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Research article

A single range-expanding species reshapes alpine ecosystems and their belowground diversity

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Species around the globe are shifting their ranges into new territories at an unprecedented rate. In particular, the spread of foundation species can transform recipient communities and ecosystems, however, the effects on belowground processes and diversity remain poorly documented. Belowground fungi are well suited for taking the 'pulse' of changing ecosystems given their rapid turnover and implication in a wide variety of ecosystem processes. To better understand the belowground effects of range-expanding species, we leveraged an ongoing invasion of a foundation tree species *Pinus contorta* into alpine tundra to study the impacts on belowground abiotic conditions and the fungal communities associating with the roots of resident plants. We found that individual range-expanding trees create distinct abiotic 'islands' with wetter soils and altered soil nutrients compared to the surrounding alpine tundra ecosystem. Potentially driven by these abiotic changes, we observed a decrease in the α diversity of mutualistic fungi and an increase in the α-diversity of pathogenic fungi during later stages of range expansion. Changes in γ-diversity mirrored patterns of α diversity while β-diversity was only minorly affected by range-expanding trees, suggesting that local habitat amelioration/deterioration rather than changes in among-patch heterogeneity underpin trends in belowground diversity. In sum, our results show that range-expanding foundation species can modify ecosystems by altering belowground abiotic

Synthesis

Tracking climate change, species around the world are shifting their ranges, expanding into new communities, and creating novel ecosystems. In particular, the upslope spread of novel trees is quickly transforming vulnerable unforested alpine ecosystems. Starting only a few years after establishment, novel trees impact soil conditions, creating distinct "islands" of microhabitat with altered soil chemistry and wetter soils. In turn, these abiotic changes are associated with deterministic shifts in communities of fungi interacting with the roots of native plants. These findings fill an important gap in our understanding of the initiation and structuring of novel ecosystems in the context of global change.

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conditions and diversity across scales. These impacts begin only a few years after initial range-expansion and establishment and scale rapidly over time, indicating the need for preventative or swift conservation action to prevent long-term consequences.

Keywords: belowground fungi, biological invasions, climate change, community assembly, foundation species, range shifts, soil microbes, species composition

Introduction

As a result of human-driven changes to the biosphere, species around the world are shifting their geographic ranges, expanding into new territories, and interacting with native taxa to form novel communities and ecosystems (Lenoir et al. 2008, Hobbs et al. 2009, Chen et al. 2011, Steinbauer et al. 2018). Biological invasions have led to world-wide range-expansions for thousands of plant and animal species (Hobbs 2000, Meyerson and Mooney 2007, van Kleunen et al. 2015). Climate change is also leading to range expansions, in both native (Dukes and Mooney 1999, Carlton 2000, Ward and Masters 2007, Hellmann et al. 2008, Colautti and Barrett 2013) and exotic species (Parmesan et al. 1999, Hickling et al. 2006, Harsch et al. 2009, Pinsky et al. 2013), often in upslope and poleward directions (Chen et al. 2011, Masson-Delmotte et al. 2018, Steinbauer et al. 2018, Warren et al. 2018). But despite the ability of range expanding species to rapidly form novel interactions, communities, and ecosystems (le Roux and McGeoch 2008), the ecological impacts of range-expanding species remain poorly explored (Wallingford et al. 2020).

Range-expanding foundation species in particular may have disproportionately large impacts on the construction and organization of novel ecosystems (Franklin et al. 1971, Vitousek et al. 1987, Sturm et al. 2001, Myers-Smith et al. 2011). Foundation species influence local community diversity through non-trophic interactions and structure ecosystems by creating microhabitat and influencing ecosystem processes (Dayton 1972, Callaway 1995, Stachowicz 2001, Ellison et al. 2005). The disappearance of foundation species can impact a variety, of ecosystem processes as seen during the loss of American chestnut from temperature forests and resulting changes in decomposition, nutrient cycling, diversity and productivity (Ellison et al. 2005). Similar to their disappearance, the spread of foundation species into new territories can also impact diversity and ecosystem functioning (Báez and Collins 2008, Collins et al. 2018, García et al. 2023), but these impacts remain understudied.

While range-expanding foundation tree species have the potential to affect a variety of ecosystems, alpine and tundra biomes, comprising an estimated ~ 13% of the earth's surface, face a unique threat. The rapid forestation of these biomes by range-expanding trees is creating novel communities, with altered biodiversity potentially governed by different ecological processes (Franklin et al. 1971, Suarez et al. 1999, Moore and Huffman 2004, Sturm et al. 2005, Harsch et al. 2009, Myers-Smith et al. 2011, Trant and Hermanutz 2014). Past work has started to explore the ecological impacts of range-expanding trees on these biomes, but much of the focus has been on aboveground, rather than belowground, patterns and processes (Lazzaro et al. 2015, Siles and Margesin 2016). For example there is strong evidence that novel foundation tree species alter abiotic conditions where they establish (García et al. 2023), reduce the area covered by herbaceous alpine and tundra plants (Moiseev and Shiyatov 2003), reduce plant diversity (Moore and Huffman 2004, Bravo-Monasterio et al. 2016, García et al. 2023), and impact ecosystem processes (Dickie et al. 2011, Rundel et al. 2014). However, the belowground consequences of novel trees remain comparatively understudied.

Belowground root-associated fungi are useful systems to study the rapid response of ecological communities and the formation of novel ecosystems. Belowground fungi exhibit strong patterns of abiotic and biotic selection (Tedersoo et al. 2014, Glassman et al. 2017), due to short generation times and ability to rapidly respond to change (Hagenbo et al. 2017). Belowground fungi also mediate critical ecosystem processes and small changes in diversity can impact broad-scale ecosystem functioning (Parker et al. 2015). For example, fungal decomposers mediate decomposition rates and carbon cycling, which is especially relevant in alpine and tundra biomes where > 90% of ecosystem carbon is stored belowground (Körner 2003). Mutualistic fungi serve as critical facilitators in alpine and tundra ecosystems, forming important and often obligate interactions with host plants that help them overcome abiotic stress (Harley and Smith 1983, Cairney and Meharg 2003). Finally, pathogenic fungi play important roles in the maintenance of aboveground diversity and coexistence by parasitizing dominant and abundant species (Bagchi et al. 2010, 2014). While past work reveals that belowground fungal diversity is influenced by a variety of factors including aboveground plant diversity (Grayston et al. 1998, Tedersoo et al. 2014, Cline et al. 2018, Koyama et al. 2019), soil moisture (Delgado-Baquerizo et al. 2020), and soil chemistry (Erlandson et al. 2016, Glassman et al. 2017), it remains unclear if and how novel range expanding trees impact belowground fungal communities.

In this study, we investigate how novel foundation-species impact belowground fungal diversity by studying an ongoing range-expansion of introduced lodgepole pine *Pinus contorta* into an alpine ecosystem in Patagonia, Argentina. Pines have invaded ecosystems around the world and provide a useful system to study the impacts of novel foundation species given their ability to establish across a range of abiotic and biotic conditions (Pauchard et al. 2016) and the high context dependency of their impacts (Sapsford et al. 2020). Past work demonstrates that pine invasions decrease abiotic heterogeneity in treeless ecosystems (García et al. 2018) and experimental evidence confirms that pine growth alters soil microclimate

and chemistry (García et al. 2023). As such, we hypothesized that range-expanding pines would change and homogenize soil conditions where they establish (H1). Although our primary focus was belowground, we also expected pine establishment and growth to reduce aboveground plant diversity, as observed in other pine invasions (Bravo-Monasterio et al. 2016, Taylor et al. 2016), experiments (García et al. 2023), and other tree range-expansion in general (Mogashoa et al. 2021). Since fungal diversity is positively influenced by abiotic heterogeneity and plant diversity (Maltz et al. 2017, Tedersoo et al. 2020) we hypothesized that pine establishment and growth would decrease the α- (H2a), β- (H2b) and γ- (H2c) diversity of belowground fungi in general. We predicted this effect would be strongest for fungal mutualists and pathogens, since they are more sensitive to abiotic conditions and plant hosts compared to decomposers (Grayston et al. 1998, Cline et al. 2018, Koyama et al. 2019).

Material and methods

Study site

To investigate the belowground impacts of novel foundation species, we studied an invasion of *P. contorta* (Lodgepole Pine) into an alpine plateau on Cerro Piltriquitron (41°58'08.904''S, 71°27'36.432''W), in the northern Patagonian Andes, Argentina (Supporting information). The site is 44 ha and 1690 m a.s.l. (min = 1654 m, max = 1743 m). *Pinus contorta* was introduced to the lower western slope of Piltriquitron in 1978 and has since spread upwards, reaching the alpine plateau in 1995.

Study design

We sampled a total of 45 2 × 2 m plots, 15 of each expansion-stage (Supporting information). Expansion stages were defined as *controls* (plots with no focal pine) representing pre-range-expansion communities, saplings (plots with a single focal pine ~ 0.5 m tall) representing communities beneath recently established pines, and adult pines (plots with a single focal pine > 1 m tall) representing communities beneath mature pines (Fig. 1A). We classified range-expanding pines based on size (a proxy for age) since both size and age determine pine impact on abiotic conditions and resident communities (Franzese et al. 2017). Plots were established using a 2 × 2 m quadrat, centered around focal pines in the case of sapling and adult pine plots or around the centre of control plots. We chose plots of this size to capture only the belowground communities of fungi associating with the roots of

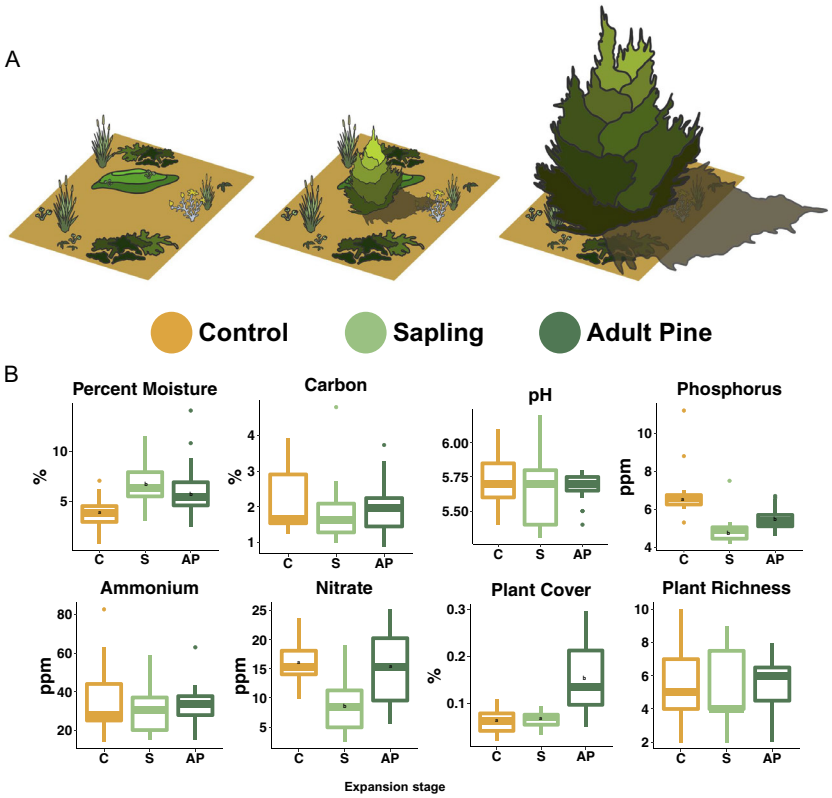


Figure 1. (A) Illustration of the differences between expansion-stage and (B) differences in micro-climatic (soil moisture and), soil (carbon, phosphorous, ammonium, nitrate and pH) and biotic plant (richness, total cover and β-diversity) variables across expansion-stages. Significant differences are indicated with letters and were assessed using GLMs based on gaussian distributions with identity link functions and spatial block as a covariate (Supporting information). No difference in plant composition between expansion stage was detected using PERMANOVA (Supporting information).

plants growing immediately adjacent to focal pines and thus experiencing potential microclimate effects of pine establishment and growth. All plots were established randomly and isolated by at least a 10 m distance from other (non-focal) pines to ensure the independence of data and avoid capturing neighbourhood effects (Glassman et al. 2017). Plots were grouped into spatial blocks, with each block containing one plot of each expansion-stage, to control for fine-scale spatial patterns in fungal diversity. Blocks were established along a directional transect that covered the study area but avoided the centre of the plateau where high pine density made sampling isolated trees impossible. Plots were georeferenced using a handheld GPS (Supporting information).

Pine age and morphology

After the study period, all 30 focal pines (15 adults and 15 saplings) were felled to measure tree height, basal diameter, canopy radius and trunk circumference. Pines in sapling plots differed from those in adult pine plots in terms of height, diameter and canopy cover (Supporting information), indicating we effectively captured the temporality of range-expansion.

Soil moisture and chemistry

We collected soil samples, consisting of 6 × 100 ml, taken at 10–15 cm belowground, obtained evenly throughout each plot (using a gridded approach) and pooled to produce one soil sample for each plot. Soil moisture was calculated as the percentage difference between wet and dry weights and represents the amount of water retained as moisture in the soil as a percentage of its weight. Dried soil samples were sent to Soil, Plant and Environment Analytical Services (LABSPA) at the Agriculture department of the Universidad Nacional Del Sur (Bahía Blanca, Argentina) where soil parameters pH, C (carbon as %), P (phosphorous as parts per million), NO_3^- (nitrate as ppm) and NH_4^+ (ammonium as ppm) were measured using LECO TruSpec CN Carbon-Nitrogen Determinator and standard procedures outlined in the Supporting information.

Extant plant community

We identified down to the lowest taxonomic level possible, all plant species within each 2 × 2 m plot. Plants were identified on site using field guides and area-specific taxonomic keys, notably Flores de Alta Montaña (Ferreyra et al. 2006), and verified by local experts and online records to ensure accurate identification (Supporting information). Plants that could not be identified were assigned morphospecies, an approach that is common in fungal ecology since many root-associated mycorrhizal fungi associate at the genus or family level with host plants (Gao et al. 2013). We quantified total plant cover by creating composite photographs of each plot and measuring the percentage of area in each plot covered by vegetation using ImageJ (Supporting information).

Plant root samples and DNA extraction

We sampled, to our best efforts, the complete fungal communities associating with the roots of each species of plant per plot. Over the course of a single week in March 2019, up to three individuals of each species of plant in each plot were uprooted and placed in sterile bags for transport. If plots contained fewer than three individuals, all individuals of that species were sampled. The roots of each plant sample were rinsed with distilled water, dried at room temperature, and fine root tips were collected and frozen at −20°C until DNA extraction. Plants whose roots broke during uprooting or didn't contain fine root material were discarded. From fine root samples, 250 mg was isolated and placed inside a QIAGEN DNeasy PowerSoil PowerBead tube (QIAGEN Inc.) containing three sterilized metal beads, ceramic QIAGEN beads, and sterilized water. Samples were beat using a vortex for 10 min to break root material. Bulk DNA was extracted using QIAGEN DNeasy PowerSoil kits, following the manufacturer's protocol. In the final step, we isolated DNA using a low concentration EDTA (5%)-TE buffer to stabilize the DNA extract. DNA was preserved in low (0.1 mM) EDTA -TE buffer and stored at −20°C.

Amplification, sequencing and bioinformatics

DNA was amplified using fungal specific primers ITS-1F (CTTGGTCATTTAGAGGAAGTAA, (Gardes and 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 ver. 2 system, with Index (i5) on the forward primer and Index (i7) on the reverse primer. PCR amplification and Illumina MiSeq sequencing procedures refer to DeBellis et al. (2019). A total of 321 samples, including negative controls were sequenced. Sequences were processed into amplicon sequence variants (ASVs) in the DADA2 pipeline (Callahan et al. 2016, 2017) for R ver. 3.6.3 (www.r-project.org), assigned taxonomy using the UNITE ver. 8.2 fungal database (Nilsson et al. 2019), and rarefied to an equal depth across all samples (10 000 reads).

Instead of using samples, representing unique species per plot, as the replication unit for our analyses, and because sampled plant richness only varied slightly across plots (mean = 5.29, SD = 2.09, max = 10, min = 2), we pooled our rarefied fungal communities at the plot level (n = 45, 15 per expansion-stage) to quantify plot-level root-associated fungal communities and to incorporate biotic variables (plant richness, cover and composition) into our analyses to explicitly test the influence of biotic variables in this system. Since we pooled fungal communities by plot, plots with more plant species were expected to harbor greater fungal richness and abundance owing to differences in sampling efforts, biasing our estimates of alpha-, beta- and gamma-diversity. To account for this, we rarefied each plot-level community of root-associated fungi to a standard sequencing depth of 18 000 reads based on plot rarefaction curves (Supporting information) and because it allowed for the retention of all 45 plots.

Statistical analysis

H1: effect of range-expansion on the abiotic environment

To investigate abiotic effects (H1), we tested for the effect of expansion stage on soil moisture and chemistry using generalized linear models (GLMs) based on gaussian distributions and identity link functions. We used GLMs with expansion stage as the predictor, spatial block as a covariate (to account for spatial patterns), and individual environmental variables as the response. To estimate expansion stage means and test significance we used the 'emmeans' package (Searle et al. 1980) for R with Tukey corrected p-values. To test whether range expansion homogenized abiotic conditions, we used Bartlett tests in the 'stats' package for R.

We also assessed whether range-expanding pines influenced the biotic habitat, by testing the influence of expansion stage on plant α- and β-diversity. For α (i.e. plant richness and cover), we used the same GLM approach described above. For β-diversity, we used Bray–Curtis dissimilarity (Baselga 2010) and tested the effect of expansion stage using PERMANOVA, incorporating the same spatial covariate, over 9999 permutations (Anderson 2001). Analyses were preformed using the 'stats' package and 'vegan' package (Oksanen et al. 2013) for R unless stated otherwise.

H2a: effect of range-expansion on the α diversity of root-associated fungi

To test if the range expansion of pines leads to an increase in the α-diversity of root-associated fungi, we quantified the richness of all fungi, as well as the richness and relative abundance of decomposers, mutualists and pathogens to test for changes in specific functional guilds. Richness was calculated as the total number of unique ASVs per plot, with decomposer, mutualist and pathogens richness being the number of unique ASVs identified to each respective functional guild with a high degree of probability (labeled Probable or Highly Probable in the FUNGuild database (Nguyen et al. 2016)). Relative abundance was calculated as the portion of reads within each plot that identified to each functional guild.

To test for differences in richness and relative abundance among expansion stages (control, sapling, adult pine), we used GLMs. Richness data was analyzed using a negative binomial distribution with a log-link function (Stoklosa et al. 2022) fit using the 'MASS' package for R (Venables and Ripley 2002). Relative abundance was log transformed to achieve normality and analyzed using Gaussian distributions with identity-link functions. Since range expansion did not impact plant richness, we included plant richness as a covariate in our GLMs to isolate the effect of expansion stage and included spatial block to account for potential spatial structuring in fungal diversity. We assessed model fit using AIC and R^2 (Kullback–Leibler in the case of negative binomial GLMs) 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.

H2b: effect of range-expansion on β-diversity of root-associated fungi

To test if range expansion increases fungal β-diversity, we quantified fungal β-diversity using Bray–Curtis dissimilarity (Bray and Curtis 1957), which represents the composition turnover between fungal communities (Baselga 2010) using Hellinger-transformed (Legendre and Gallagher 2001) abundance data, visualized using non-metric multidimensional scaling ($k=3$). We used PERMANOVA ($nperm=9999$) to test for significance differences in fungal composition and PERMDISP ($nperm=9999$) to test for changes (increases or decreases) in fungal β-diversity among expansion stages. We tested if the effects were functional-guild-specific by performing separate tests on derived assemblages of decomposers, mutualists and pathogens. Again, we included plant richness and spatial block, as covariates in the PERMANOVA analyses to isolate the effect of range-expansion on fungal β-diversity (Anderson 2014).

H2c: effect of range-expansion on γ-diversity of root-associated fungi

To test if range expansion increases fungal γ-diversity, we compared species accumulation curves between expansion stages (control, sapling, adult pine). 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. We chose Chao1 to account for the presence of rare ASVs in our dataset represented by only a few reads. This process was repeated 1000 times to create a margin of confidence. The height of each curve represents the Chao estimated γ-diversity within each expansion-stage for this study site. To assess if the observed effects were functional-guild-specific, we also performed this analysis separately for decomposers, mutualists and pathogens.

Examining the drivers of fungal diversity

To link the abiotic and biotic impacts of pine range expansion to changes in fungal communities, we also identified which abiotic and biotic variables were influencing fungal diversity in our system. To do this we used model selection to isolate the variables that best explained variation in fungal α-and β-diversity and partitioned explained variation into individual variable contributions (full methods are available in the Supporting information).

Results

H1: effect of range-expansion on the abiotic and biotic environment

We found altered abiotic soil conditions beneath novel pines (Fig. 1B, Supporting information). Soil in adult pine and sapling plots contained 66 and 78% more moisture and 20 and 28% less phosphorous (respectively) compared to control

plots. Sapling plots had 43 and 39% less nitrate compared to control and adult pine plots. Soil carbon, pH, ammonium and plot altitude did not differ significantly among expansion-stages. However, variation in soil moisture, phosphorous and pH decreased with expansion stage, indicating that range expansion homogenized abiotic conditions (Supporting information).

In terms of the effect of novel trees on the biotic environment, we observed no significant statistical effects of expansion stage on plant richness or β-diversity (Fig. 1B, Supporting information). We did however observe a significant increase (> 200%) in plant cover associated with late stages of range expansion.

H2a: effect of range-expansion on α-diversity of root-associated fungi

While the local richness of all root-associated fungi and decomposers remained consistent among expansion stages, mutualist richness decreased and pathogen richness increased as range expansion proceeded (Fig. 2, Supporting information). Specifically, mutualist richness was 11 and 15% lower in sapling and adult pine plots (respectively) compared to controls, mainly driven by changes in ectomycorrhiza (notably *Endogone* spp.) and endophyte (notably *Serendipita* spp.) richness, though this was marginally significant (Supporting information). Pathogen richness was 19 and 32% higher in sapling and adult pine plots (respectively) compared to controls, driven by changes in *Allantophomopsiella*, *Devriesia*, *Gremmenia* and *Phacidium* (Supporting information).

Changes in fungal richness were mirrored by changes in relative abundance (Fig. 3, Supporting information). While the relative abundance of decomposers did not change over expansion stages, mutualists decreased and pathogens increased as range expansion proceeded. In fact, we observed a 50% decrease in mutualist abundance in adult pine plots compared to saplings and controls, while pathogen abundance increased 250 and 725% in adult pine plots compared to saplings and controls respectively.

H2b: effect of range-expansion on β-diversity of root-associated fungi

Range expanding pines did not increase or decrease fungal β-diversity (Supporting information) and expansion stage only explained a small amount of variation in fungal β-diversity (Supporting information), varying from 5.3% for all fungi to 7.3% for pathogens, and was non-significant for decomposers and mutualists (Supporting information).

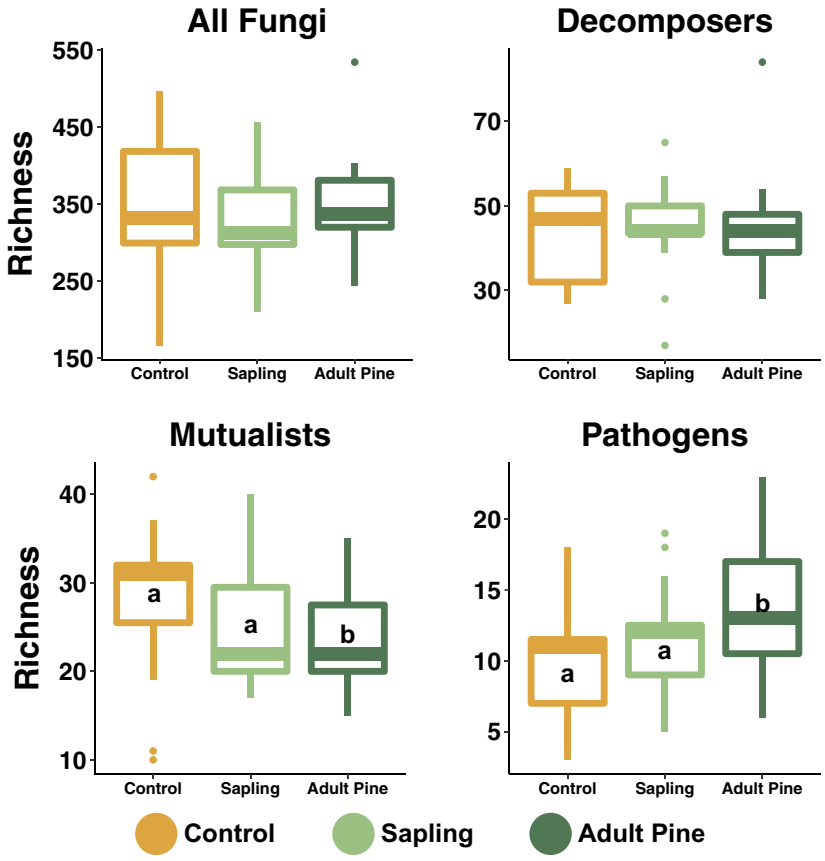


Figure 2. All fungi, decomposer, mutualist and pathogen richness across expansion-stages. Significant differences across expansion-stages, represented by differing letters, were assessed using GLMs based on gaussian distributions with identity link functions, including spatial block and biotic variables (plant richness) as covariates (Supporting information). Note that this visualization does not incorporate those covariates.

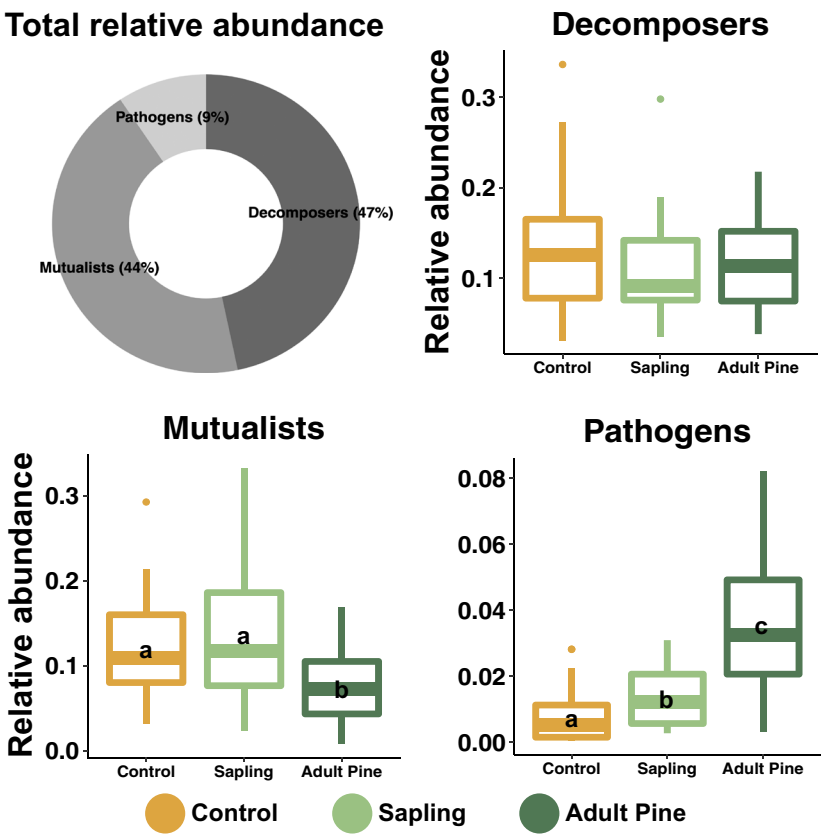


Figure 3. Decomposer, mutualist and pathogen relative abundance across expansion-stages. Significant differences in log-transformed relative abundance across expansion-stages, represented by differing letters, were assessed using GLMs based on gaussian distributions with identity link functions, including spatial block and biotic variables (plant richness) as covariates (Supporting information). Note that this visualization does not incorporate those covariates.

H2c: effect of range-expansion on γ-richness of root-associated fungi

The regional γ-richness of all root-associated fungi and decomposers did not differ among expansion-stages (Fig. 4). On the other hand, mutualist γ-richness was lower and pathogen γ-richness was higher in adult pine communities, compared to sapling and controls, with error bars suggesting potential significant differences. Mutualist γ-richness was highest in control plots (113) compared to saplings (105) and considerably lower in adult pine plots (96). Pathogen γ-richness was highest in adult pine plots (61) compared to saplings (54) and controls (51).

Drivers of fungal diversity

Fungal richness was largely driven by variation in soil moisture and host plant richness across all trophic modes, except for mutualists, where soil moisture did not explain variation (Supporting information). In terms of relative abundance, host plant richness, cover and composition, as well as soil carbon, explain variation in mutualists and pathogens, with soil moisture also explaining variation in pathogen relative abundance (Supporting information).

Interestingly, although plant richness was consistently important across expansion stages, its relative importance to explaining variation in fungal richness decreased as range expansion progressed, indicating a potential shift in assembly rules from biotic to environmental (Supporting information). Model estimates for predictors are available in the Supporting information. Drivers of root-associated fungal β-diversity varied in identity and strength among expansion stage and among functional guilds (Supporting information), although this variation was minor compared to trends in α-diversity.

Discussion

Past work clearly demonstrates the ability of novel trees to create microhabitat where they establish, altering abiotic conditions and reducing the diversity of understory plants (Bravo-Monasterio et al. 2016, Taylor et al. 2016, García et al. 2018, 2023, Mogashoa et al. 2021). Here, we show that the belowground abiotic impacts begin only a few years after establishment and are associated with concurrent shifts in the richness, abundance and composition of belowground fungal communities, potentially driven by key changes in soil moisture. Specifically, we observed a decrease

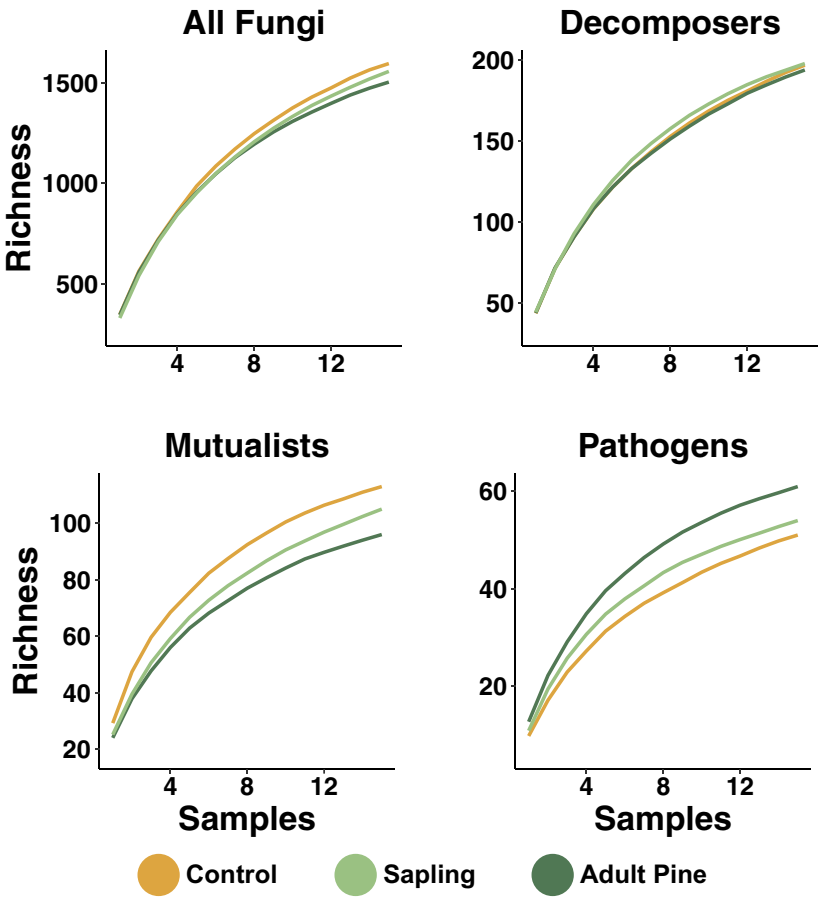


Figure 4. All fungi, decomposers, mutualist and pathogens γ-diversity by expansion-stage. γ-diversity is represented using species accumulation curves, where sites were randomly sampled and the accumulated Chao1 index calculated. Confidence intervals were produced by repeating this procedure 1000 times.

in fungal mutualists and an increase in fungal pathogens as range expansion progressed. Since mutualists and pathogens play critical roles in both facilitating plant persistence and mediating diversity (Harley and Smith 1983, Bagchi et al. 2014), our results point towards a potential belowground mechanism by which foundation species are restructuring the communities they invade.

Tree establishment alters environmental conditions creating distinct abiotic islands

The spread and establishment of the foundation trees created abiotic 'islands' with distinguishable soil conditions relative to the surrounding landscape. Consistent with our hypothesis (H1), we found wetter soils beneath range-expanding trees, likely driven by canopy shading and root growth (van Dijk and Keenan 2007), an effect that has been long associated with tree establishment (Dancette and Poulain 1969, Belsky et al. 1993). The spread and establishment of novel trees also led to decreases in soil nitrate and phosphorus, although this effect was strongest in earlier stages (sapling plots) likely due to tree-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 and Krause 1973, Jumpponen et al. 1998, Hayward et al. 2015b). Alongside the creation of distinct abiotic islands, we also observed a drastic increase in plant cover associated with novel tree growth. This could be facilitation driven by more tolerable abiotic conditions (i.e. wetter soils) under older trees (Callaway 1995), or it could be a result of light limitation beneath larger canopies, causing understory plants to prioritize aboveground biomass (Shirley 1945, Brouwer 1963).

Novel tree establishment leads to changes in belowground diversity across scales

In support of our hypothesis (H2), our results show that the establishment and growth of novel foundation species affects the α-diversity of belowground fungi, possibly as a consequence of changes in the abiotic environment. Specifically, range expansion led to a decrease in the α-diversity of fungal mutualists such as *Endogone* and *Serendipita*, who enable plant fitness and defense against pathogens (Daft and Nicolson 1966, Oberwinkler et al. 2013, Sarkar et al. 2019).

Contrary to the general pattern, *Suillus*, a genus of fungi co-introduced with exotic pines that enables them to grow and reproduce outside of their native range (Dickie et al. 2010, Nuñez and Dickie 2014, Hayward et al. 2015a), increased in diversity accompanying range-expansion. Past research shows the ability of tree-mutualists like *Suillus* to outcompete, replace, and homogenize mutualist communities of understory plants (Becklin et al. 2012), suggesting a possible mechanism behind these patterns. Given the critical role fungal mutualists play in enabling plant persistence, especially in harsh environments, that the loss of these mutualists may negatively impact the fitness of plants growing adjacent to novel trees, potentially eventually leading to the loss of plant species and the commonly observed decrease in plant diversity associated with novel tree invasion (García et al. 2018, 2023).

In contrast to fungal mutualists, local pathogen diversity was positively influenced by plant richness and cover, and by increases in soil moisture, resulting in greater richness and abundance of pathogens in later stages of range expansion. Previous studies document similar relationships (Liu et al. 2020, Strengbom et al. 2006) and the accumulation of local pathogens by invading plants is considered an important mechanism that influences the success of exotic plant invasions and contributes to the eventual exclusion of native flora (Eppinga et al. 2006). While our results suggest that soil moisture may facilitate pathogenic fungi, it is also possible that we observed increased pathogen diversity due to the parallel reduction in mutualist diversity, as mutualists play key roles in mediating plant-pathogen defense (Singh et al. 2011). This idea is supported by our observed decrease in the fungal mutualist *Serendipita* following range-expansion, as past work has demonstrated *Serendipita's* ability to enhance plant defense against fungal pathogen infection (Oberwinkler et al. 2013, Sarkar et al. 2019). Similar to the loss of mutualists, an increase in the diversity of fungal pathogens could decrease the fitness of native plants. Taken together, these changes in root-associated fungal communities point towards a potential belowground mechanism by which novel foundation species are restructuring the communities they invade, which could contribute to the commonly observed reductions in aboveground plant diversity associated with novel tree spread and establishment (Bravo-Monasterio et al. 2016, Taylor et al. 2016, García et al. 2018, 2023, Mogashoa et al. 2021).

Implications

The spread and establishment of range-expanding trees threatens biodiversity and ecosystem functioning in alpine and tundra ecosystems around the world, however the belowground consequences remain understudied (Suarez et al. 1999, Harsch et al. 2009, Greenwood and Jump 2014, Collins et al. 2020). Here, we show that the initiation of novel ecosystems by novel foundation tree species alters the belowground abiotic environment and is coupled with guild-specific responses in fungal diversity. These belowground impacts may determine the fate of vulnerable alpine and tundra ecosystems threated by range expansion, as long-term shifts in the diversity of belowground mutualistic and pathogenic fungi can have ecosystem-wide impacts on nutrient cycling, aboveground diversity and ecosystem services (Körner 2003, Parker et al. 2015, Kivlin et al. 2017). Although the changes we outline in this study reflect the early stage impacts of single range-expanding individuals, over time, these effects will likely strengthen as trees multiply and spread, reshaping and transforming alpine and tundra landscapes into forested ecosystems (García et al. 2018, 2023), with novel water and fire regimes, increased competition among native species for limited resources, and decreased biodiversity (Simberloff et al. 2010, Taylor et al. 2016, 2017, Bravo-Monasterio et al. 2016, Franzese et al. 2017, Mogashoa et al. 2021).

As trees continue to shift upward and poleward (Harsch et al. 2009), the future of alpine and tundra ecosystems grows increasingly uncertain. Understanding how these initial impacts of range-expanding foundation species 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. 2018). Our study fills an important gap in our knowledge of how these biomes are changing and highlights the importance of considering the belowground component of ecosystem response to global change.

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Author contributions

Jean-Philippe Lessard and **Martin A. Nuñez** contributed equally to this publication. **Isaac Eckert**: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (equal); Visualization (lead); Writing – original draft (lead); Writing – review and

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Data availability statement

Data are available from figshare: https://doi.org/10.6084/m9.figshare.14675814.v2 (Eckert et al. 2023).

Supporting information

The Supporting information associated with this article is available with the online version.

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