prevent (e.g. competition, predation, parasitism) persistence in local communities (Hutchinson 1957; Paine 1966, 1969; Connell 1970; Janzen 1970). Dispersal regulates the rates at which species colonize new communities (Leibold *et al.* 2004; Kraft *et al.* 2011). Ecological drift implies random fluctuations in the abundance of species, leading to patterns of community composition that are unpredictable and stochastic (Chesson 2000; Hubbell 2001). The relative influence of these processes can be inferred by partitioning beta diversity into relative environmental, spatial and stochastic components (Myers *et al.* 2013).

Although it remains unclear if the spread of foundation species impacts community assembly processes (Sanders *et al.* 2003; Myers *et al.* 2015; Frrenberg *et al.* 2016), past research suggests this is likely the case. Cushion forming plants, considered foundation species in high alpine habitats, have been shown to release recipient communities from strong abiotic selection and impose regime shifts by creating more tolerable environment conditions (Schob *et al.* 2012). Similar facilitative effects have also been observed between isolated savanna trees (foundation species) and grass communities, where trees facilitated grass biomass production likely by creating more favorable abiotic conditions (Moustakas *et al.* 2013). Foundation species may also decrease the relative influence of biotic selection by creating less stressful conditions for resident taxa, thereby reducing the dependence of these taxa on mutualistic interactions (Stachowicz 2001; Maestre *et al.* 2009; Butterfield *et al.* 2013). Less stressful abiotic conditions could also reduce the rate of extinction due to fluctuating environments, leading to communities that are temporally and spatially homogeneous in terms of composition and driven more by stochastic processes (dispersal and drift) than selection (Chesson & Warner 1981; Warner & Chesson 1985). Temporal and spatial homogenization may also interact directly with dispersal by affecting the mortality of dispersed individuals and the persistence of rare taxa, by limiting the probability that certain dispersed taxa, dependent on specific and rare environmental conditions for persistence, encounter suitable habitat upon arrival (Thomas 2000).

While range-expanding foundation species have the potential to impact a variety of ecosystems, alpine and tundra biomes, comprising an estimated ~13% of the earth's surface, face a unique threat. Settele *et al.* (2014) reported that the geographic distributions of many terrestrial taxa have shifted ~17km poleward and ~11km upslope in the last decade, and Freeman *et al.*

(2020) confirmed that, in mountainous regions, these climate-driven range shifts are most prevalent in alpine taxa. Of these range-expanding species, trees, considered quintessential foundation species (Ellison *et al.* 2005), are colonizing treeless alpine and tundra ecosystems at an unprecedented rate, forming novel communities with resident species, and potentially impacting assembly processes (Franklin *et al.* 1971; Suarez *et al.* 1999; Moore & Huffman 2004; Sturm *et al.* 2005; Harsch *et al.* 2009; Myers-Smith *et al.* 2011; Trant & Hermanutz 2014). Alpine and tundra ecosystems are typically treeless and exhibit both a unique hydrology typical of arid biomes as well as distinct local communities adapted to life in these harsh environments (Slaymaker 1974; Körner 2003). As such, rapid, human-driven forestation or shrubification has catastrophic long-term consequences for these communities and ecosystems (Greenwood & Jump 2014). Over long time scales, range-expanding trees reduce the area of treeless alpine and tundra biomes (Moiseev & Shiyatov 2003), often reducing biodiversity (Moore & Huffman 2004) and displacing alpine taxa (García-Romero *et al.* 2010). However, past research focuses on long-term consequences, likely driven by density-dependent impacts of tree spread and not the effects of individual tree establishment (Franzese *et al.* 2017; García *et al.* 2019). Therefore, it is possible that the initial impacts of the spread and establishment of isolated foundation species in alpine and tundra biomes are facilitative, as suggested by foundation species literature (Ellison *et al.* 2005; Butterfield *et al.* 2013; Moustakas *et al.* 2013; Kikvidze *et al.* 2015). Nonetheless, the underlying ecological processes mediating the short and long-term impacts of tree range expansion through community re-assembly remain largely obscure.

Root-associated fungi are a useful system to study the rapid and ongoing impact of range-expanding tree species on biological diversity, as well as on the assembly and ecosystem processes leading to these changes. Root-associated fungi have short generation times and

therefore respond quickly to abiotic changes (Hagenbo *et al.* 2017). They are also influenced by dispersal dynamics on extremely fine spatial scales (Peay *et al.* 2012; Feinstein & Blackwood 2013) and exhibit strong patterns of abiotic and biotic selection on both global (Tedersoo *et al.* 2014) and local (Glassman *et al.* 2017) scales. Furthermore, root-associated fungi mediate a suite of ecosystems processes, such that small changes in their diversity can impact whole ecosystem functioning (Parker *et al.* 2015). For example, saprotrophic fungi (decomposers) are responsible for a large component of carbon cycling, especially in alpine and tundra biomes where >90% of ecosystem carbon is stored underground (Körner 2003). Symbiotrophic (mutualistic) fungi are critical facilitators in alpine and tundra ecosystems, forming important, and often obligate, mutualistic interactions with host plants that help them overcome abiotic stress (Harley & Smith 1983; Cairney & Meharg 2003). Pathotrophic (parasitic) fungi are critical maintainers of community diversity (Bagchi *et al.* 2010, 2014). In sum, root-associated fungi are both a useful system to elucidate rapid changes in diversity and assembly processes, and an indicator of impact on resident plant diversity, food web structure, and carbon cycling.

In this study, we quantify the response of fungal communities associating with the roots of resident plants to the establishment and growth of a single range-expanding foundation species. Specifically, we quantify the impact of the recent (approximately ~25 years old) and ongoing range-expansion by introduced Lodgepole Pine (*Pinus contorta*) into a treeless alpine ecosystem in Patagonia, Argentina. We achieve this through a natural experiment, which entails comparing the diversity and assembly processes of root-associated fungi (associating with resident plants) under isolated pines of different expansion stage (saplings and adults). Specifically, we classified range expanding pines by size (as a proxy for age) to simulate different stages of range expansion over time, focusing on the isolated impacts of individual trees

to investigate the initial impacts of range expansion. Past research suggests that foundation species in stressful environments facilitate biodiversity by creating more tolerable microhabitats and releasing communities from abiotic selection (Schob *et al.* 2012; Moustakas *et al.* 2013). Isolated pines may achieve similar effects in this system, likely providing shade and shelter and increasing water retention (Simberloff *et al.* 2010) in what is normally a harsh arid environment characteristic of the high alpine (Slaymaker 1974; Körner 2003). Therefore, based on their potential to create more-tolerable microhabitat where they establish, we hypothesize that isolated range-expanding pines will (i) increase the β diversity of root associated fungi, by (ii) releasing resident communities from strong patterns of selection, thereby weakening the relative influence of deterministic assembly processes (abiotic and biotic selection) and increasing the relative influence of stochastic processes (dispersal and drift). Furthermore, we hypothesize that due to the more tolerable conditions and weaker patterns of selection, fungal communities assembling beneath isolated range-expanding pines will (iii) exhibit higher α diversity and (iv) increased regional γ diversity.

METHODS

Study Site

To investigate the impacts of a range expanding foundation species, we studied an invasion of *Pinus contorta* (Lodgepole Pine) into a treeless alpine plateau beneath the summit of Cerro Piltriquitron (-41.96914°N; -71.46012°W), a mountain in northern Patagonia, Argentina (Fig. 1). The site was 44 hectares in area, with an average elevation of 1690m above sea level (min=1654m, max=1743m). The site is delimited on north and south sides by steep ridges and on east and west sides by slopes leading down the mountain to the native *Nothofagus pumilio* treeline. This alpine plateau is home to a diverse community of alpine plants, such as the

abundant *Gaultheria pumila*, *Festuca sp.*, and *Perezia fonkii*. Lodgepole pines were introduced to the lower-altitude western slope of Piltriquitron as part of a reforestation effort, after a fire in the late 1970s left the slope bare. Since then, pines have spread upwards, reaching the alpine plateau in ~1995 (based on oral histories and tree age data).

Study Design

We randomly sampled a total of 45 plots, 15 of each expansion stage. Expansion stages were defined as *controls* (plots with no focal pine) representing pre-range-expansion communities, *saplings* (plots with a single focal pine ~0.5m tall) representing communities beneath young, recently established pines, and *adult pines* (plots with a single focal pine >1m tall) representing communities beneath mature pines. All plots were isolated by at least a 10m distance from other (non-focal) pines to ensure that the affects captured in our data represent those of a single range expanding individual and not a neighborhood effect (Glassman *et al.* 2017). Over the course of 4 weeks in February of 2019, we measured the microclimatic conditions of each plot (soil moisture and maximum ground temperature), and identified, down to the lowest taxonomic level possible, the species of plant within a 2m x 2m quadrat established around the focal pine in the case of sapling and adult pine plots and the center of the plot in the case of controls. We chose a quadrat of this size in order to include only the resident plants growing immediately adjacent to the focal trees, and therefore, likely experiencing potential microhabitat effects of pine establishment and growth. After the sampling period, cores were obtained from each of the 30 focal pines to measure tree age, and height and diameter-at-base measurements were taken. Plots were georeferenced using a handheld GPS (Garmin GPSMAP 64s) to obtain latitude and longitude. Altitude was measured using Google Earth, however, variation between plots proved negligible and thus, altitude was not included in statistical analyses. Illustrated examples of plots are

available in Fig. 2a and photographic examples of plots are available in Fig. S1 in the supplementary material.

Soil Chemistry

Soil samples, consisting of 6 x 100mL, taken at roughly 10-15cm belowground, were obtained evenly throughout each plot and pooled to produce a single composite sample for each plot. Soil samples were transferred to a sterile plastic bag for transport and stored at ~2°C overnight. In a laboratory setting, soil was weighed to obtain a wet weight, dried over the course of a week using a space-heating element, and reweighed to obtain a dry weight. Soil moisture was calculated as the percentage difference between wet and dry weights. Dried samples were packaged and sent to Departamento de Agronomía at Universidad Nacional Del Sur (Bahía Blanca, Argentina) where soil parameters pH, C (carbon), P (phosphorous), NO3 (nitrate) and NH4 (ammonium) were measured.

Resident Plant Community Composition

Plants were identified on site using field guides and area-specific taxonomic keys, notably Flores de Alta Montaña (Ferreyra *et al.* 2006). Identification was performed on-site by Eckert and Muñoz. Samples of each plant morphospecies were pressed and mounted for preservation and future reference. Identified species were cross referenced with online databases of plant distributions and online herbarium specimens to ensure correct identification (Table S1 in supplementary material).

Plant Root Samples

During the final week of sampling, up to 3 individuals of each species of plant in each plot were uprooted and placed in sterile bags for transport. We chose 3 individuals in order to capture the

complete fungal communities associating with the roots of each species of plant. If plots contained fewer than 3 individuals, all individuals of that species were sampled. In a laboratory setting, the roots of each plant sample were washed with distilled water, excess liquid removed by drying at room temperature, and fine root tips were obtained and frozen at -20°C until genetic analysis. Plants whose roots either broke during uprooting, or didn't contain fine root material, were discarded.

Root Sample Preparation and DNA Extraction

From each fine root sample, 250mg was isolated and placed inside a QIAGEN DNeasy PowerSoil PowerBead tube (QIAGEN Inc.) containing 3 sterilized metal beads in addition to the ceramic QIAGEN beads and sterilized water. Samples were beat using a vortex for 10 minutes to break root material.

Bulk DNA was extracted from each fine root sample using QIAGEN DNeasy PowerSoil kits, following the manufacturer's protocol. We modified the final step of the protocol, isolating DNA using a low concentration EDTA (5%)-TE buffer instead of the C6 solution provided in the kit in order to stabilize DNA extract for transport. DNA was preserved in EDTA-TE buffer, and frozen at -20°C before being thawed and shipped, at room temperature, back to Canada for PCR. Upon arrival, DNA was refrozen at -80°C.

Amplification, Sequencing and Bioinformatics

DNA was amplified using fungal specific primers ITS-1F (CTTGGTCATTTAGAGGAAGTAA; Gardes & Bruns 1993) and ITS2 (GCTGCGTTCTTCATCGATGC; White *et al.* 1990). Fungal ITS primers used Illumina P5 and P7 adaptors for the forward and reverse primers respectively,

in conjunction with the Nextera XT v2 system, with Index (i5) on the forward primer and Index (i7) on the reverse primer.

Amplification through PCR was performed using Phusion Hot Start II High-Fidelity DNA polymerase and accompanying 5X Phusion HF Buffer (ThermoScientific) with 1μL of undiluted DNA. The reactions were carried out in a final volume of 25 μL in the presence of 200 μM dNTPs, 200 pmols of each primer, and 0.75 μL of DMSO. The reaction was run on an Eppendorf MasterCycler thermocycler with a 30s initial denaturation step at 98°C and 35 cycles of 98°C for 15s, annealing at 64°C for 30s, and extension at 72°C for 30s, with a final extension at 72°C for 10 min. Resulting PCR products were loaded on a 1% agarose gel to verify amplification success. Controls without DNA and positives with target DNA were run with every series of amplifications to test for the presence of contaminants. We also used negative extraction controls to assess contamination. Water from the same source as what we used to wash and clean root tips, was added to a blank extraction tube, and treated to the same extraction/amplification procedure. All amplified samples (including controls) were cleaned and normalized using the Invitrogen Sequalprep PCR Cleanup and Normalization Kit. Multiplexed amplicon libraries were prepared by mixing equimolar concentrations of amplified DNA, cleaned with AMPure magnetic beads (Beckman Coulter Inc., Brea, CA, USA). Libraries were quantified using Qubit dsDNA High Sensitivity kit Life Technologies Inc., Gaithersburg, MD, USA) and sequenced on an Illumina MiSeq Sequencer with the 2 × 300 bp (base pair) paired end platform using the Illunima MiSeq Reagent Kit v3. A total of 321 samples, including negative controls, were sequenced.

Illumina sequences were processed using the DADA2 pipeline (Callahan *et al.* 2016) for R 3.6.3 (R Core Team 2013). Raw sequences were pre-filtered, removing sequences containing

ambiguous bases (Ns). Primers were identified, ensuring correct orientation, and removed by trimming (22 and 20 bp respectively for forward and reverse). Sequences were filtered using default parameters, enforcing a maximum number of errors per read (maxEE=2), and discarding all reads shorter than 50bp. Reads were truncated based on quality profiles (260 and 240 bp for forward and reverse reads respectively). Sequences were dereplicated, and alternative sequence variants were inferred based on learned error rates. Sequences were merged (minimum overlap = 15 bp with zero mismatches allowed), filtered for chimeras using a denovo approach and used to generate amplicon sequence variants (ASVs) (Callahan *et al.* 2017). We assigned taxonomy based on the UNITE fungal database (Nilsson *et al.* 2018). To derive functionally specific communities, identified sequences were assigned functional guild (saprotroph, symbiotroph, pathotroph) using FUNguild (Nguyen *et al.* 2016). These functional classifications are based on the following definitions: saprotroph = receiving nutrients by breaking down dead host cells, symbiotroph = receiving nutrients by exchanging nutrients with host cells, and pathotroph = receiving nutrients by harming host cells (*sensu* Tedersoo *et al.* 2014). From the all-fungi community, only taxa that could be identified down to genus and matched a single specific functional guild with a high degree of confidence (Probable or Highly Probable) were retained. Finally, each sample was rarefied to a standard depth (10000 reads), in order to control for differences in sequencing depth between samples. Rarefaction was repeated 1000 times. Of the 321 total samples, 45 (including 7 negative controls and 38 plant samples) contained less than 10000 reads and were excluded at this step, leaving 276 samples representing the fungal communities associating with the roots of 24 different species of plant, for downstream analysis. Negative extraction and PCR controls produced no fungal reads after filtering, suggesting that contamination is not an issue in this data.

Instead of using each of the sampled plant individual as a replication unit for our analyses, we pooled fungal communities at the plot level (n=45, 15 per expansion-stage), removing rare ASVs that occurred in less than 3 plots a common and widespread approach to account for potentially spurious sequences in genetic data from complex communities (Zhan *et al.* 2014). We decided to use this approach in order to simulate plot-wide fungal communities as a whole, and incorporate biotic variables (plant richness and plant composition) into our analyses to explicitly test the role of biotic selection in this system. In addition, because our sampling design involved the extraction of fungal community data for each species of plant occurring in a given plot, plots with more plant species were expected to harbor greater richness and abundance of fungi owing to differences in sampling efforts. This sampling issue could affect our estimates of alpha, beta and gamma richness. To account for this sampling bias, we rarefied each plot community to a standard sequencing depth of 18000 reads 1000 times. We chose 18000 based on plot rarefaction curves (Fig. S2 in supplementary material), and because it allowed for the retention of all 45 plots in our analysis.

Statistical Analysis

All analyses were preformed using the 'vegan' package (Oksanen *et al.* 2013) for R, unless stated otherwise. Normality and heteroscedasticity were assessed in all applicable cases using a combination of visual and statistical hypothesis-testing approaches.

1. Effect of range-expansion on the biotic and abiotic environment

To assess whether pines influenced abiotic conditions, which could in turn affect the structure of fungal communities, we tested for the effect of expansion-stage (control, sapling, adult pine) on micro-climate (soil moisture and maximum ground temperature) and soil characteristics (pH, C, P, NO3, NH4) using ANOVA. Specifically, we used separate ANOVAs with expansion stage as

the predictor and individual environmental variables as the response. When ANOVA suggested statistically significant differences in means between expansion stages, we used Tukey tests to evaluate all pairwise comparisons and assign significance.

To further examine the effects of range-expanding pines on the abiotic environment, we quantified and compared the spatial structuring of abiotic variables between expansion stages. To do this, we quantified Moran's I measures of spatial autocorrelation for each environmental variable within expansion stage. Moran's I was calculated using a matrix of inverse geographic distance weights, and significance (suggesting the presence of spatial autocorrelation) was tested using the 'ape' package (Paradis & Schliep 2019) for R. In addition to the previous approach, we also quantified and compared the spatial structuring of our fungal communities between expansion stages, in order to better understand how geographic distance influences community turnover as range expansion proceeds. To do this, we used Mantel correlograms. A Mantel correlogram plots the spatial correlation in community composition as a function of geographic distance between plots. First, data is binned into distance classes based on Sturge's rule. A Mantel test is then preformed on each distance class, and significance (suggesting the presence of spatial autocorrelation) is assessed against a null model over 9999 permutations. Both Moran's I and Mantel R measures of spatial autocorrelation fall on the same scale, with +1 indicated positive spatial autocorrelation or clustering, where near sites are more similar than expected due to change, while -1 indicates negative spatial autocorrelation, or spatial evenness and zero indicates no spatial autocorrelation.

To assess whether pines influenced biotic conditions, which could in turn affect the structure of fungal communities, we assessed the influence of range expansion stage on plant richness and plant composition. We tested for the statistical effect of expansion-stage on plant α

richness using the same ANOVA/Tukey approach described for abiotic conditions. Then, we tested for the statistical effect of expansion-stage on plant β diversity using PERMANOVA over 9999 permutations (Anderson 2001). PERMANOVA works by comparing the centroids of groups of objects, in our case communities in plots grouped by expansion stage, using sum of squares partitioning on distance matrices. Linear models can be fit to explain variation and using permutations of the objects and their distances from one another, a pseudo-F statistic can be generated to statistically test the null hypothesis; that the distances between the centroids of two or more groups of objects is zero. This approach is robust to non-normal distributions, and colinear predictors, and similar to ANOVA, partitioning can be conducted marginally to isolate individual predictor contributions.

2. Effect of range-expansion on β diversity of root-associated fungi

To test our hypothesis that (i) root-associated fungal communities would be affected by expansion stage (control, sapling, adult pine), we first quantified fungal β diversity across all plots and expansion stages using Bray-Curtis dissimilarity (Bray & Curtis 1957) of Hellinger-transformed (Legendre & Gallagher 2001) abundance data. Specifically, we calculated and compared fungal β both between expansion stages (control, sapling and adult pine) as well as between functional guilds (all fungi, saprotrophs, symbiotrophs and pathotrophs). We then tested the statistical effect of expansion stage on fungal β diversity using non-metric multidimensional scaling (k=3), PERMANOVA to test for differences in fungal composition and PERMDISP to test for homogenization of species composition. Both of these analyses were based on 9999 permutations. To account for the influence of plant community structure and isolate the effect of range-expansion on fungal β diversity, we included biotic variables (plant richness and plant community composition, represented by the first two principal components of the plant

community matrix) as covariates into PERMANOVA analyses (Anderson 2014). Since PERMDISP in 'vegan' does not allow for the inclusion of covariates into PERMDISP analyses, it is a common approach to include covariates in PERMANOVA, but omit them from PERDISP (Donohue *et al.* 2009; Ciccolini *et al.* 2016; Hill & Pawley 2019; Ramos *et al.* 2020).

To test our hypothesis that (ii) expansion stage mediates the relative influence of selection, dispersal, and drift on fungal communities, we modelled fungal turnover (i.e. β diversity) within each expansion-stage in relation to variation in abiotic (microclimate and soil), biotic (plant community richness and composition) and spatial (geographic distance) predictor variables. By summing the scaled individual contributions of each predictor into appropriate partitions (microclimate, soil, biotic and spatial) we were able to compare the relative influence of these community assembly processes across expansion stages. To do this, we used generalized dissimilarity modelling (GDM) in the 'gdm' package (Ferrier *et al.* 2007; Manion *et al.* 2017) for R. In GDM, predictor variables are transformed into a series of I-spline base functions and used to explain deviations in β diversity from a null model (β deviance), hereafter referred to as turnover. The maximum height of spline curves represents the maximum contribution of that variable to explaining turnover, and the contributions are tested for significance by permutating (permutations=99) the matrix of predictor variables (n=11) to remove the trend in one variable while holding all other constant. GDM is computationally heavy, and since it is working with distance matrices, requires less permutation than other approaches in this study. Nonetheless, we chose a relatively high number of permutations (compared to other studies employing the same approach) to ensure accuracy of the results (Bongalov *et al.* 2019). GDM has been shown to accurately model the non-linear response of microbial communities to fine environmental and spatial gradients (Glassman *et al.* 2017), and has been used for answering similar questions

regarding community assembly (Bongalov *et al.* 2019). Pairwise geographic distance can easily be incorporated into GDM as demonstrated in Fitzpatrick *et al.* (2013) and Glassman *et al.* (2017a).

Using GDM, we performed model selection separately for each expansion stage in order to identify the combination of variables best explaining variation in fungal turnover between plots. First, we constructed a global GDM containing all predictor variables (soil moisture, maximum ground temperature, C, P, NO3, NH4, pH, plant richness, plant composition PC1 and PC2, and geographic distance), and calculated the percent turnover explained, model p-value and the individual contributions and significance of each predictor. From this model, the least important predictor was then dropped, a new model containing the remaining predictors was constructed, and model parameters calculated. This process was repeated until a model containing only the single most important predictor was generated. Then, from all models generated, the model that explained the most turnover with a p-value less than 0.05 was selected as the best model, and reported in the results.

In order to partition the turnover explained by each best model into abiotic, biotic and spatial components, we summed the individual contributions to explained turnover of each significant predictor based on their class (abiotic, biotic, spatial). We chose to report abiotic variables as two groups (climate and soil) in order to generate meaningful inferences on differing community assembly processes in our root-associated fungal communities. The remaining portion of explained turnover, not allocated into any distinct partition, represents variation explained by the interaction between variables (Bongalov *et al.* 2019). Variation in community turnover not explained by the model represents a combination of local stochastic processes such as ecological drift and unmeasured environmental/spatial variables (Myers *et al.* 2013).

Note that in addition to running these analyses separately for each expansion stage, we also performed them across expansion stages but between functional guilds (all fungi, saprotrophs, symbiotrophs and pathotrophs) to examine whether community assembly processes differ depending on fungal functioning in this study system.

3. Effect of range-expansion on the α diversity of root-associated fungi

To test our hypotheses that (iii) range expansion leads to an increase in the α diversity of root-associated fungi, we quantified fungal richness and relative abundance per plot for all fungi as well as for functional guilds of saprotrophs (decomposers), symbiotrophs (mutualists) and pathotrophs (parasites). Fungal richness was calculated as the total number of unique ASVs per plot, with saprotroph, symbiotroph and pathotroph richness being the number of unique ASVs that identified to each respective functional guild with a high degree of probability. Relative abundance was calculated as the number of reads corresponding to a specific functional guild divided by the total number of reads (n = 18000) per plot.

To test for significant differences in fungal richness and relative abundance between expansion stages (control, sapling, adult pine), we used generalized linear models (glms) fitted based on gaussian distributions and identity link functions. Relative-abundance was log-transformed to achieve normality, and along with the normally distributed richness data, permitted the use of gaussian distributions. To account for the influence of plant community structure and isolate the effect of range-expansion on fungal α diversity, we included biotic variables (plant richness and composition) as covariates in the GLMs. Outliers were identified based on Cook's distance, and removed from this analysis. We assessed model fit using AIC and R^2 and estimated marginal expansion-stage means and pairwise p-values using the 'emmeans' package (Searle *et al.* 1980) for R, with Tukey-corrected p-value adjustments.

To assess the influence of biotic and abiotic selection on the relative abundance and richness of fungi, we constructed glms based on gaussian distributions with identity link functions, using the same predictors as in the GDM analysis with the exception of dispersal predictors, since pairwise distance (distance matrix) cannot be included in our univariate glms. First, we constructed a global model containing all predictors. Then we used AIC-based model selection in the 'MuMln' package (Barton 2009) for R to select the model which best fit our data. Using these best models, we then calculated the individual contribution of predictor variables to explained variation using lmg (the R^2 contribution averaged over orderings among regressors) in the 'relaimpo' package for R (Grömping 2006). Again, similar to our approach to partitioning GDM results, we summed the relative individual contributions of significant predictors based on class (climate, soil, biotic) in order to identify the processes influencing fungal α diversity. The contribution of predictors included in the best models that did not significantly explain variation in our response variable represents variation explained by the interaction between variables, and was labeled as such. This lmg approach as demonstrated in Li *et al.* (2020) and Gavish *et al.* (2019), is robust to colinear variables can be used to partition variation in α diversity, calculating the independent relative contributions of individual predictors, before summing based on partition. Note that similar to our GDM analysis, this analysis was performed both between expansion stages (on all fungi) as well as between functional guilds.

4. Effect of range-expansion on γ diversity of root-associated fungi

To assess the impact of range-expanding pines on fungal γ diversity (iv), we used species accumulation curves. Plots were randomly selected within expansion stage and the Chao1 index (Chao 1987) was calculated after each plot addition until all of the samples had been added. This

process was repeated 1000 times to produce confidence intervals. The maximum height of each curve represents the Chao estimated γ diversity within each expansion-stage for this study site.

RESULTS

Trees in adult pine plots were significantly older and taller, with thicker trunks and larger canopies compared to sapling plots (Table S2 in supplementary material), indicating our assignment of expansion-stage accurately represents trees at different stages of growth, and thus at different stages of range expansion. Specifically, average tree age was 7 and 16 years old for saplings and adult pines respectively.

We generated a total of 2627 fungal ASVs from 9,842,980 raw reads. Of these ASVs, 588 identified as saprotrophic fungi, 178 as symbionts, and 113 as pathogens. The most common genus was *Mycena* (saprotrophic) followed by *Cadophora* (pathotropic), *Phialocephala* (symbiotrophic), *Serendipita* (symbiotrophic) and *Lachnum* (saprotrophic).

1. Effect of range-expansion on the biotic and abiotic environment

Abiotic conditions differed between expansion-stage both in mean and in variance (Fig. 2b, Table 1, Table S3). Maximum ground surface temperature was 7°C and 10°C lower in adult pine plots compared to saplings and controls respectively. Soil in adult pine and sapling plots contained 43-46% more moisture and 20-29% less phosphorous (respectively) compared to control plots. Adult pine plots also had increased variation in soil moisture. Sapling plots had 43-49% less nitrate compared to control and adult pine plots. Soil carbon, pH, ammonium, and plot altitude did not differ significantly between expansion-stages. However, variation in phosphorous, carbon, ammonium and pH decreased with expansion stage, with later stages of expansion exhibiting the least variation.

In order to further investigate the abiotic impacts of range expansion, specifically the potential of range expanders to homogenize the abiotic environment over space, we quantified the spatial structuring of abiotic variables by expansion stage (Fig S3, Table S4). We found that pH was significantly spatially autocorrelated in control (Moran's I=0.12, p=0.002) and sapling (Moran's I=0.09, p=0.02) plots, but not in adult pine plots (p=0.7). Ammonium was also spatially autocorrelated, but only in control plots (Moran's I=0.19, p=0.001). Soil moisture exhibited significant spatial autocreation in adult pine plots only (Moran's I=0.06, p=0.01).

To investigate the impact of range expansion on the biotic environment (i.e. resident plant communities) we tested for the statistical effect of range expansion on resident plant richness, plant composition, and homogeneity of plant composition, however we observed no significant statistical effects (Fig. 2b, Fig. S4, Tables S5-S7).

In order to further investigate the biotic impacts of range expansion, specifically the potential of range expanders to homogenize biotic communities over space, we quantified the spatial structuring of biotic variables by expansion stage (Fig S3, Table S8). Plant community composition (PC1) was significantly spatially autocorrelated in control (Moran's I=0.09, p=0.003) and sapling (Moran's I=0.08, p=0.02) plots, but not in adult pine plots (p=0.6). Finally, Mantel correlograms, used to examine the spatial structuring and dependence of fungal communities revealed that fungal β diversity was positively spatially autocorrelated in sapling (Mantle R=0.201, p=0.014) and control plots (Mantle R=0.296, p=0.001) at low distance classes (200-300m), while communities in adult pine plots exhibited no spatial autocorrelation, indicating that range expansion may lead to spatial homogenizing of fungal communities (Fig. S5).

2. Effect of range-expansion on β diversity of root-associated fungi

Supporting our hypothesis that (i) range expansion increases fungal β diversity, expansion stage explained a significant amount of variation in fungal β diversity (Fig. 3), varying from 5% for all fungi and saprotrophs to 10% for pathotrophs (non-significant for symbiotrophs; Table S9). However, contradicting this hypothesis, there was no significant increase in β diversity as range expansion progressed, suggesting that although range expansion influenced fungal community composition, communities did not become more similar or dissimilar as a result (Table S10).

In partial support of our second hypothesis (ii) that range expansion weakens the influence of abiotic and biotic selection, the relative influence of both processes varied between expansion stages (Fig. 4a, Table 2). Abiotic selection (driven by soil carbon availability) explained 3.67% of variation in fungal turnover in control plots, 4.61% in sapling plots and 0% in adult pine plots. However, contrasting with our prediction, the influence of soil moisture, another abiotic filter, was only significant in adult pine plots (6.16%), suggesting a stronger influence of abiotic selection in later stages of range expansion, but driven by a different abiotic factor. Additionally, the influence of biotic selection, specifically host plant richness, was twice as high in control plots (40.77%) as it was in saplings (19.92%) and adult pine (26.05%) plots.

We hypothesized that the relative influence of selection would decrease with expansions stage, which implies that the relative influence of other processes should increase. Contrary to this prediction, the influence of dispersal (pairwise geographic distance) was greatest in control plots, explaining 6.02% of the variation in β deviance. This effect was lower in sapling plots (4.01%) and even more so in adult pine plots (0.06%). On the other hand, the portion of unexplained variation, which may indicate the influence of stochastic processes, was greater in adult pine plots (48.5%), followed by sapling (41%) and control (37.5%) plots.

Note that we completed the same analysis across expansion stages but between functional guilds (all fungi, saprotrophs, symbiotrophs, and pathotrophs) and results are available in Fig 4b and Table S11 in the supplementary material.

3. Effect of range-expansion on α richness of root-associated fungi

Contradicting our third hypothesis that (iii) range expansion would increase fungal, the richness of all root-associated fungi remained consistent between expansion stages (Fig. 5, Table S12). Additionally, while saprotroph richness also remained consistent between expansion stages, symbiotroph richness decreased and pathotroph richness increased as range expansion proceeded. Specifically, symbiotroph richness was 15-11% lower in sapling and adult pine plots compared to controls and pathotroph richness was 25-33% higher in sapling and adult pine plots compared to controls, providing mixed evidence that range expansion leads to richer communities of fungi and instead suggesting that different functional guilds respond differently to the impacts of range expansion. The relative abundance of root-associated fungi varied greatly between plots, however we found no evidence that expansion stage had any effect on the relative abundance of root-associated fungi (Table S13).

Investigation into the abiotic and biotic variables influencing the observed patterns in fungal α diversity, revealed changes in the strength of abiotic and biotic selection between expansion stages (Fig. 6a, Table 3). Specifically, the strength of abiotic selection increased in sapling and adult pine plots compared to controls, driven by micro-climatic factors. Temperature explained 10% of variation in richness in sapling plots and soil moisture explained 10% of the variation in adult pine plots, while neither variable explained significant variation in control plots. The influence of soil factors on fungal richness was consistently important between expansion stages, specifically pH (4%) and ammonium (3%) in control plots, pH (7%) and

carbon (3%) in sapling plots and pH (6%) in adult pine plots. Biotic selection, specifically host plant richness, strongly influenced fungal richness within expansion stages, explaining 88% of the variation in richness in control plots, 74% in sapling plots and 70% in adult pine plots.

Note that we completed the same analysis across expansion stages but between functional guilds, which indicated that the richness of different functional guilds is influenced by different variables, notably plant richness in the case of symbiotrophs and soil moisture and maximum ground temperature in the case of pathotrophs (Fig. 6b, Table S14). Furthermore, although there was no evidence that range expansion impacted the relative abundance of root-associated fungi, the relative abundance of different functional guilds was influenced by abiotic variables, notably soil moisture (Table S15).

4. Effect of range-expansion on γ richness of root-associated fungi

Contradicting our hypothesis that (iv) range expansion would lead to increased regional (γ) diversity of root-associated fungi, estimated γ diversity of all root-associated fungi did not differ between expansion-stages (Fig. 7). For all fungi, γ diversity was highest in sapling plots (1931) compared to controls (1884) and adult pine (1832).

Patterns of γ diversity differed greatly among functional guilds (Fig. 7). The γ diversity of saprotrophs was again highest in sapling plots (458) compared to controls (447) and adult pine (446), however, similar to all fungi, there is likely no significant difference between expansion stages. On the other hand, symbiotroph γ diversity lower and pathotroph γ diversity was higher in adult pine communities, compared to sapling and controls, with error bars suggesting potential significant differences in γ diversity between expansion stages. For symbiotrophs, γ diversity was highest in sapling plots (143) compared to controls (140) and considerably lower in adult

pine plots (123). For pathotrophs, γ diversity was highest in adult pine plots (92) compared to saplings (86) and controls (81).

DISCUSSION

In this study, we show that a single range expanding foundation species can significantly impact belowground diversity and alter the processes governing community assembly through the creation of novel microhabitat. Range expansion altered environmental conditions, creating spatially explicit microhabitats in terms of soil chemistry and microclimate. Contrary to our predictions, range expansion led to increased variation and spatial autocorrelation in soil moisture, increasing the relative influence of abiotic selection, while the influence of biotic selection and dispersal on fungal community composition decreased. Whereas these changes in assembly processes only lead to minor differences in the composition of fungal communities, fungal communities in these novel microhabitats also exhibited a decrease in the richness of symbionts (mutualistic fungi) and an increase in the local richness of pathogens (parasitic fungi). These changes in fungal richness also led to broader-scale changes in regional richness. In sum, our results suggest that range-expanding foundation species reroute the assembly trajectory of root-associated microbial communities resulting in shifts in community composition and taxon-specific changes in richness, which has important implications for the future biodiversity and functioning of these ecosystems.

Creation of microclimatic islands in alpine ecosystems

The ability of foundation species to alter environmental conditions through habitat creation has traditionally been assessed through the removal or loss of foundation species (Ellison *et al.* 2005; Peters & Yao 2012). Here we found evidence that, similar to their removal, the spread of foundation species into new territory alters environmental conditions and creates distinct islands

of microhabitat. As predicted, we found wetter and cooler soils beneath range-expanding trees, likely a result of canopy shading and root growth (van Dijk & Keenan 2007), an effect that has been long associated with tree establishment (Dancette & Poulain 1969; Belsky *et al.* 1993). Contrary to our predictions however, range expansion also increased the variation in soil moisture. This pattern could be due to variation in tree size within expansion stage with larger trees retaining more water in soils due to increased canopy cover or root growth. However, when we evaluated this theory, only basal diameter (thickness of the trunk at the base of the tree) significantly correlated with soil moisture (Pearson's $r = 0.14$, $p=0.05$), suggesting that variation in root system size or depth and not variation in canopy cover, may be responsible for increased variation in soil moisture.

For some resident taxa, these conditions may be more tolerable compared to the adjacent rocky habitat, prone to the high winds, extreme temperatures, and constant sun typical of alpine ecosystems. This facilitative effect of foundation species has been previously observed in tundra plants (Carlsson & Callaghan 1991), savanna grasses (Moustakas *et al.* 2013), belowground alpine fungi (Roy *et al.* 2013), and cushion forming plants/mosses (Schob *et al.* 2012; Butterfield *et al.* 2013). Range-expanding pines also altered the availability of key soil nutrients. Soil nitrate and phosphorus concentrations were negatively related to expansion stage possibly due to age-dependent physiological and metabolic requirements (Ovington 1959; Brockley 2001). Lower concentrations of phosphorus could also result from obligate associations between pines and mycorrhizae, which leads to higher rates of phosphorus uptake (Mejstrik & Krause 1973; Jumpponen *et al.* 1998; Hayward *et al.* 2015).

Range expansion also altered the spatial structuring of abiotic and biotic factors across the study region. Most notably, the presence of a novel foundation species removed the spatial

autocorrelation of soil ammonium and pH, and increased the spatial autocorrelation of soil moisture, perhaps due to variation in root systems size as previously suggested, or by amplifying a preexisting spatial structuring of runoff and catchment in this system, or modifying hydrological regimes (Farley *et al.* 2005; Little *et al.* 2008). Range expansion also removed the spatial autocorrelation of plant composition, which could be a response of the plant community to more spatially homogeneous and stable environmental conditions (Astorga *et al.* 2014). Recent work has demonstrated the positive relationship between the spatial structuring of flowering-plant diversity and spatial variation in temperature in alpine systems (Ohler *et al.* 2020), and the indirect consequences on the richness and specialization of plant-insect interaction networks. Our results suggest that range-expanding foundation species can change the spatial distribution of variation in abiotic variables, which could in turn impact the relative influence of assembly processes such as abiotic selection and ecological drift.

Changes in the relative influence of assembly processes

Our data indicates that range expansion increases the importance of abiotic selection, contradicting our predictions and suggesting that the initial impacts of range expansion are not facilitative. Furthermore, different environmental variables were responsible for abiotic selection, with range expansion leading to selection driven by variation in soil moisture as opposed to soil carbon (Fig. 4). For microbes in particular, who exhibit rapid turnover, abiotic selection has been shown to heavily influence community assembly (Szekely & Langenheder 2014; Glassman *et al.* 2017). The abiotic changes we observed, specifically wetter and cooler soils, have been previously observed during tree range expansion/invasion into treeless ecosystems (Pierce & Reich 2010). Therefore, it is likely that our finding that range expansion

impacts abiotic selection and the relative influence of assembly processes is general, at least for treeless ecosystems.

In support of our predictions, the influence of biotic selection appears weaker as range expansion proceeds, as evidenced by the decreasing dependence on biotic selection in later stages of range expansion. Specifically, we found that as range expansion proceeds, the influence of plant community richness on fungal β diversity weakens (Fig. 4). Since stressful environments promote a higher frequency of mutualistic interactions (Nuñez *et al.* 1999; Stachowicz 2001; Kivlin *et al.* 2017), we expected biotic selection in harsh alpine environments to exert a strong influence prior to the invasion. This phenomenon is known as the stress gradient hypothesis (Bertness & Callaway 1994; Maestre *et al.* 2009). In accordance with this hypothesis, the observed decrease in the influence of biotic selection as range expansion proceeds could result from host plants depending less strongly on mutualistic interactions with fungal symbionts, due to decreased abiotic stress. Recent work on plant-fungi interactions in alpine communities supports this idea (Lynn *et al.* 2019). If this hypothesis is indeed as general as past studies suggest (Callaway 2007), then it is likely that worldwide range expansions will lead to a general decrease in mutualisms and increase in competition within impacted communities (García-Cervigóna *et al.* 2013), with biotic selection playing a smaller role in determining community assembly.

As range expansion proceeds, the relative influence of dispersal in determining community assembly decreased, possibly due to the spatial homogenization of environmental conditions limiting the recruitment of rare taxa. Past research has shown that dispersal has a strong influence in species-rich communities with high numbers of rare taxa, due to the reduced likelihood that many rare species reach all suitable habitat (Hubbell *et al.* 1999; Hubbell 2001).

In this study, while we found no evidence suggesting that range expansion limited the number of rare taxa, we did find strong evidence that it led to spatially homogeneous environmental conditions. Environmental heterogeneity can be crucial in providing suitable conditions for rare taxa and promoting biodiversity on fine scales (Astorga *et al.* 2014; Bergholz *et al.* 2017). As such, spatial homogenization of environmental conditions may limit the ability of physical distance to resolve patterns in fungal turnover, by curating spatially homogeneous or independent communities of fungi. On the other hand, the reduction in the influence of dispersal following range expansion may simply be an effect of overriding selection, where the influence of strong spatial variation in soil moisture eliminated any preexisting spatial patterns in fungal turnover. Regardless, we found clear evidence that range expansion alters the importance of dispersal in driving both environmental and community similarity, indicating that homogeneity may be a long term consequence for ecosystems facing the threat of forestation (Harsch *et al.* 2009; García *et al.* 2019).

In general, range expansion may increase the relative influence of ecological drift, although directly testing the role of drift in community assembly is challenging in observational studies (Gilbert & Levine 2017), especially for microbial data (Zhou & Ninga 2017). In this study, variation in fungal turnover not explained by our models represents some combination of drift and unmeasured environmental variation (Myers *et al.* 2013). As such, it is unclear as to whether the increase in unexplained turnover as range expansion proceeds is due to an increase in the relative influence of drift or other unmeasured variables structuring fungal communities. Explicitly modelling the influence of drift in microbial community data remains a challenge, and current approaches are limited by both conceptual and statistical issues (Zhou & Ninga 2017).

Changes in the local richness of symbiotrophs and pathotrophs

We found strong evidence that range expansion led to changes in the richness of root-associated microbes. Specifically, range expanders decreased the richness of fungal symbiotrophs and increased the richness of fungal pathotrophs, potentially leading to shifts in the functioning of belowground communities. The presence of mutualistic biotic interactions is common in alpine ecosystems, typically in the form of plant-plant (e.g. cushion plants) or plant-mutualist (e.g. symbiotrophic fungi) associations (Nuñez *et al.* 1999; Stachowicz 2001; Callaway *et al.* 2002; Butterfield *et al.* 2013; Hupp *et al.* 2017). Indeed, mutualistic fungal symbionts play a crucial role in alleviating abiotic stress, allowing plants to persist in harsh alpine conditions by aiding in nutrient acquisition and pathogen defense and conferring thermotolerance (Haselwandter & Read 1982; Redman *et al.* 2002; Kivlin *et al.* 2013; Lenoir *et al.* 2016). Pathotrophic fungi, on the other hand, parasitize plants, resulting in infection, decreased fitness, increased mortality (Hawksworth 2001), and leading to their role as critical maintainers of high diversity in competitive ecosystems (Bagchi *et al.* 2010, 2014). As such, changes in the local diversity of these fungal functional guilds may have serious repercussions for plant communities, ecosystem functioning and biodiversity.

The observed decrease in symbiont richness and increase in pathogen richness as a result of range expansion likely reflect different selection pressures, as biotic variables (specifically host plant richness) positively influenced symbiont richness whereas microclimate factors (specifically soil moisture) positively influenced pathogen richness (Table S14). Aside from host plant richness, symbiont richness was also influenced by soil nitrate, which was significantly lower during early stages of range expansion, but was at similar levels both before and during later stages. As such, it remains unclear if abiotic selection plays a role in determining fungal

symbiont diversity and if/how this relationship is impacted by range expansion. On the other hand, it is possible that symbiont richness was lower after range expansion due to a decreased dependence of plants on fungal symbiosis to overcome abiotic stress (Bertness & Callaway 1994). If this is the case, it indicates that the abiotic impacts of range expansion create more tolerable conditions for resident plants. This idea aligns well with our finding that biotic interactions are less influential on community assembly after range expansion, and is supported by previous research looking at the relationship between fungal symbiosis and abiotic stress (Kivlin *et al.* 2017).

The increased pathogen richness accompanying range expansion is likely a result of positive abiotic selection. Whereas we usually think of selection as a filtering process preventing maladapted species to establish and persist, the converse can also be true. By changing and stabilizing environmental conditions where they establish, range expanders likely reduced abiotic stress, potentially leading to facilitation or "positive filtering" (Schob *et al.* 2012). These conditions may promote persistence of many fungal pathogens, who depend on antagonistic relationships with host plants for survival (Hawksworth 2001). Cooler and wetter soil thus increased pathogen richness either due to these novel conditions increasing fungal fitness or decreasing host plant resistance to pathogenic infection, possibly due to plants being more tolerant to infection due to decreased abiotic stress (Wiese *et al.* 2004; Atkinson & Urwin 2012). Or, richer communities of pathogens may be related to decreased symbiont diversity, as past work has shown that fungal symbionts play important roles in pathogen defense (Pozo *et al.* 2009; Zeilinger *et al.* 2016).

Long term consequences for communities

While the impacts we outline in this study reflect the impact of a single, range-expanding individual, long-term range-expansion will likely exacerbate and modify these effects, as range expanding trees form secondary and old growth forest stands (García *et al.* 2019). For native biodiversity, the long-term spread of novel trees has severe consequences, permanently altering fire and water regimes, increasing competition among native species for limited resources, and decreasing biodiversity (Simberloff *et al.* 2010; Franzese *et al.* 2017). Our results indicate that some of these impacts, specifically on hydrological and community assembly processes, likely began within the first few years following the initial establishment of the range expanding species. Understanding how these initial impacts of range expansion work to structure and organize novel ecosystems, and how these processes change over time, may hold the key to designing effective and successful conservation/restoration strategies (Wainwright *et al.* 2017).

Implications

In response to rapid environmental change caused by humans, foundation species around the world are shifting their ranges and expanding into new territories at an unprecedented rate (Hobbs *et al.* 2009; Boivin *et al.* 2016). For many alpine and tundra ecosystems, the establishment and spread of woody species threatens biodiversity and ecosystem functioning, however the specific consequences for these systems are generally unknown (Suarez *et al.* 1999; Harsch *et al.* 2009; Greenwood & Jump 2014). Aboveground plant and belowground microbial community response to range-expansion may determine the fate of these ecosystems, and carry disproportionate consequents for global nutrient cycling (Parker *et al.* 2015).

Here we show that range expansion of a foundation tree species leads to shifts in community assembly processes, influencing microbial β diversity, decreasing the richness of fungal symbionts, and increasing the richness of fungal pathogens. It is important to note that the

changes we outline in this study reflect the impacts of single range-expanding individuals. As such, our findings suggest severe long-term impacts for alpine and tundra ecosystems threatened by range expansion. An increased dependence on climatic assembly processes, combined with a decreased dependence on biotic selection and the spatial homogenization of soil and biotic variation, will likely lead to strong micro-climate selection associated with range expansion. Over time, this intense selection will likely lead to uniform, depauperate communities of plants, especially tolerable of the micro-climate conditions created by range expanders. Indeed, this pattern has already been identified in impacted ecosystems across much of South America (Abreu & Durigan 2011; Bravo-Monasterio *et al.* 2016; Franzese *et al.* 2017; García *et al.* 2019).

For alpine and tundra ecosystems, defined by a lack of tall vegetation, and heavily structured by landscape heterogeneity and abiotic gradients, the homogeneity accompanying range-expanding foundation species represents more than just a biotic disturbance. The transformation of treeless alpine and tundra biomes into homogeneous forest will have a direct impact on the diversity and function of resident taxa (Carnus *et al.* 2006), and thus represents one of the most direct threats facing native biodiversity today (Potton 1994; Larsson & Danell 2001). As treelines in general continue to shift upward and poleward (Harsch *et al.* 2009), the future of alpine and tundra ecosystems grows increasingly uncertain. These findings fill an important gap in our understanding of how these systems are changing, and highlight the need for a rapid conservation strategy to conserve and protect these threatened biomes.

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TABLES

Table 1 — Mean and standard deviation of environmental variables by expansion-stage (Control, Sapling, Adult Pine). Significant differences between expansion stages are indicated with letters. ANOVA results are available in Table S3 in supplementary material.

Predictor	Unit	Control Plots		Sapling Plots		Adult Pine Plots	
		Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev
Maximum Ground Temperature	°C	43.07[a]	4.35	40.23[a]	4.85	33.19[b]	3.92
Soil Moisture	%	3.57[a]	1.84	6.62[b]	2.34	6.22[b]	3.30
Imputed Soil Moisture	%	3.72[a]	1.75	6.62[b]	2.34	6.62[b]	3.19
Phosphorous	ppm	6.87[a]	1.41	4.92[b]	0.79	5.53[c]	0.62
Nitrate	ppm	15.97[a]	4.01	9.11[b]	5.32	14.86[a]	6.54
Ammonium	ppm	36.68	18.58	31.36	12.27	34.44	10.69
Carbon	%	2.20	0.90	1.88	0.95	1.98	0.76
pH	pH	5.7	0.21	5.68	0.29	5.67	0.12
Altitude	m	1689.93	19.87	1688.87	21.66	1690.80	19.72

Table 2 — Results from GDM analysis of β-deviance (calculated from Bray-Curtis dissimilarity of the entire fungal community) of root-associated fungal communities by expansion-stage (Control, Sapling and Adult Pine). First, global models containing all predictors were constructed and assessed for significance over 99 permutations. Then the predictor deemed least important to explaining variation (assessed through matrix permutation) was dropped and the next model constructed containing all remaining predictors. This process was completed until a model containing only the most important predictor was assessed. From all of these models, the one that explained the most variation in β-deviance and was significant ($p<0.05$) is reported below. Variable contribution represents the individual contribution of each variable to the total explained deviance in this best model. Variable contribution significance was further assessed over 99 permutations. (Significance codes: 0.0 *** 0.001 ** 0.01 * 0.05 ˙ 0.1)

Predictor	Partition	Control	Sapling	Adult Pine
Host Plant Richness	Biotic	34.77*	19.92**	25.05*
Plant Composition (PC1)	Biotic	-	3.01	0.79
Plant Composition (PC2)	Biotic	6.00*	-	-
C (%)	Soil	3.67*	4.61*	1.93
pH	Soil	-	2.27	-
P (ppm)	Soil	-	-	-
NH4 (ppm)	Soil	-	1.04	-
NO3 (ppm)	Soil	-	0.06	1.69
Soil Moisture (%)	Micro-Climate	-	0.47	6.16*
Maximum Ground Temperature (°C)	Micro-Climate	5.49	0.72	-
Spatial (m)	Space	6.02**	4.01**	0.06**
Model Deviance		**0.672**	**0.44**	**0.68**
Model p-value		**<0.001**	**<0.001**	**0.02**
Total Deviance Explained (%)		**62.54**	**59.00**	**51.50**

Table 3 — Results from generalized linear model analysis of all-fungal richness by expansion-stage (Control, Sapling and Adult Pine). We fit models based on gaussian distributions using identity link functions due to the normality of our richness data. First, a global model containing all predictors was constructed. We then used dredge in the 'MuMln' package for R to select the model that best fit the data based on AIC. Finally, we partitioned the variation explained by this best model into individual variable contributions using lmg in the 'relaimpo' package for R. This approach is robust to colinear variables as models are first selected based on AIC before partitioning. (Significance codes: 0.0 *** 0.001 ** 0.01 * 0.05 ˙ 0.1).

Predictor	**Partition**	**Control**	**Sapling**	**Adult Pine**
Host Plant Richness	Biotic	0.876***	0.743***	0.695***
Plant Composition (PC1)	Biotic	-	-	-
Plant Composition (PC2)	Biotic	-	-	-
C (%)	Soil	-	0.028*	-
pH	Soil	0.042**	0.069***	0.058*
P (ppm)	Soil	-	-	-
NH4 (ppm)	Soil	0.025*	-	-
NO3 (ppm)	Soil	-	-	-
Soil Moisture (%)	Micro-Climate	-	-	0.097*
Maximum Ground Temperature (°C)	Micro-Climate	-	0.098**	-
R^2		**0.943**	**0.937**	**0.850**
R^2 adj		**0.926**	**0.910**	**0.809**
AIC		**151.66**	**149.99**	**161.32**

FIGURES

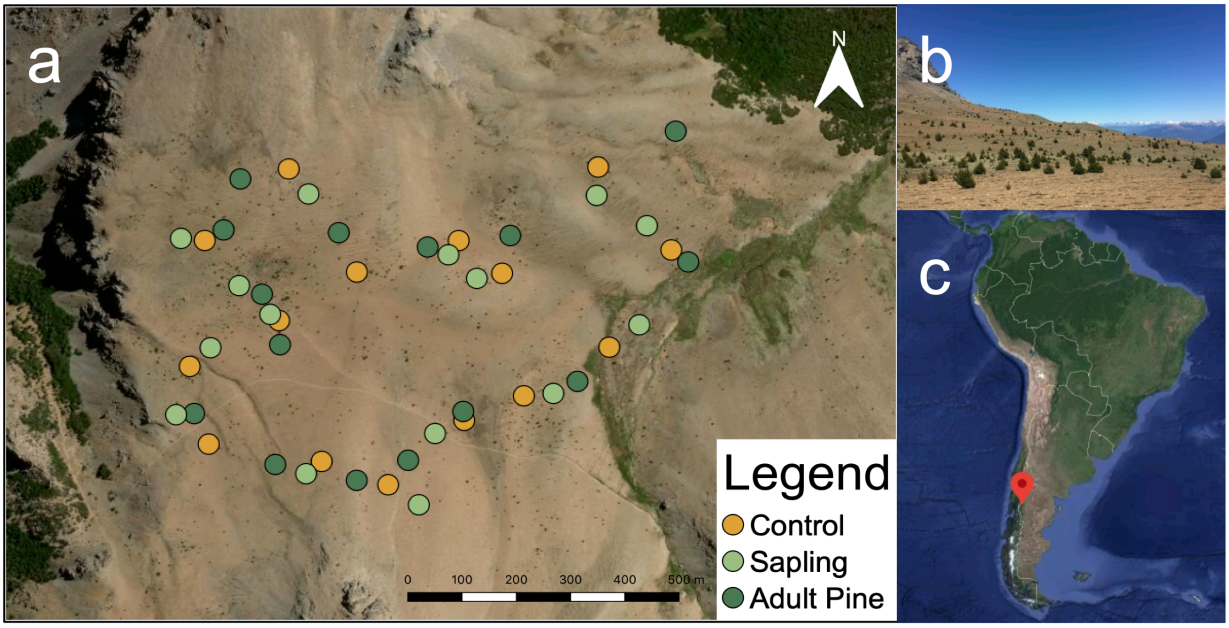

Fig. 1 — (a) Map of study area with sampled plots, (b) photo of study area and (c) geographic location of study area.

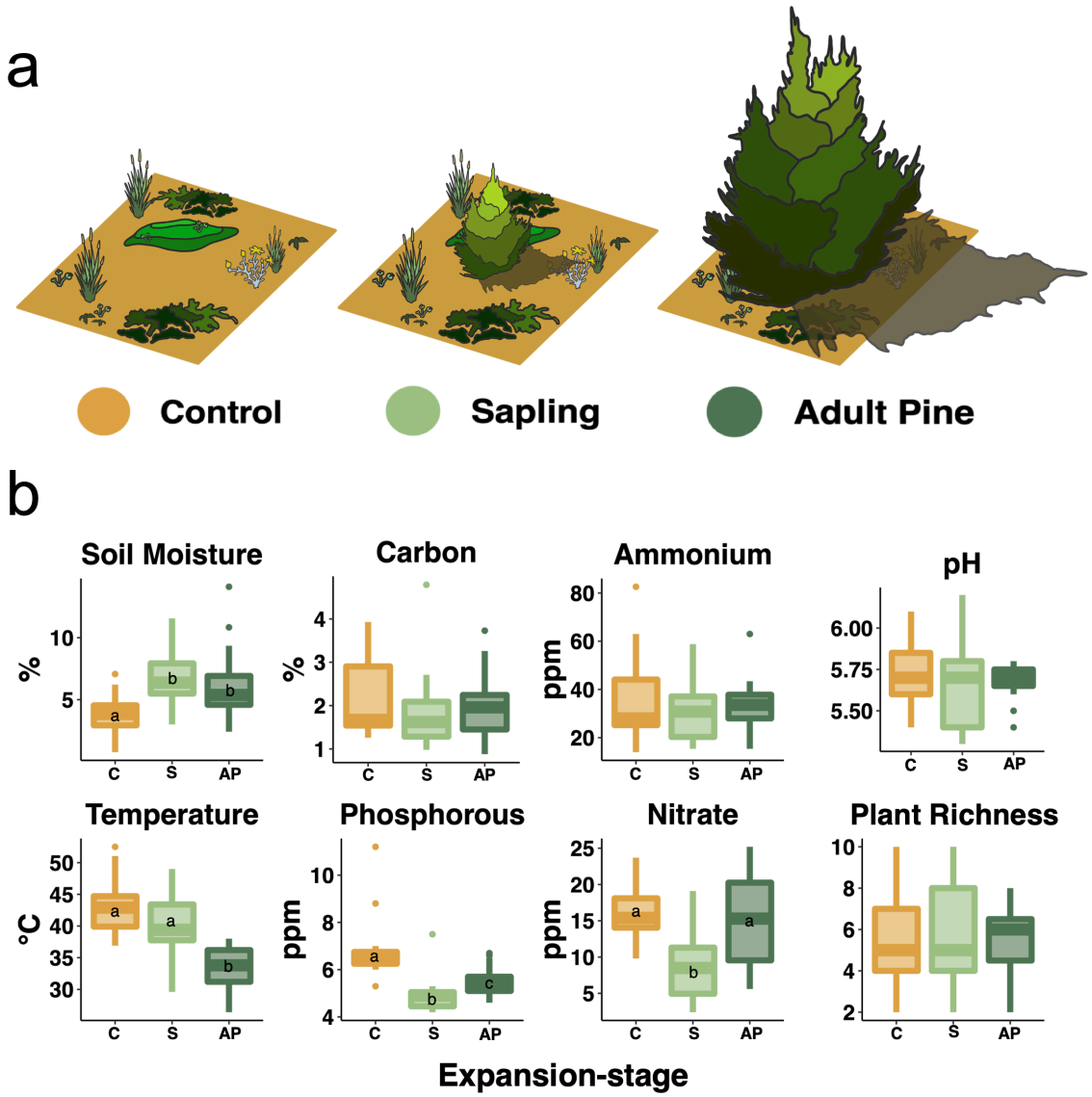

Fig. 2 — (a) Illustration of the differences between expansion-stage and (b) differences in micro-climatic (soil moisture and maximum ground temperature), soil (carbon, phosphorous, ammonium, nitrate and pH) and biotic (plant richness) variables between expansion-stage. Significant differences are indicated with letters, and were tested using ANOVA. Control, sapling and adult pine means for each variable are reported in Table 1.

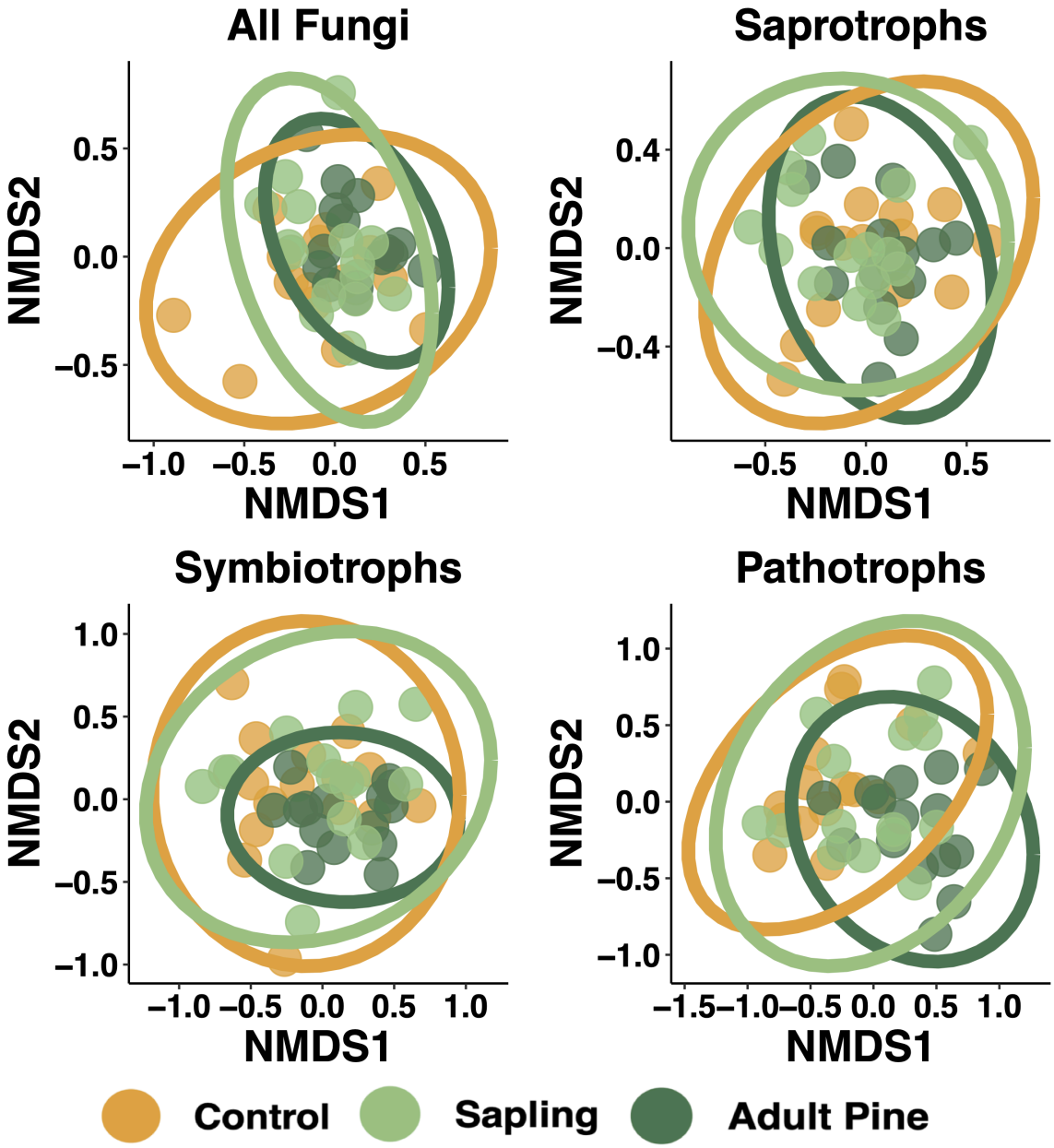

Fig. 3 — Fungal β diversity (Bray-Curtis dissimilarity) visualized in 3D non-metric multidimensional space. Plots are coloured by expansion stage with ellipses representing 95% confidence intervals. Note that while our PERMANOVA analyses incorporate biotic variables as covariates to account for differences in plant diversity between plots, this visualization does not. Stress values were 0.166, 0.186, 0.167 and 0.163 for All Fungi, Saprotrophs, Symbiotrophs and Pathotrophs respectively.

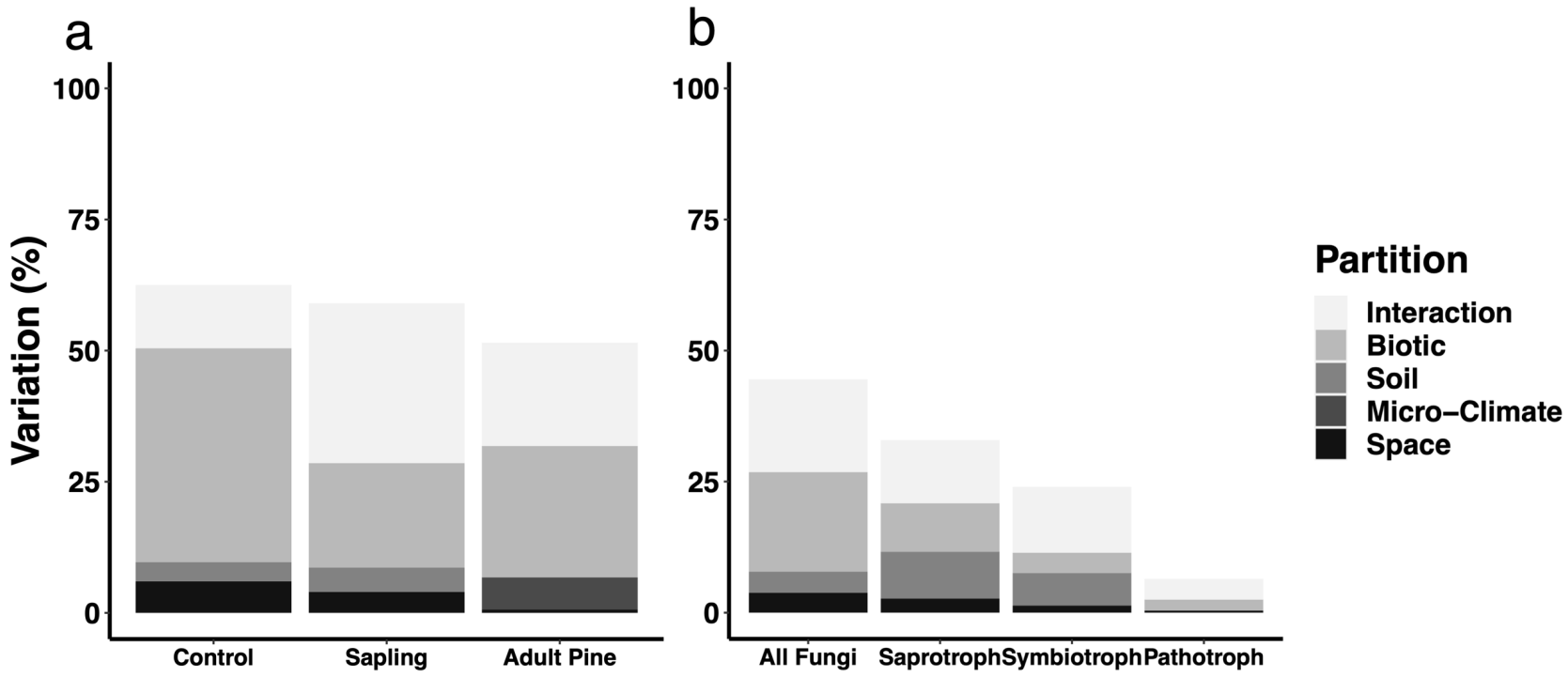

Fig. 4 — GDM Partitioning of significant variable contributions to explained variation in β-deviance (calculated with Bray-Curtis dissimilarity) between expansion stages (a) and between fungal communities (b). Model selection was conducted using matrix permutation to eliminate variables that did not contribute to explained variation, with the best model representing the model that explained the most variation and was overall significant. Non-significant variable contributions were included in the interaction component, which represents variation explained by the model that cannot be significantly assigned to a single variable.

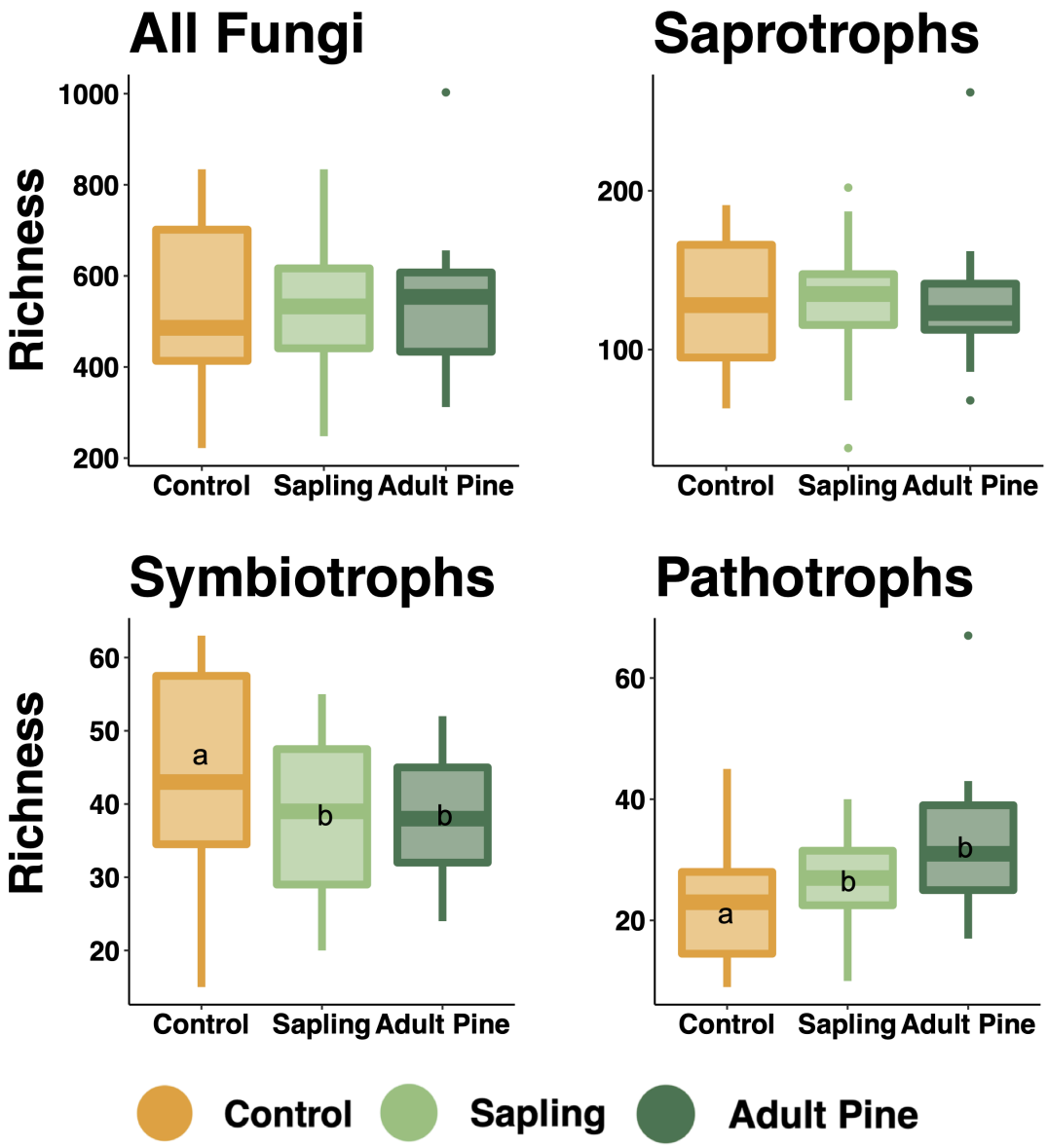

Fig. 5 — All Fungi, Saprotroph, Symbiotroph, and Pathotroph richness across expansion-stages. Significant differences between expansion-stages, represented by differing letters, were assessed using glms based on gaussian distributions with identity link functions, including biotic variables (plant richness and composition) as covariates to account for differences in plant diversity between plots and expansion stage. Note that this visualization does not incorporate covariates and instead illustrates the patterns in fungal richness between expansion stages before accounting for plant diversity.

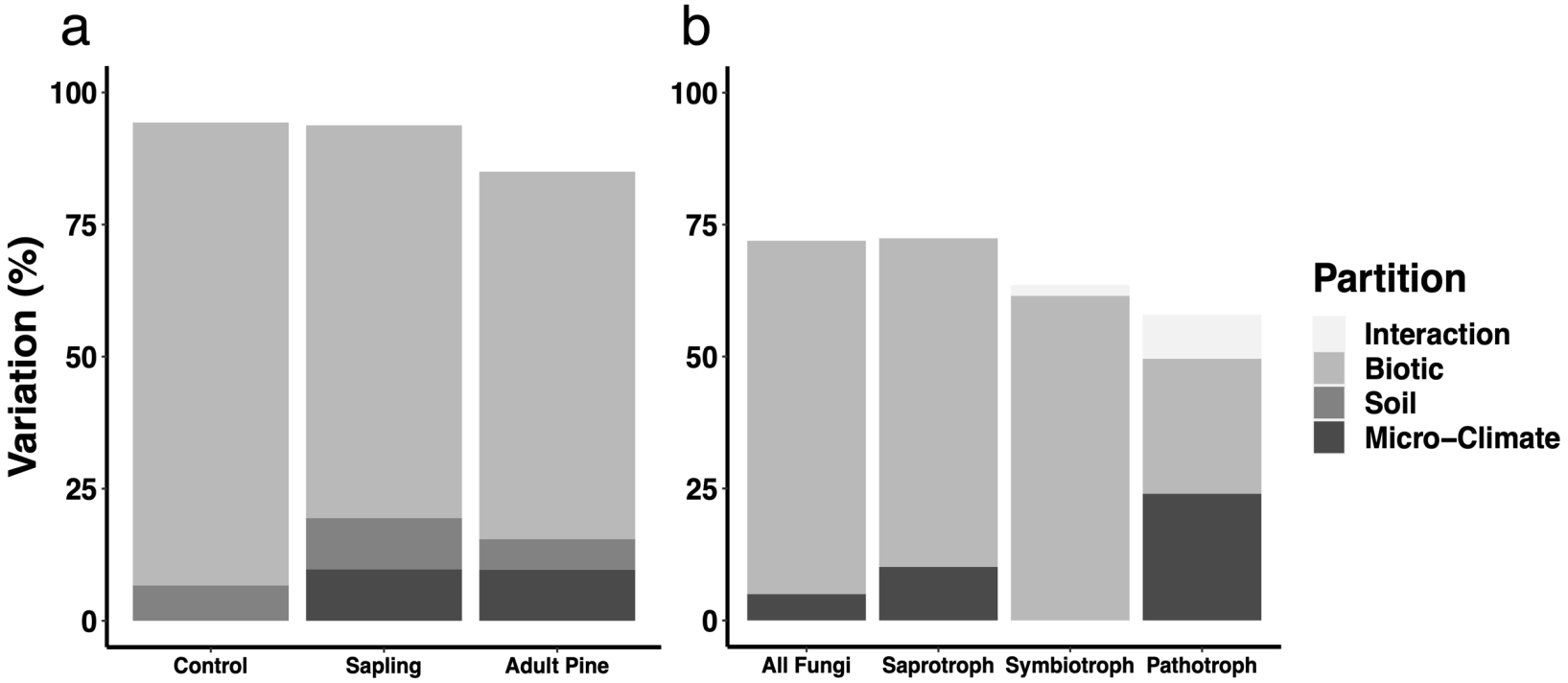

Fig. 6 — Partitioning of significant variable contributions to explained variation in fungal richness between expansion stages (a) and between fungal communities (b). Partitioning was conducted using Relative Importance of Regressors in generalized linear models based on gaussian distributions with identity link functions. Models were selected based on AIC using the 'MuMIn' package for R. Model R^2 value is partitioned based on individual variable contribution, and summed based on partition (biotic, soil, micro-climate). Non-significant variable contributions were included in the interaction component, which represents variation explained by the model that cannot be significantly assigned to a single variable.

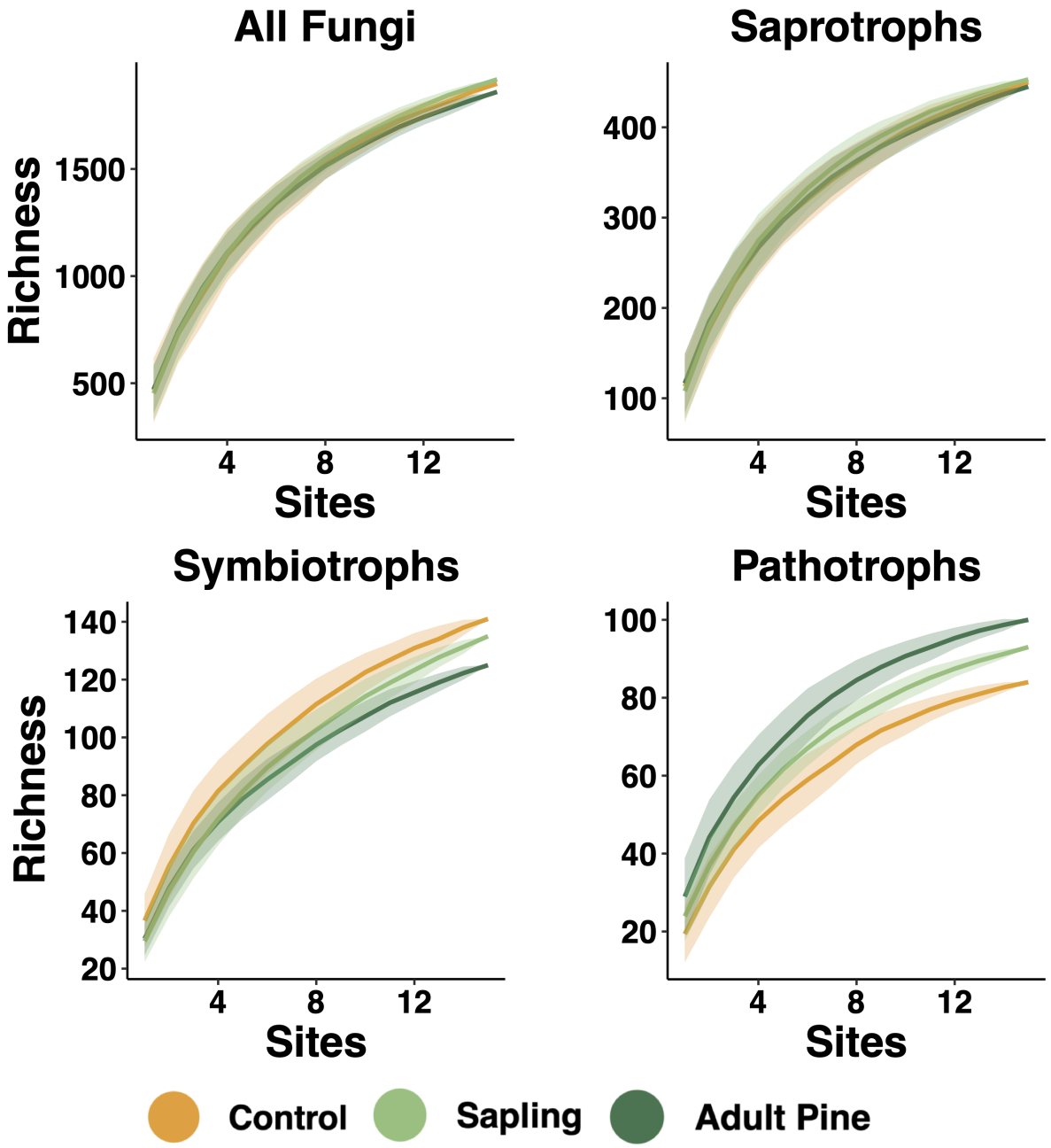

Fig. 7 — All Fungi, Saprotroph, Symbiotroph and Pathotroph γ diversity by expansion-stage. γ diversity is represented using species accumulation curves, where sites were randomly sampled and the accumulated chao1 index calculated. Confidence intervals were produced by repeating this procedure 1000 times.

SUPPLEMENTARY MATERIAL

Tables

Table S1 — List of plants identified in the study area.

Plant Name
Azorella sp.
Baccharis magellanica
Bolax gummifera
Empetrum rubrum
Erigeron sp.
Euphrasia meiantha
Festuca sp.
Gaultheria pumila
Hypochaeris sp.
Mulinum echinus
Nassauvia revoluta
Nasthantus patagonicus
Oxalis adenophylla
Perezia bellidifolia
Perezia fonkii
Poa sp.
Senecio agyreus
Viola sp.

Table S2 — t-Test results for differences in tree properties between sapling and adult pine plots.

Variable	Expansion stage	Group Mean	df	t	p
Basal Diameter	Sapling	4.36	19	8.0427	<0.001
	Adult Pine	17.04			
Half Tree	Sapling	48.86	19	10.074	<0.001
Cover	Adult Pine	128.06			
Tree Height	Sapling	102.60	19	10.114	<0.001
	Adult Pine	310.53			
Trunk	Sapling	13.71	19	8.042	<0.001
Circumference	Adult Pine	53.53			
Age	Sapling	6.86	19	2.093	<0.001
	Adult Pine	15.93			

Table S3 — ANOVA results for differences in environmental variables by expansion stage. Pairwise p-values represent Tukey corrected pairwise tests for differences between groups.

Variable		Df	Sum Sq	Mean Sq	F	p	Pairwise p
Soil Moisture	Expansion-stage	2	72.95	36.48	5.827	0.006	P-C=0.028 S-C=0.007 S-P=0.86
	Residuals	42	262.94	6.36			
Maximum Ground Temperature	Expansion-stage	2	775.1	387.5	20.14	<0.001	P-C<0.001 S-C=0.19 S-P<0.001
	Residuals	42	808.2	19.2			
C (log)	Expansion-stage	2	0.263	0.117	0.765	0.472	
	Residuals	42	6.47	0.154			
P (log)	Expansion-stage	2	0.603	0.301	39.59	<0.001	P-C<0.001 S-C<0.001 S-P<0.001
	Residuals	42	0.297	0.007			
NO3	Expansion-stage	2	406.6	203.3	6.99	0.002	P-C=0.839 S-C=0.003 S-P=0.015
	Residuals	42	1220.5	29.06			
NH4	Expansion-stage	2	214	107	0.626	0.595	
	Residuals	42	8541	203.3			
pH	Expansion-stage	2	0.005	0.003	0.061	0.94	
	Residuals	42	1.973	0.0469			
Altitude	Expansion-stage	2	28	14.1	0.034	0.96	
	Residuals	42	17543	417.7			

Table S4 — Moran's I measures of spatial autocorrelation for environmental variables by expansion-stage. Significant spatial autocorrelation is indicated in **bold**.

Variable	Partition	Moran's I	Adjusted p	Expansion-stage
Ammonium	**Soil**	**0.19**	**0.001**	**Control**
Ammonium	Soil	-0.029	0.53	Sapling
Ammonium	Soil	-0.049	0.66	Adult Pine
Carbon	Soil	-0.029	0.4	Control
Carbon	Soil	-0.083	0.8	Sapling
Carbon	Soil	-0.079	0.88	Adult Pine
Nitrate	Soil	0.006	0.144	Control
Nitrate	Soil	-0.12	0.48	Sapling
Nitrate	Soil	-0.035	0.53	Adult Pine
pH	**Soil**	**0.119**	**0.002**	**Control**
pH	**Soil**	**0.085**	**0.02**	**Sapling**
pH	Soil	-0.091	0.7	Adult Pine
Phosphorous	Soil	-0.099	0.4	Control
Phosphorous	Soil	-0.025	0.3	Sapling
Phosphorous	Soil	-0.06	0.8	Adult Pine
Soil Moisture	Climatic	-0.011	0.26	Control
Soil Moisture	Climatic	-0.188	0.08	Sapling
Soil Moisture	**Climatic**	**0.058**	**0.01**	**Adult Pine**
Maximum Ground Temperature	Climatic	-0.014	0.275	Control
Maximum Ground Temperature	Climatic	-0.029	0.5	Sapling
Maximum Ground Temperature	Climatic	-0.066	0.9	Adult Pine

Table S5 — ANOVA results for differences in plant richness between expansion stages.

Variable	Df	Sum Sq	Mean Sq	F	p
Expansion stage	2	2.53	1.267	0.264	0.769
Residuals	42	201.47	4.797		

Table S6 — PERMANOVA results for differences in plant composition between expansion stages over 9999 permutations.

Variable	Df	Sum Sq	R2	F	p
Expansion stage	2	0.4588	0.04	0.989	0.476
Residuals	42	0.955	0.95		
Total	44	1	1		

Table S7 — PERMDISP results to test for differences in plant compositional homogenization between expansion stages over 9999 permutations.

Variable	Df	Sum Sq	Mean Sq	F	p
Expansion stage	2	0.132	0.006	0.482	0.633
Residuals	42	0.575	0.014		

Table S8 — Moran's I measures of spatial autocorrelation for plant variables by expansion-stage. Significant spatial autocorrelation is indicated in **bold**.

Variable	Partition	Moran's I	Adjusted p	Expansion-stage
Plant Composition (PC1)	**Biotic**	**0.087**	**0.003**	**Control**
Plant Composition (PC1)	**Biotic**	**0.08**	**0.02**	**Sapling**
Plant Composition (PC1)	Biotic	-0.09	0.6	Adult Pine
Plant Composition (PC2)	Biotic	-0.049	0.67	Control
Plant Composition (PC2)	Biotic	-0.15	0.2	Sapling
Plant Composition (PC2)	Biotic	-0.034	0.5	Adult Pine
Plant Richness	Biotic	-0.108	0.49	Control
Plant Richness	Biotic	-0.07	0.41	Sapling
Plant Richness	Biotic	-0.096	0.66	Adult Pine

Table S9 — PERMANOVA table of the effect of expansion stage on All Fungi, Saprotroph, Symbiotroph and Pathotroph beta-diversity measured as Hellinger-transformed Bray-Curtis dissimilarity of abundance data. Estimates were obtained over 9999 permutations.

Community	Factor	Df	Sum Sq	R^2	Pseudo-F	p
All Fungi	Plant Richness	1	0.3488	0.02738	1.2701	0.025
	Plant PC1	1	0.5221	0.04098	1.9009	<0.001
	Plant PC2	1	0.3912	0.03071	1.4245	0.004
	Expansion-stage	2	0.6803	0.0534	1.2385	0.008
	Residual	39	10.7113	0.84074		
	Total	44	12.7404	1		
Saprotrophs	Plant Richness	1	0.3989	0.03117	1.4577	0.007
	Plant PC1	1	0.5096	0.03981	1.8619	<0.001
	Plant PC2	1	0.393	0.0307	1.4359	0.011
	Expansion-stage	2	0.6494	0.05074	1.1865	0.056
	Residual	39	10.6733	0.83382		
	Total	44	12.8005	1		
Symbiotrophs	Plant Richness	1	0.2949	0.02943	1.3436	0.103
	Plant PC1	1	0.2834	0.02829	1.2913	0.129
	Plant PC2	1	0.2768	0.02763	1.2612	0.154
	Expansion-stage	2	0.5002	0.04993	1.1396	0.208
	Residual	39	8.5587	0.85432		
	Total	44	10.0181	1		
Pathotrophs	Plant Richness	1	0.2864	0.02384	1.1872	0.227
	Plant PC1	1	0.5371	0.04472	2.2267	0.005
	Plant PC2	1	0.5235	0.04359	2.1705	0.004
	Expansion-stage	2	1.1824	0.09846	2.4512	<0.001
	Residual	39	9.4067	0.78325		
	Total	44	12.0098	1		

Table S10 — Permdisp results to test for difference in variation of beta-diversity (Bray-Curtis dissimilarity) between expansion-stage in All Fungi, Saprotroph, Symbiotroph and Pathotroph communities.

Community	Variable	Df	Sum Sq	Mean Sq	F	p
All Fungi	**Expansion-stage**	2	0.002743	0.002743	0.8036	0.463
	Residuals	42	0.071687	0.071687		
Saprotrophs	**Expansion-stage**	2	0.00011	0.0000549	0.0316	0.971
	Residuals	42	0.072897	0.0017356		
Symbiotrophs	**Expansion-stage**	2	0.008224	0.004112	0.7402	0.454
	Residuals	42	0.23331	0.005555		
Pathotrophs	**Expansion-stage**	2	0.016117	0.0080584	1.3498	0.26
	Residuals	42	0.250751	0.0059703		

Table S11 — In order to examine potential differences in community assembly processes between fungal functional guilds, we performed an additional GDM analysis across all expansion stages but separately for each functional guild (all fungi, saprotrophs, symbiotrophs, pathotrophs — Fig. 4b). Below are the results from GDM analysis of β-deviance (calculated from Bray-Curtis dissimilarity) of root-associated fungal communities by functional guild (all, saprotroph, symbiotroph, pathotroph). First, global models containing all predictors were constructed and assessed for significance over 99 permutations. Then the predictor deemed least important to explaining variation (assessed through matrix permutation) was dropped and the next model constructed containing all remaining predictor. This process was completed until the model containing only the most important predictor was assessed. From all of these models, the one that explained the most variation in β-deviance and was significant ($p<0.05$), is reported below. Variable contribution represents the individual contribution of each variable to the total explained deviance in this best model. Variable contribution significance was further assessed over 99 permutations. (Significance codes: 0.0 *** 0.001 ** 0.01 * 0.05 ˙ 0.1)

Predictor	**Partition**	**All Fungi**	**Saprotroph**	**Symbiotroph**	**Pathotroph**
Host Plant Richness	Biotic	16.26*	6.22*	3.87*	2.05*
Plant Composition (PC1)	Biotic	1.04	2.62	0.36	-
Plant Composition (PC2)	Biotic	2.74*	3.02*	0.66	1.102
C (%)	Soil	4.04*	8.96*	1.08	0.63
pH	Soil	1.16	0.37	6.22*	-
P (ppm)	Soil	0.43	0.08	1.81	0.76
NH4 (ppm)	Soil	0.00	0.12	0.26	-
NO3 (ppm)	Soil	0.32	0.17	1.16	0.21
Soil Moisture (%)	Micro-Climate	0.51	0.27	0.34	-
Maximum Ground Temperature (°C)	Micro-Climate	0.00	0.14	0.00	0.43
Spatial (m)	Space	3.81*	2.68*	1.35*	0.39*
Model Deviance		**8.13**	**13.36**	**33.83**	**61.20**
Model p-value		**<0.001**	**<0.001**	**<0.001**	**0.04**
Total Deviance Explained (%)		**44.51**	**32.92**	**23.99**	**6.43**

Table S12 — Estimated marginal means of all, saprotroph, symbiotroph and pathotroph fungal richness by expansion-stage. Means were estimated using a generalized linear model including expansion stage and biotic variables to account for differences in plant diversity between plots and its ability to explain variation in fungal richness. Models were fitted with a gaussian distribution using an identity link function after normality had been assessed. Outliers were removed based on Cook's distance. (Significance codes: *** 0.001 ** 0.01 * 0.05 ˙ 0.1)

Community	AIC	Expansion Stage	Estimated Marginal Mean	Confidence Interval	Pairwise p-value
All Fungi	548.44	Control	475	438-512	C-P=0.878
		Sapling	462	425-500	C-S=0.890
		Adult Pine	488	450-525	S-P=0.625
Saprotrophs	436.81	Control	119	109-129	C-P=0.886
		Sapling	116	109-125	C-S=0.889
		Adult Pine	116	108-125	S-P=0.999
Symbiotrophs	269.16	Control	41.7	39.1-44.4	C-P=0.044*
		Sapling	35.4	32.6-38.1	C-S=0.004**
		Adult Pine	37.1	34.3-39.8	S-P=0.675
Pathotrophs	255.03	Control	18.2	15.4-21	C-P<0.001***
		Sapling	24.5	21.7-27.2	C-S=0.006**
		Adult Pine	26.9	24.2-29.7	S-P=0.427

Table S13 — Estimated marginal means of all, saprotroph, symbiotroph and pathotroph fungal relative abundance by expansion-stage. Means were estimated using a generalized linear model including expansion stage and biotic variables to account for differences in plant diversity between plots and its ability to explain variation in fungal richness. Models were fitted with a gaussian distribution using an identity link function after normality had been assessed. Outliers were removed based on Cook's distance. (Significance codes: *** 0.001 ** 0.01 * 0.05 ˙ 0.1)

Community	AIC	R^2 Adj	Variable	Chisq	Df	Pr(>Chisq)
Saprotrophs	48.329	-0.057	Plant Richness	0.52	1	0.471
			Plant PC1	0.86	1	0.353
			Plant PC2	0.39	1	0.528
			Expansion Stage	0.42	2	0.809
Symbiotrophs	89.87	0.018	Plant Richness	0.47	1	0.491
			Plant PC1	2.04	1	0.153
			Plant PC2	0.02	1	0.895
			Expansion Stage	3.87	2	0.144
Pathotrophs	133.9	0.008	Plant Richness	2.13	1	0.144
			Plant PC1	1.37	1	0.240
			Plant PC2	1.79	1	0.180
			Expansion Stage	1.87	2	0.391

Table S14 —Results from generalized linear model analysis of fungal richness by functional guild (all, saprotroph, symbiotroph, pathotroph — Fig. 6b). We fit models based on gaussian distributions using identity link functions due to the normality of our richness data. First, a global model containing all predictors was constructed. We then used dredge in the 'MuMln' package for R to select the model that best fit the data based on AIC. Finally, we partitioned the variation explained by this best model into individual variable contributions using lmg in the 'relaimpo' package for R. This approach is robust to colinear variables as models are first selected based on AIC before partitioning. (Significance codes: 0.0 *** 0.001 ** 0.01 * 0.05 ˙ 0.1)

Predictor	**Partition**	**All Fungi**	**Saprotroph**	**Symbiotroph**	**Pathotroph**
Host Plant Richness	Biotic	0.669***	0.519***	0.557***	0.256***
Plant Composition (PC1)	Biotic	-	-	0.058˙	0.033˙
Plant Composition (PC2)	Biotic	-	0.104*	-	-
C (%)	Soil	-	-	-	-
pH	Soil	-	-	-	-
P (ppm)	Soil	-	-	-	-
NH4 (ppm)	Soil	-	-	-	-
NO3 (ppm)	Soil	-	-	0.020	0.050˙
Soil Moisture (%)	Micro-Climate	0.050*	0.102***	-	0.163*
Maximum Ground Temperature (°C)	Micro-Climate	-	-	-	0.077*
R^2		**0.719**	**0.724**	**0.635**	**0.579**
R^2 adj		**0.706**	**0.704**	**0.609**	**0.525**
AIC		**523.54**	**404.37**	**302.24**	**296.3**

Table S15 — Important variables driving patterns of relative abundance. No variables significantly explained variation in saprotroph relative abundance (Significance codes: *** 0.001 ** 0.01 * 0.05 ˙ 0.1)

Community	R2	R2 adj	Variable	lmg	p
Saprotrophs	-	-	-	-	-
Symbiotrophs	0.08	0.06	Soil Moisture	-	0.058
Pathotroph	0.215	0.178	C	0.096	0.018*
			Soil Moisture	0.119	0.009**

Figures

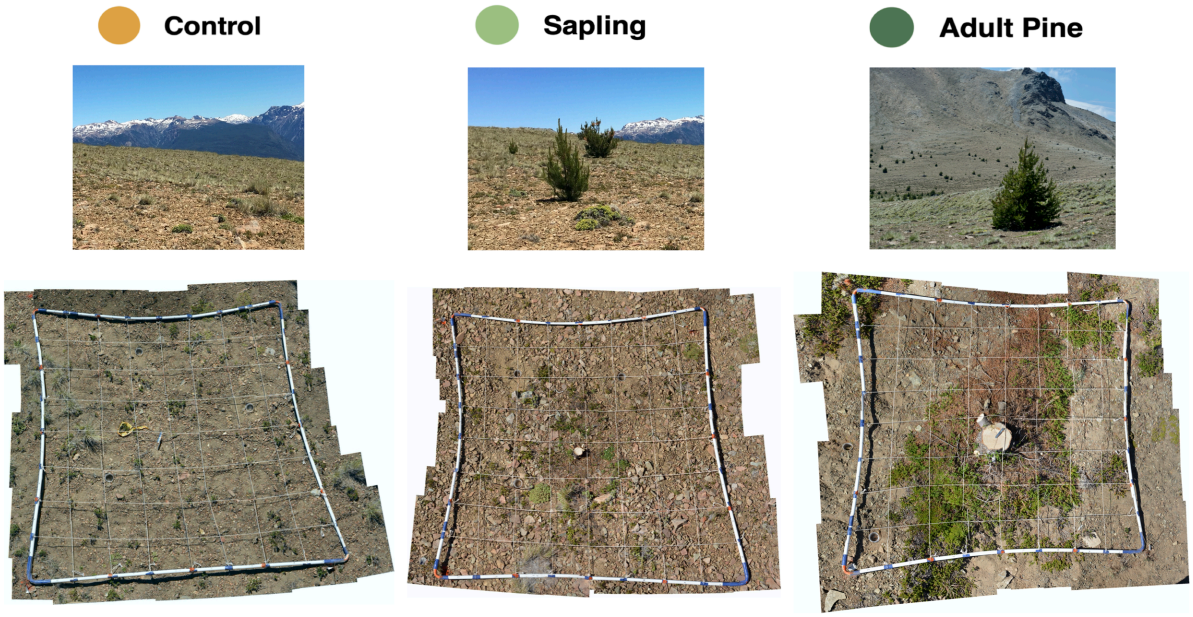

Fig. S1 — Examples of control (left), sapling (middle) and adult pine (right) plots. Bird's eye view pictures are collages of multiple photos taken after the focal tree was removed (cut down) to show the differences between plots.

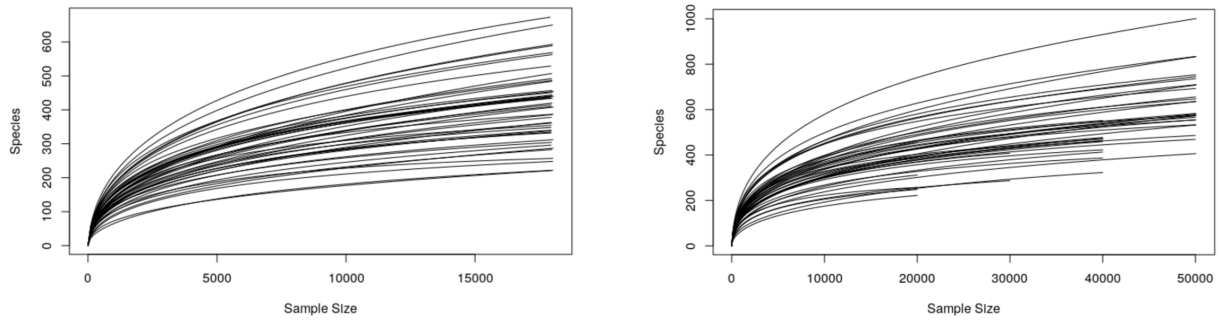

Fig. S2 — Difference between rarefaction curves for plots rarefied to 18000 reads (left) and 50000 reads (right)

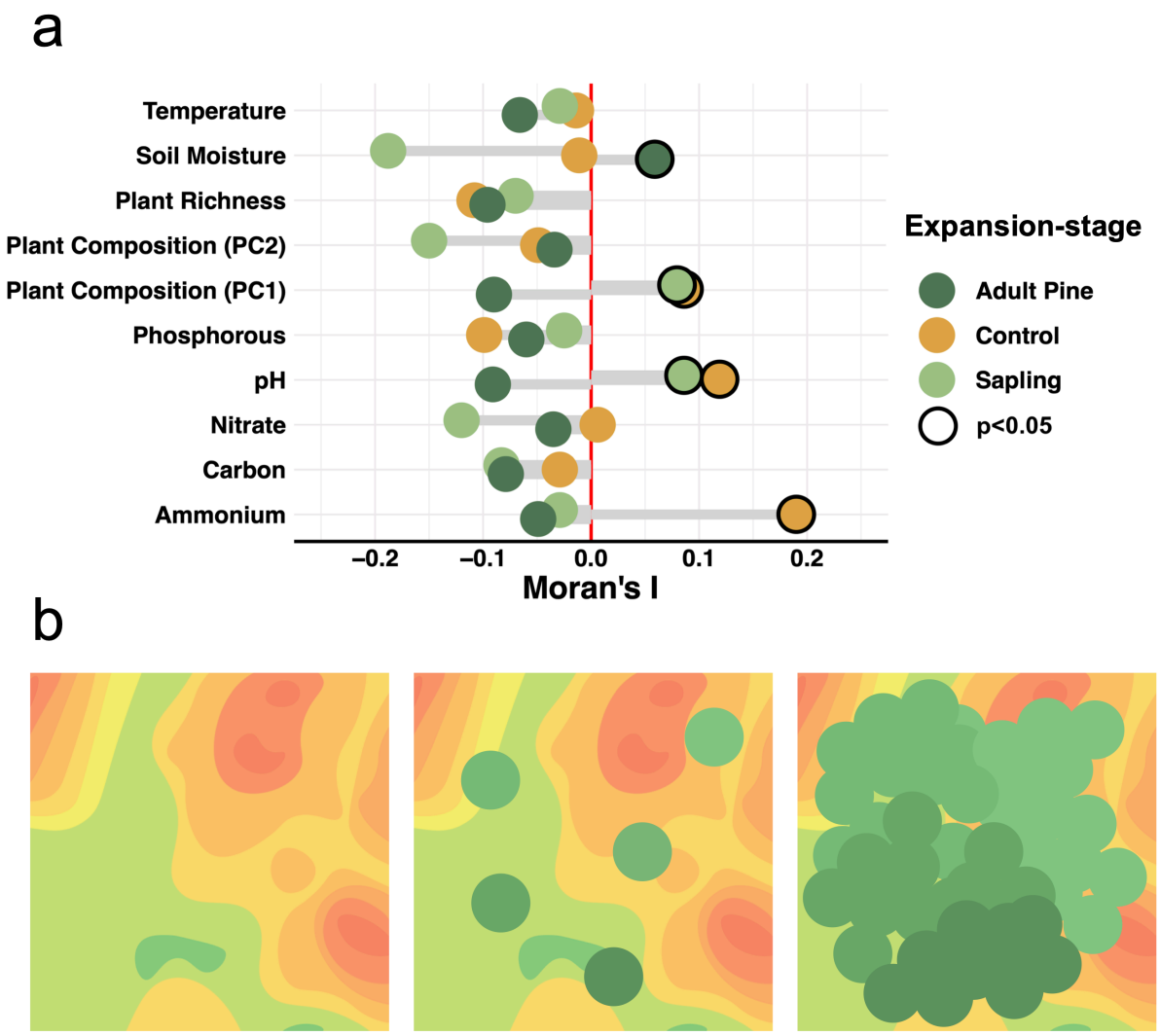

Fig. S3 — (a) Differences in Moran's I measure of spatial autocorrelation of environmental variables by expansion-stage. Positive Moran's I indicate spatial clustering (near sites are more similar), while negative Moran's I indicate spatial evenness (near sites are more different) and 0 indicates no spatial autocorrelation (spatial randomness). Significance was tested against a null model and is indicated by a black border. (b) Illustrated change in the spatial organization of environmental variation as a result of range expansion. Before establishment of a range-expanding foundation species (left) ecosystems exhibit strong spatial structuring of environmental variation. Isolated range-expanding foundation species alter environmental conditions where they establish (middle) creating distinct "islands" of micro-climate. Further colonization (right) over time increases the spatial coverage of this novel microhabitat, homogenizing environmental conditions and reducing variation across space.

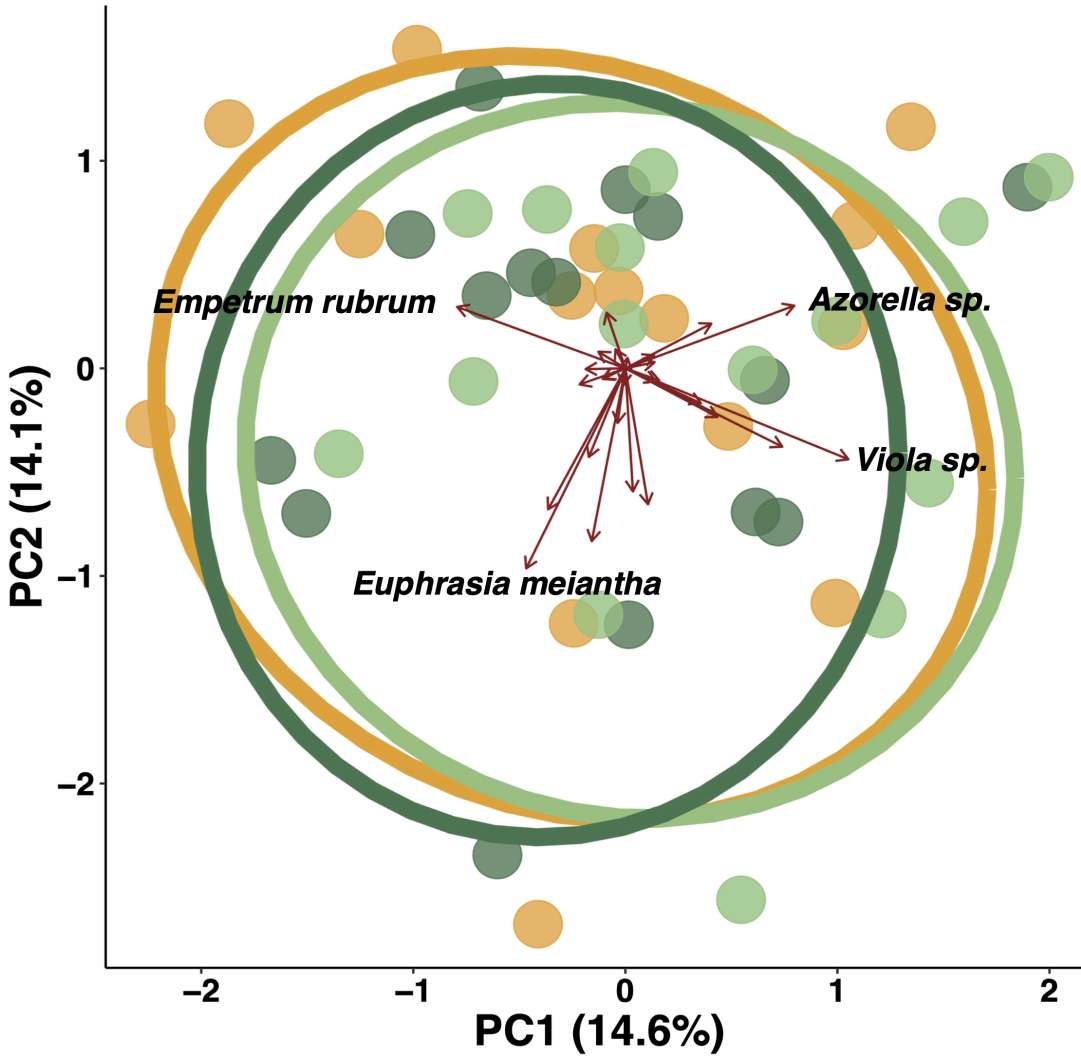

Fig. S4 — Plant composition by expansion-stage represented in principal component space. Notable species driving differences between plots are named, and ellipses were calculated based on 95% confidence.

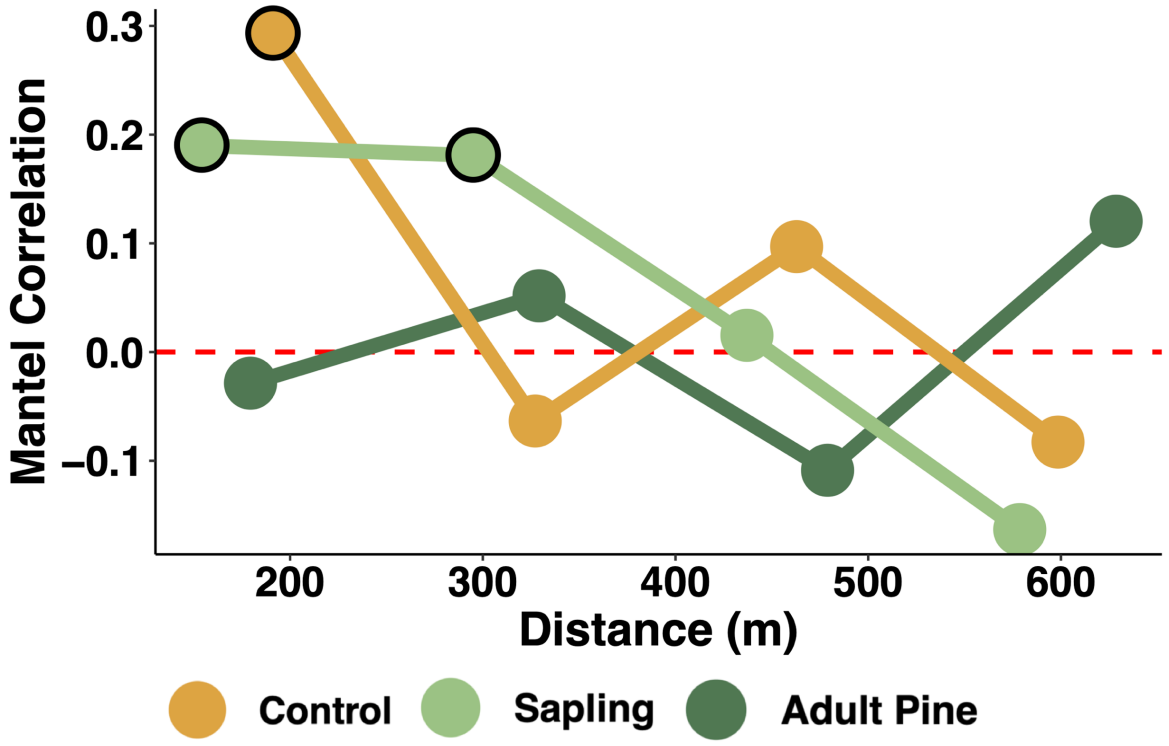

Fig. S5 — Mantel correlogram of spatial autocorrelation in fungal β diversity (Bray-Curtis dissimilarity) by expansion-stage. Positive mantel correlation suggests that sites at that distance class are more similar than expected by chance, while negative correlation suggests sites are more different. Black outlines around points represent spatial autocorrelation significantly ($p<0.05$) different from zero.