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RESEARCH ARTICLE

Plant-soil feedback from non-native communities increases pine invasion and re-invasion potential

Joanna L. Green · Lauren P. Waller · Warwick J. Allen · Kate H. Orwin · Pieter B. Pelser · Simeon Smaill · Ian A. Dickie

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Abstract

Background and aims Individual plants and plant communities leave legacies in the soil that influence the future growth and success of subsequent plants grown in those soils However, direct tests of plant community legacies are rare. Understanding these is especially relevant for sites where invasion or re-invasion of non-native plants threatens restoration success.

Methods We used soils modified by experimental plant communities to test which aspects of plant community structure create legacies that affect the initial growth and mycorrhizal community of *Pinus contorta,* a common invasive pine species in the Southern Hemisphere. These plant communities varied in several factors including presence of *Pinus* species and the proportion of other non-native species, nitrogen-fixers, and grasses.

Results *Pinus contorta* seedlings grew largest in soils that were previously conditioned by *Pinus* individuals and by soils conditioned by communities of other non-native plants, or grasses. Ectomycorrhizal fungal diversity on *P. contorta* roots was 19.7% less in soil with a *Pinus* legacy than in soil without *Pinus* legacy. Fungal communities were generally one of three types: dominated by *Wilcoxina,* or *Inocybe,* or without a dominant fungal taxon.

Conclusion Our results demonstrate that legacy effects of plant communities on the diversity of mutualistic fungi and soil abiotic conditions can change the growth of *P. contorta* seedlings. This suggests some restoration sites, and particularly those with a previous history of *Pinus* species or high percentage of grasses or other non-native species, could be more vulnerable to invasion or reinvasion by *P. contorta.*

Keywords Soil legacies · *Pinus contorta* · Biological invasions · Non-native trees · Plant-soil feedback · Ectomycorrhizal

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J. L. Green (✉) · W. J. Allen · P. B. Pelser · I. A. Dickie
Te Kura Pūtaiao Koiora | School of Biological Sciences, Te Whare Wānanga o Waitaha, University of Canterbury, Canterbury, New Zealand
e-mail: Joanna.Green@lincoln.ac.nz

J. L. Green · L. P. Waller
Agriculture and Life Sciences, Te Whare Wānaka o Aoraki, Lincoln University, Lincoln, New Zealand

L. P. Waller · W. J. Allen · K. H. Orwin · I. A. Dickie
Bio-Protection Aotearoa, Lincoln, New Zealand

W. J. Allen · K. H. Orwin
Manaaki Whenua | Landcare Research, Lincoln, New Zealand

S. Smaill
Scion, Christchurch, Christchurch, New Zealand

Introduction

Understanding the drivers of invasion and re-invasion is critical for optimal management of invasive species and successful restoration efforts. Plants leave legacies in soils that can influence the establishment and growth of plant species in subsequent communities (Jordan et al. 2008; Reynolds et al. 2017; Van der Putten et al. 2013). These soil legacies often vary by species, but can be similar within functional groups, such as grasses or woody species (Teste et al. 2017; Wang et al. 2023). Provenance (native or non-native) can also influence the types of legacies created (Bowen et al. 2017; Callaway et al. 2008; Waller et al. 2024). Soil legacies comprise both biological (e.g. through associated microbiota) and abiotic (e.g. via nutrient modification or pH changes) components. In some cases, biological legacies have a more persistent impact than abiotic legacies (Heinen et al. 2020), possibly due to the fact that many abiotic changes provoke lasting changes in the microbial community (Heinen et al. 2020; Afzal et al 2023). This is particularly relevant with invasions involving nitrogen-fixing species, where increases in nitrogen availability is compounded by associated shifts in microbial communities (Nsikani et al. 2018). Non-native plants often associate with different below-ground fungal and bacterial communities than native plants (Bowen et al. 2017; Dickie et al. 2017; Zobel & Öpik 2014). Biological legacies created by non-native plants could therefore facilitate re-invasion of non-native species previously present in a community or the invasion of different non-natives, particularly if those legacies promote mutualists associated with the non-native plant (Callaway et al. 2008; Dickie et al. 2017; McLeod et al. 2016). Re-invasion could also be facilitated if a non-native plant has evolved resistance or tolerance to pathogens present in the legacy soil as through previous exposure (Jordan et al. 2008; Day et al 2016). Though plant pathogens are often host-specific many are generalists and can infect a range of species. Non-native grasses have been noted to host a variety of pathogens potentially facilitating invasion or co-invasion (Flory et al. 2011; Wilson et al. 2014). This type of facilitation between species is often termed an invasional meltdown or secondary invasion (Richardson et al. 2000; Simberloff & Von Holle 1999; Pyšek et al. 2004).

Plant-soil feedback experiments provide an opportunity to understand how soil legacy effects can influence the success or failure of secondary plant invasions. These studies involve conditioning soil by growing plants for a period of time and then testing how changes in the soil created by those plants affect the growth of subsequent plants. Most studies are pairwise in design and test the legacy effects of one species on another (Bartelt-Ryser et al. 2005; Shannon et al. 2012), or compare a series of pairwise legacies (Bezemer et al. 2018, 2023). While this approach provides valuable insights, single-species communities are rare in nature, suggesting that studying the impact of plant community soil legacies could provide a more realistic assessment of the role of legacies in driving invasion or re-invasion. Plant community legacies have only been considered in a few recent studies (Baxendale et al. 2014, Bezemer 2020, Heinen et al. 2020, Kostenko & Bezemer 2020, Ren et al. 2023).

Pinus species are ideal model plants to test impacts of plant community legacies on the success of invasion or re-invasion. Invasion of these trees is a major problem for many countries in the Southern Hemisphere including Brazil (de Abreu & Durigan 2011), Argentina (Torres et al. 2018), Chile (Langdon et al 2010), South Africa (Holmes et al. 2020), Australia (Calviño-Cancela & van Etten 2018) and New Zealand (Sapsford et al. 2020; Dickie et al.2022). In addition, *Pinus* species are ectomycorrhizal (ECM) and often do not associate with native ECM fungi (Policelli et al. 2019). This suggests that legacies created via impacts on mutualists may be particularly relevant for *Pinus* species. Large investments of time and money have been made in the removal of invasive pines for native restoration and pastureland reclamation (Peck et al. 2022). In many places, cleared areas do not return to the original pre-invasion state. For example, areas with a pine-invasion history are regularly re-invaded by pine or become dominated by other introduced plants (Dickie et al. 2014, 2022; Edwards et al. 2020; Sapsford & Dickie 2023). This outcome suggests that legacies created by invasive species could have long-lasting effects that promote re-invasion by the original invader or dominance of the community by other non-native plant species, although these effects can vary across ecosystems. Legacy effects could be a potential mechanism for much of invasive pine spread in New Zealand,

particularly given the high rates of re-invasion after control efforts (Peltzer 2018; Dickie et al 2022).

Here, we studied the impacts on variation in plant community soil legacies on the growth and mycorrhizal community of the invasive tree species *Pinus contorta*. We focus on *P. contorta* as it is one of the more problematic *Pinus* species (Langdon et al 2010; McAlpine & Howell 2024). The plant communities used to create different legacies varied in the presence of *Pinus* and other ectomycorrhizal (ECM) associated plants, and the proportion of nitrogen-fixing plants, non-native plants, and grasses. These plant communities were also associated with abiotic soil legacies that will created biological legacies such as changes in the fungal community. The growth response of the *Pinus contorta* seedlings was used as an indicator of potential invasion strength.

We hypothesized that:

1. Soil legacies of plant communities containing *Pinus* or other ECM plants (*Alnus glutinosa* and *Leptospermum scoparium*) or communities dominated by non-native plants, nitrogen-fixers, or grasses, yield larger *P. contorta* seedlings.
2. Plant community soil legacy effects are mediated by both abiotic (e.g., nutrients) and biological changes (e.g. soil-borne mutualists and potential pathogens) changes to the soil.

Materials and methods

Soil legacy establishment

We used soils from a large-scale mesocosm experiment (Allen et al. 2021; Waller et al. 2020) comprised of 20 different plant communities, created from a mixture of native and non-native plant species (Supplementary Table S1) that commonly occur in areas invaded by *P. contorta* in New Zealand. This experiment provided soils conditioned by known plant communities in two phases (see Fig. 1 for an experimental design schematic focusing, and Supplementary Figure S1 for photographs of the experiment). Full methods describing the mesocosm experiment can be found in Allen et al. (2021) and Waller et al. (2020), with the relevant details briefly described below.

For Phase 1, soils were created from a single-species plant-soil feedback experiment in a glasshouse. These soils were conditioned by growing 39 plant species (19 native and 20 non-native, including *P. contorta* and *P. radiata*) in a common, field-collected soil in individual pots. After growing for 9–10 months, above ground plant material was discarded, roots were coarsely cut and reincorporated into the soil and these soils were used in Phase 2.

For Phase 2,20 different plant communities, each consisting of eight species, were planted into 160 mesocosms containing different combinations of eight Phase 1 soils. The soils used for Phase 2 consisted of "home soils" (where the Phase 1 soils mixed together were the same as the plant species planted in the Phase 2 community) and "away soils" (where the Phase 1 soils mixed together were different to the plant community used for Phase 2). The composition of Phase 2 plant communities was designed to range from 0–100% non-native species in five levels (0%, 25%, 50%, 75%, and 100%), (Waller et al. 2020). Pine species were present in seven of the 20 communities. Although not explicitly part of the experimental design, nitrogen-fixing plants comprised 0–50% of each plant community; one community at 50%, three at 37.5%, three at 25%, eight at 12.5%, and five with no nitrogen-fixers. Depending on which soil community plants were grown in, this plant community legacy establishment period could range from a minimum of nine months if only in the first growth phase (i.e., home soils followed by away soils), or up to 23 months, if the community was the same for both phases (i.e., both home soils). This two-phase approach allowed us to test whether legacies left by different non-native plant species influenced the growth of subsequent plant species, and whether a previous legacy (Phase 1) could remain influential even after additional plants were grown in the soil (Phase 2). Each plant community was replicated eight times with a $2 \times 2x2$ factorial combination of the soil treatment (home/away, home meaning the same community was re-planted into a home soil), and the presence/absence of invertebrate herbivores, and Scotch broom (*Cytisus scoparius*) seedlings. Both herbivore treatment and broom seedling invasion did not impact in soil legacies in our experiment, so we excluded these variables from our analyses for the sake of simplicity.

Soil samples for nutrient analysis were taken from each of the mesocosm pots at harvest and measured

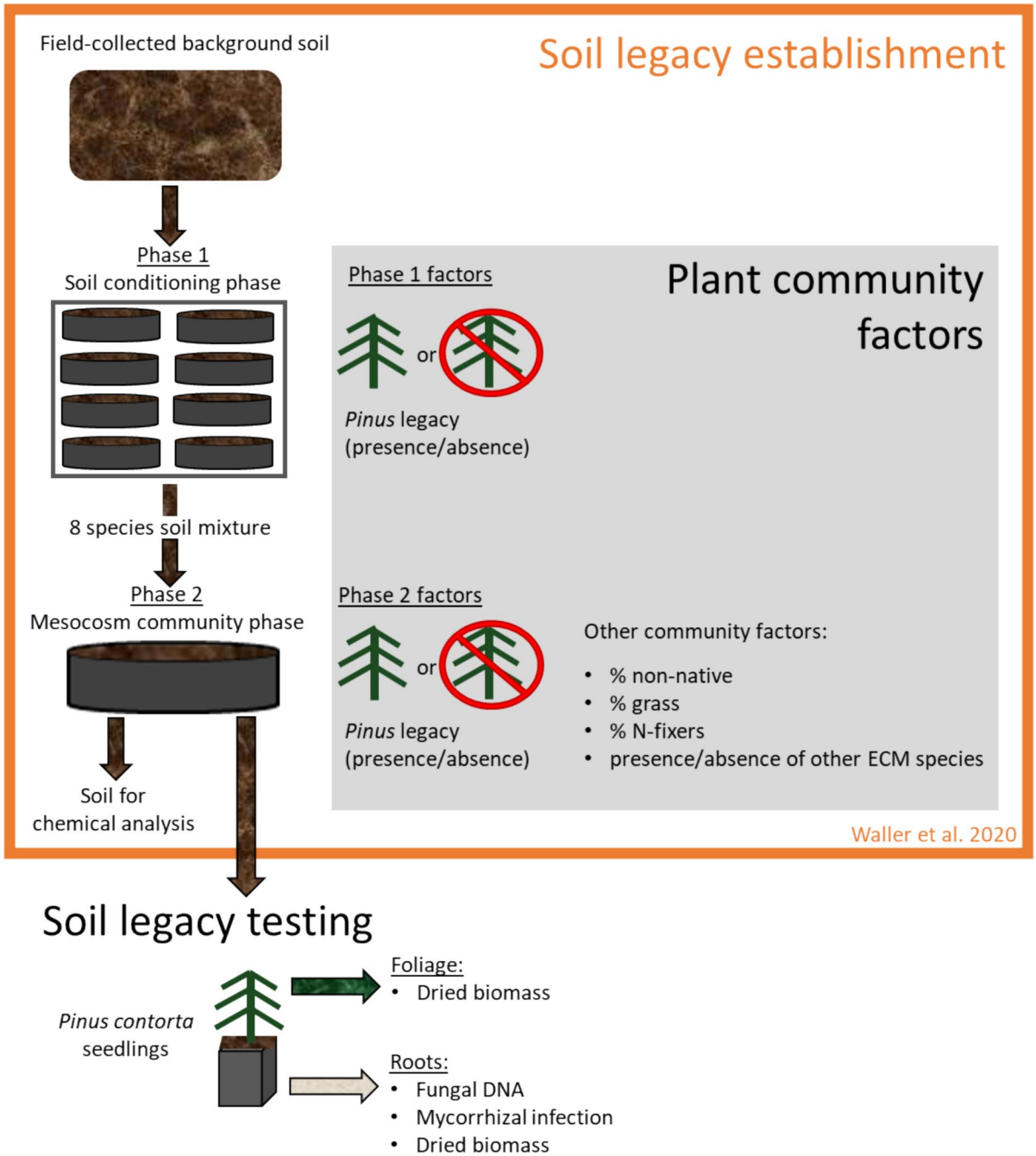


Fig. 1 Schematic of the experimental design showing the process to obtain data from one soil legacy establishment pot. The legacy establishment experiment is presented in the orange box; showing the soil conditioning for one mesocosm community. Plant community legacy factors that were manipulated in each phase are indicated in the grey box. For photos of the legacy establishment experiment see Supplementary Figure S1

by Hill Laboratories, Christchurch, New Zealand, using a Dumas type combustion with an Elementar Vario-Max CN Elemental Analyzer (Waller et al. 2020). Specific nutrients measured included available nitrogen, anaerobically mineralizable nitrogen, total nitrogen, organic matter, total carbon, carbon to

nitrogen ratio, and anaerobically mineralizable nitrogen to total nitrogen ratio (Waller et al. 2020).

Concurrent with soil sampling for nutrient analysis, we harvested soils for our experiment. (apx 5 L per mesocosm pot) from the 160 mesocosms aseptically using sterilised trowels. Soils were stored at 4˚C for less than a month and samples were selected in a random order for processing.

Legacy testing experiment

To test the impact of these soil legacies on *P. contorta* growth and their root-associated fungal communities,160 pots (0.5 L) were sterilised with 10% bleach before each was filled with soil sampled from each pf the 160 mesocosm communities. *Pinus contorta* seeds were surface sterilised to remove any microbial contaminants and were then germinated in a sterile perlite and vermiculite mixture (1:1). We planted two seedlings per pot to mitigate transplant mortality, and we randomly thinned each pot to a single seedling after 1 month. Pot placement in the glasshouse was fully randomised. Pots were watered every other day and kept at 25–30 ℃. Plants were harvested after six months. Above ground biomass was dried at 65 ℃ until reaching a constant mass and weighed; the below-ground (roots) were dried in the same manner after mycorrhizal scoring and sampling for DNA. Total biomass was used for the analyses.

Before root drying, percent mycorrhizal infection of each entire *P. contorta* root system was measured using the grid-line intercept method (Giovannetti & Mosse 1980); scoring all roots that intersected the grid as either mycorrhizal or not.

After scoring all the roots, a single pooled root sample was taken from each seedling for DNA extraction to characterise mycorrhizal biodiversity. This sample included three separate 2 cm sections of fine root with at least 10 individual root tips of 1–3 cm length and 10 additional randomly selected root tip fragments of <1 cm to ensure sampling of a variety of mycorrhizal morphotypes across various parts of the root system. These were stored at −80 °C until DNA extraction.

We extracted DNA from *P. contorta* root samples using the Extract-N-Amp™ Plant PCR Kit by Sigma-Aldrich. PCR amplification of the Internal Transcribed Spacer (ITS) region was performed using primers ITS1 F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) modified with unique molecular identification (MID) tags and adapters to allow for multiplex sequencing downstream. PCR was performed as follows: denaturation of 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 57 °C and 30 s at 72 °C, with a final step at 72 °C for 7 min (and held at 4 °C). All PCRs were completed with positive and negative controls. To confirm amplification, we performed agarose gel electrophoresis with the PCR product, using a 1% agarose gel stained with RedSafeTM (iNtRON). No PCR products were seen in negative controls and samples that showed poor amplification were rerun. After gel electrophoresis verification, products were cleaned using the QIAquik PCR Purification Kit by QIAGEN, then pooled and sent for Illumina MiSeq, 2×250 bp sequencing at the Otago Genomics Facility at the University of Otago.

Reads were merged using USEARCH v11.0.667 (Edgar 2010). We removed sequences shorter than 200 bp and those with more than one expected error using VSEARCH 2.10.4 (Rognes et al. 2016). Sequences were clustered to a 95% similarity threshold. OTUs were identified using BLAST v2.5.0 + (Altschul et al. 1997) and the UNITE public database (Nilsson et al. 2019). We removed OTUs that were not within the kingdom Fungi and all OTUs that had a $<$ 200 bp match to any known species. Extraction buffer blanks, as well as positive and negative controls were sequenced and OTUs that were found within these controls (0.38% of OTUs) were also removed from further analysis.

Statistical analysis

Fungal community sequences were examined via ordination with the function *pcoa* in the package"ape (v5.5)"(Paradis & Schliep 2019). Principal Coordinate Analysis (PCoA) was chosen for analysing the complex, non-linear data with differences in the predictor format (two with presence/absence, three using proportions) and with the fungal DNA sequences (Legendre & Legendre 2012). To determine the functional groups of the fungal OTUs, we used the FUNGuild database (Nguyen et al. 2016) to match BLAST-identified OTUs to guilds. We then focused on potential pathogens and mutualists (concentrating on ectomycorrhizal fungi as these are obligate mutualists for *Pinus*), analysing each separately from total community. We also verified FUNGuild's

findings against the FungalTraits database (Põlme et al. 2020) erring on the side of inclusion, for example if either database classified an OTU as a pathogen it was included in our pathogen analysis. We further checked our final decisions on ectomycorrhizal (ECM) categorization based on species present in the published catalogue of known ECM species (Tedersoo et al. 2010, 2014; Tedersoo & Smith 2013).

To quantify how the soil legacy effects on *P. contorta* seedling total biomass varied with plant community characteristics, we used linear mixed-effect models with the *lmer* function of the R package "lme4 (v1.121)" (Bates et al. 2014), accounting for the plant community identity (i.e. the 20 different plant communities) as a random effect within the model. Plant community characteristics included in the models as fixed effects were: presence of *Pinus* (in either Phase 1 or in Phase 2), % N-fixer, % non-native, and % grass. Soil nutrients were included as continuous fixed effects. Due to correlations among soil nutrient data, we conducted a principal component analysis (PCA) using base R function *prcomp* and used the first two axes as predictors in the mixed-effect models. Combined, these axes explained 85% of observed variance (with axis one explaining 51.2%, and axis two explaining 33.9%) (Fig. 2; see further discussion in results section). Each effect was modelled independently as a single predictor of *P. contorta* biomass as well as being a predictor in a multiple-predictor mixed-effects model.

The fungal community of *P. contorta* seedling root tips was analysed using the R package "vegan (v2.5.7)" (Oksanen et al. 2013). To test the effects of different plant community legacy factors (pine legacy, mesocosm plant community characteristics, and soil nutrients) on fungal community composition and seedling biomass, we used a permutational multivariate analysis of variance (PERMANOVA) with the *adonis2* function and marginal sum of squares.

As most predictors were interrelated, we used mixed-effects models with stepwise model

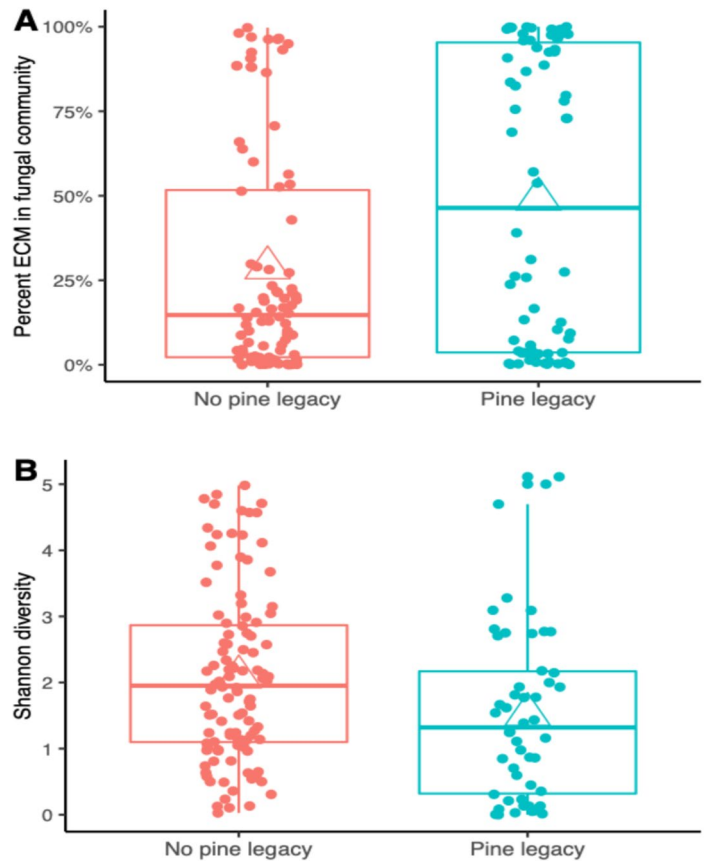


Fig. 2 Box and whisker plots showing **A** the relative abundance of ectomycorrhizal (ECM) fungal taxa and **B** Shannon diversity of the fungal community of root tips of *Pinus contorta* seedlings that were grown in soils with (teal) and without (coral) a pine legacy. Triangle symbols indicate the mean. Boxes show the upper and lower quartiles with the bold line representing the median, whiskers indicate the range of the data points

simplification (Crawley 2007) to find the best model between three major categories of predictors: 1.) plant community legacy factors from the original mesocosm study (*Pinus* presence, proportion of nitrogen fixers, proportion of non-native plants, proportion of grass species, and presence of other non-*Pinus* ECM plants), 2.) soil nutrients from at harvest (PCA axes 1 and 2), and 3.) fungal community diversity in root tips (including Shannon diversity of the whole fungal community and the three major fungal community types as identified via DNA sequence analysis including PCoA ordination and ECM identification/analysis). Following Crawley (2007), third-order interactions (or higher) were considered significant when $p < 0.01$, while main effects and second-order interactions were considered significant at $p < 0.05$. P-values for mixed effects models were calculated via the "lmerTest" package (Kuznetsova et al. 2017).

Results

Ectomycorrhizal infection rates on *P. contorta* roots were not significantly influenced by any legacy predictors (all $p > 0.05$), as all *P. contorta* seedlings showed high rates of infection, ranging from 56–98% of total root tips (Supplementary Figure S2). However, a higher proportion of ectomycorrhizal fungal DNA sequences (PERMANOVA: $p = 0.008$ $R^2 = 0.03$) and 30% fewer fungal OTUs (PERMANOVA: $p = 0.001$ $R^2 = 0.05$) were identified from root tips of *P. contorta* grown in soils with a pine legacy, relative to those without a pine legacy (Fig. 3, see Supplementary Table S2 for the 50 most abundant OTUs).

Fungal communities belonged to one of three major groupings (Figs. 3 and 4): dominated by *Wilcoxina* (> 50% of community was *Wilcoxina*), dominated by *Inocbye* (> 50% of community was *Inocbye*), or a diverse community with no single dominant taxon. There were no significant differences in the abundance or diversity of potential pathogens on *P. contorta* roots grown with or without a pine legacy. There did appear to be a weak relationship between soil nutrients and *Wilcoxina* (Supplementary Figure S3), though it was not significant in any models.

The PCA of the soil nutrient data indicated that axis 1 was associated with soil nutrient concentrations, such as organic matter, total carbon and nitrogen, and the C:N ratio (Fig. 5). Axis 2 was indicative of nutrient quality, as it was most strongly associated with the ratio of available mineralizable nitrogen to total nitrogen and other measures of available nitrogen (Fig. 5).

Pinus contorta seedling biomass was negatively related with fungal Shannon diversity ($F_{1,153} = 5.69$, $P = 0.012$) and positively related with the proportion of non-native plants ($F_{1,18} = 5.35$, $P = 0.033$) and soil nutrient PCA axis 2 ($F_{1,152} = 5.11$, $P = 0.025$) (Table 1, Fig. 6, Supplementary Figure S4). Shannon diversity, was 20% lower for seedlings grown

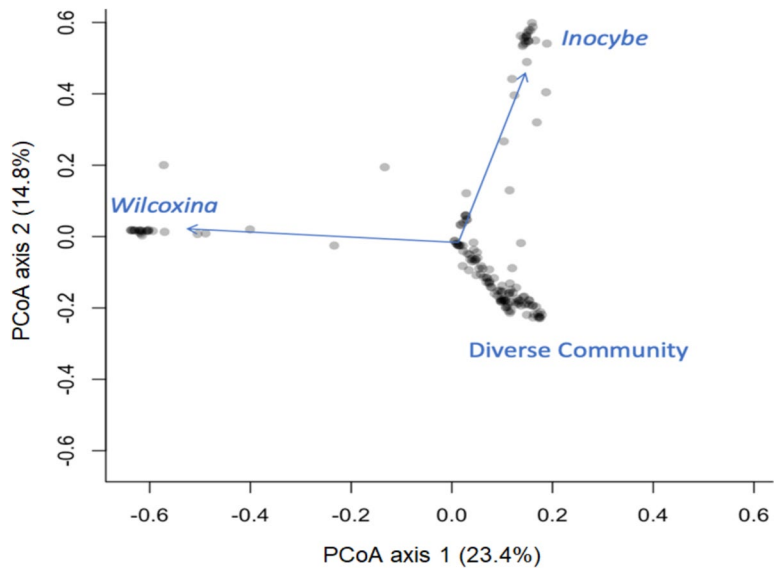


Fig. 3 Ordination plot showing the results of a Principal Coordinate Analysis (PCoA) of thefungal community data from *Pinus contorta* root tips. Points represent individual seedling samples and the closer they are in ordination space, the more similar their fungal community composition. Two major groups were dominated by *Wilcoxina* and *Inocybe* respectively, and the third group represents diverse fungal communities with no single dominant taxon

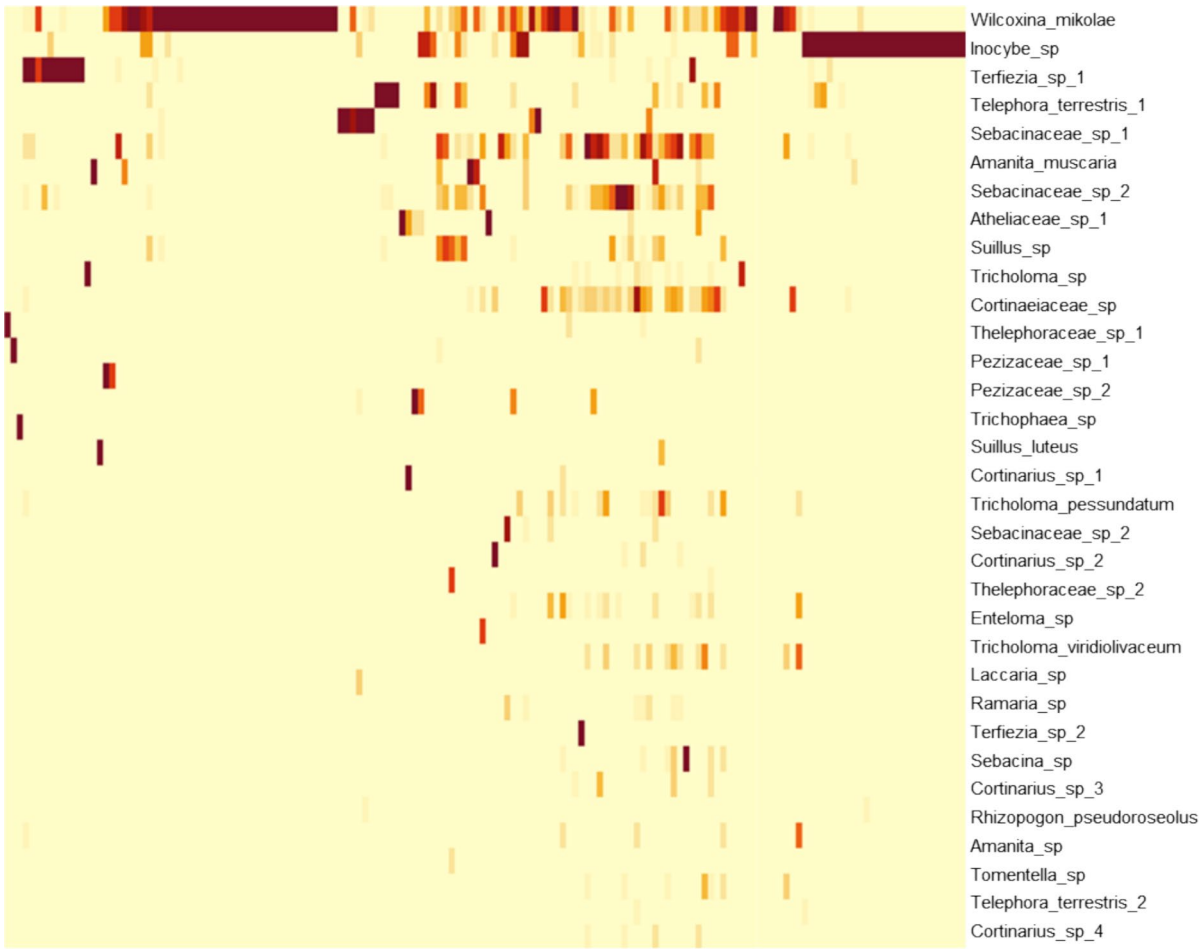


Fig. 4 Heat map of all ectomycorrhizal (ECM) fungal taxa from root tip samples from each *Pinus contorta* seedling, ranked from most (top) to least (bottom) abundant taxa in the experiment. Individual root samples are shown along the x-axis (using hierarchical clustering with complete linkage and Euclidean distance) with the identity match to each OTU on the y-axis. Darker colours indicate greater relative abundance in each sample. This figure was created using the base R function *heatmap*

with a pine legacy ($F_{1,148}$ = 12.22, P = 0.0006), and was also influenced by both a two-way interaction between nitrogen-fixers and grasses ($F_{1,148}$ = 4.55, P = 0.0346), and a three-way interaction between legacy effects created by pines, grasses, and non-native species ($F_{1,148}$ = 17.47, P = 4,975e^{-5}) (Fig. 2, Table 1, Fig. 6). Non-native legacies when grass proportions were high were associated with a positive effect ($F_{1,148}$ = 5.29, P = 0.013) on Shannon diversity when pines were absent, but non-native plants showed a strong negative effect when pines were present ($F_{1,148}$ = 13.34, P = 0.0004). When the proportion of grass was low, the effect of non-native legacies showed the opposite patterns between when pine legacies were present, and when pine legacies were absent. Soil PCA axis 2 was negatively correlated with the proportion of nitrogen-fixing plants (Figs. 3 and 6, Supplementary Figure S5). *Wilcoxina* and *Inocybe* dominated fungal communities were not associated with *P. contorta* seedling biomass but were influenced by interactions among plant community factors and soil nutrient axis PC1 respectively.

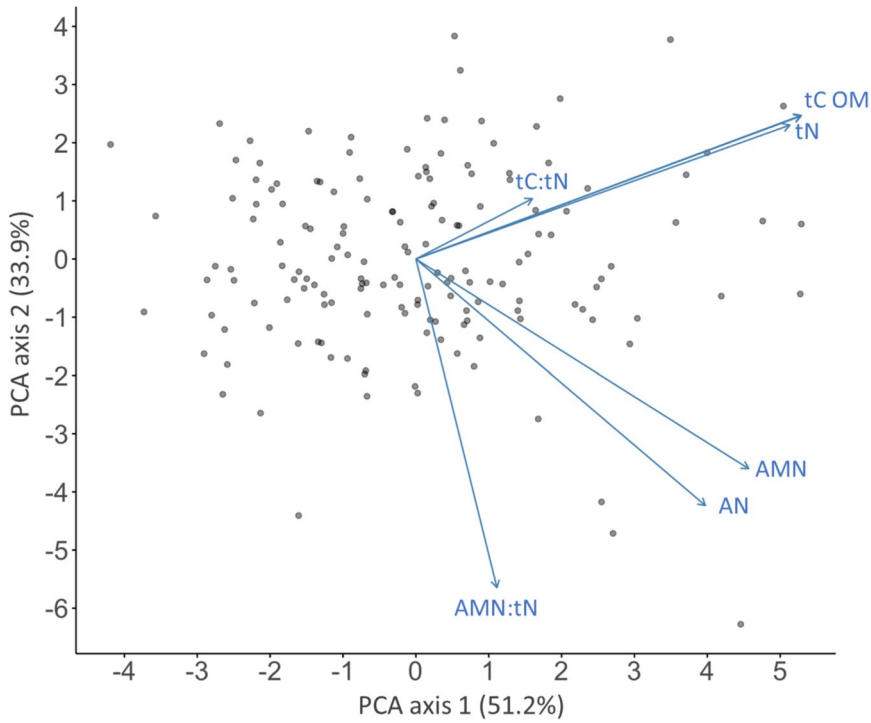


Fig. 5 Results of the principal components analysis (PCA) for soil nutrient samples, each represented by an individual data point: vectors are associated with soil nitrogen (available mineralizable nitrogen, AMN; available nitrogen, AN; total nitrogen, tN; and the ratio, AMN:tN), carbon (total Carbon, tC; and organic matter, OM), and the ratio tC:tN. The amount of variation explained by each axis is presented with the axis label

Table 1. Standardised Coefficients from Pinus contorta seedling mixed-effects models with legacy factors, soil nutrient PCA axes, and fungal community measurements as predictors

Response variable	Pine legacy	N-fixers	Non-native	Soil axis 1	Soil axis 2	*Wilcoxina* dominated	*Inocybe* dominated	Shannon diversity	N-fixers : Grass	Pine : Non-native	Pine : Grass	Non-native : Grass	Pine : Non-native : Grass	Pine : Non-native : ECM
Seedling biomass	○	○	0.235 *	○	0.174 *	○	○	-0.181 **	○	○	○	○	○	○
Shannon diversity	***	○	○	○	○				0.516 *	***	**	*	-1.86 ***	○
Wilcoxina dominated	◊	○	○	○	○				○	**	○	○	1.662 **	0.796 **
Inocybe dominated	○	○	○	0.139 ◊	○				○	○	○	○	○	○
Soil PCA axis 1	○	○	0.381 **						○	○	○	○		○
Soil PCA axis 2	○	-0.229 *	○						○	○	○	○	○	○

Significance of p-values are indicated by symbols: *** < 0.001, ** < 0.01 and * < 0.05, ◊ < 0.1, ○ indicates a non-significant term (p ≥0.1), [] filled cells indicate predictors that were not relevant for the respective response variable as either inter-related (diversity & fungal dominance) or were based on legacy soil measurements taken before legacy testing thus were only used to predict plant response and not root-associated fungal responses. Predictors with no significant p-values and not involved in higher interactions were dropped from the model. Only values for coefficients of highest-level interactions are shown here, significance still indicated for terms involved in interactions when significant. Terms with no significant values were omitted from the table

Discussion

Our research shows that even a short-term invasion can leave a legacy that affects the growth of future plants. Specifically, *Pinus contorta* growth was enhanced by soils previously conditioned by plant communities containing pines and by soils conditioned by non-native plant communities regardless of previous pine presence. This strong feedback could facilitate current *Pinus* invasions and potentially lead to an invasional meltdown as non-native plants also increased *P. contorta* growth.

The expectation that established plant legacies would alter the potential symbiotic interactions of invading pine seedlings was partially supported in that specific community factors such as *Pinus* legacy and proportions of grass, non-native plants and nitrogen-fixers impacted fungal Shannon diversity. The three-way interaction (pine: non-native: grass) found in the model, indicated that pine presence along with

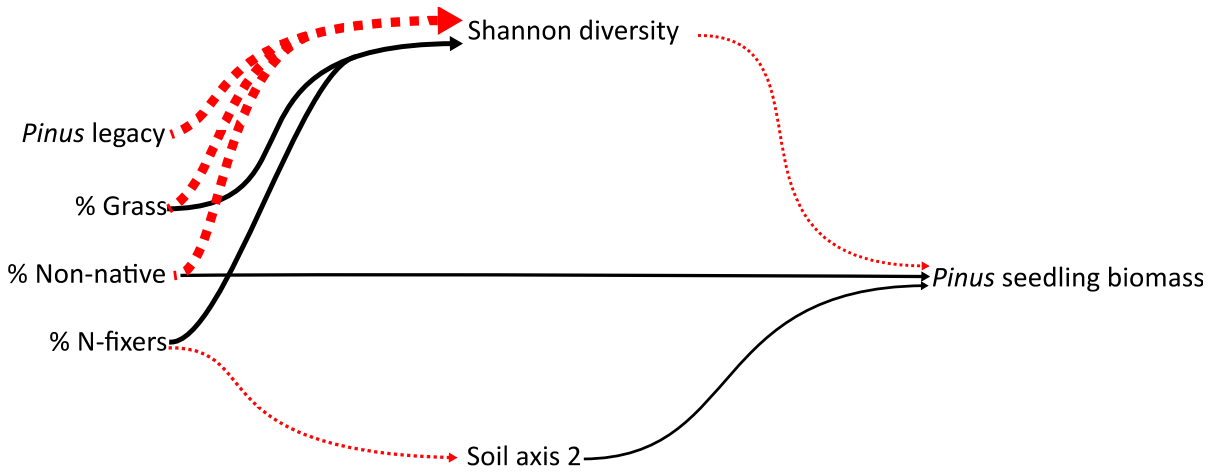


Fig. 6 Summary figure showing significant effects in the linear mixed-effects models (Table 1). Red dashed lines indicate negative coefficients, and black solid lines indicate positive coefficients; arrow thickness is scaled to the $R.^2$ value of each single variable linear mixed-effects model. Branched arrows indicate interactions, as listed in Table 1

a greater proportion of non-native and grass legacy leads to lowered mycorrhizal diversity (Table 1, Supplementary Figure S5). Pine legacies alone reduced the Shannon diversity of root tip fungi, as seen in single predictor models, causing a simplification of the fungal community. This trend may partially reflect the increased dominance of a specific few ectomycorrhizal fungi (like *Inocybe* and *Wilcoxina*) on some root tips. This finding is also consistent with a strong decline in fungal diversity in soils observed in pine invasions into grasslands (Sapsford & Dickie 2023) as well as a high background abundance of these specific taxa within New Zealand pine plantations and invasions (Walbert et al. 2010; Policelli et al. 2019; Sapsford & Dickie 2023). This could increase the probability of future pine invasions, if the fungal community post-removal could remain dominated by pine-associated fungi while supressing the diversity of other fungi.

Wilcoxina and *Inocybe* did not appear to positively influence *P. contorta* seedling biomass, despite these ECM fungi being commonly associated with pine trees (Hayward et al. 2015; Policelli et al. 2019). *Wilcoxina* has previously been found to be positively associated with invasive pine in New Zealand (Sapsford & Dickie 2023; Walbert et al 2010) and to be common in *Pseudotsuga menziesii* invasions (Wood et al. 2015), while *Inocybe* has only been reported on pines in plantations in New Zealand (Walbert et al. 2010). Overall, our results suggest that a loss of fungal diversity rather than shifts in specific ectomycorrhizal community species, could be the main drivers of *P. contorta* re-invasion after removal.

In plant-soil feedback studies, negative conspecific feedback is common and often attributed to pathogens specific to those plant species (Kulmatiski et al. 2008; Van der Putten et al. 2013). In this experiment there was no relationship between OTUs identified as potentially pathogenic and final pine seedling biomass (Supplementary Table S2). The soil legacy establishment experiment (Waller et al. 2020, 2024) did indicate an increase in potentially pathogenic OTUs on non-native plants, although this was not apparent in the pine root fungal communities of our experiment.

The effect of pine legacies on subsequent pine seedling growth appeared to be mediated primarily by biological factors rather than abiotic ones. Although seedling biomass increases were associated with the soil nutrient PCA axis 2, the proportion of nitrogen-fixing plants negatively associated with soil nutrient axis 2, both interactions were at low levels of significance (Table 1, Fig. 6).

The residence time required for plants to create a soil legacy is still unknown for most plant species. One single biological introduction event has been shown to have impacts on plant communities lasting decades (Wubs et al. 2019), though long-term legacies can have arguably greater impacts (Ke et al. 2021). In this experiment, pine presence affected fungal diversity, even if pine legacy was only from Phase 1,

suggesting that even short-term plant community soil conditioning can shape future microbial communities, and that their legacy can persist through time and through multiple different plant communities. This is particularly relevant as many pine seedlings did not survive until the end of the legacy establishment experiment (Waller et al. 2020). Pine success in the Phase 2 communities was not predicted by plant-soil feedbacks/soil legacies, as many of these pines failed regardless of whether soil was conditioned with pine or not in Phase 1 (Waller et al. 2020). However, in our legacy testing, in the absence of competition, pine benefited from any pine legacy soil, including those soils previously conditioned by pine either in Phase 1 or Phase 2 (Supplementary Figure S6), with the apparent impact showing no significant differences between Phase 2 pine legacy with pines that survived versus a legacy with pines that died within Phase 2 (Supplementary Figure S7) regardless of Phase 1.

Plant community legacies depend on a variety of factors including the traits of the invasive plant, resident community, and on abiotic site conditions. We found this legacy is mediated, and sometimes obscured by, both abiotic and biological effects. While *Pinus* legacies drive plant successional trajectories to exotic grass dominance and *Pinus* reinvasion in New Zealand (Dickie et al. 2014), the experience in South Africa suggests legacies modify but do not limit native succession following *Pinus* removal there (Holmes et al. 2020; Baard et al. 2024). Conversely, nitrogen-fixing shrubs follow the opposite pattern, with *Ulex* modifying but not preventing native succession in New Zealand (Sullivan et al. 2007) while *Acacia* in South Africa is considered highly problematic for native plant recovery (Holmes et al. 2020; Baard et al. 2024). Thus, species- and region-specific assessment of how soil legacies may affect future invasion potential should be considered in assessing ecological threat and restoration effort (Corbin and D'Antonio 2012; Corbin 2025). The presence of plant competitors (regardless of provenance) is also pivotal, as biotic resistance may reduce the risk of reinvasion. In some cases, herbicides or other weed eradication tools may actually hinder long-term remediation success following invasive plant removal. Awareness of plant community legacies, particularly biotic factors such as past pine history and non-native and grass presence along with associated fungal community dynamics, will inform restoration efforts in pine invasion sites.

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Data Availability Data files on figshare.com: DNA processing code, data inputs for models (soil nutrients, PCA outputs, DNA outputs, seedling biomass). https://figshare.com/articles/dataset/Data_used_for_manuscript_Soil_legacy_effects_of_exotic_plant_communities_Drivers_of_pine_invasion_and_reinvasion/25560615. https://doi.org/10.6084/m9.figshare.25560615.

Declarations

Competing interests No financial or non-financial competing interests to declare.

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References

Afzal MR, Naz M, Ashraf W, Du D (2023) The legacy of plant invasion: impacts on soil nitrification and management implications. Plants 12(16):2980. https://doi.org/10.3390/plants12162980

Allen WJ, Waller LP, Barratt BI, Dickie IA, Tylianakis JM (2021) Exotic plants accumulate and share

herbivores yet dominate communities via rapid growth. Nat Commun 12(1):2696. https://doi.org/10.1038/s41467-021-23030-1

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25(17):3389–3402. https://doi.org/10.1093/nar/25.17.3389

Baard JA, Grobler BA, Kraaij T (2024) Passive restoration of fynbos after afforestation with exotic pines, South Africa. Restoration Ecology 32(1):e14037. https://doi.org/10.1111/rec.14037

Bartelt-Ryser J, Joshi J, Schmid B, Brandl H, Balser T (2005) Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth. Perspectives in Plant Ecology, Evolution & Systematics 7(1):27–49. https://doi.org/10.1016/j.ppees.2004.11.002

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects model using lme4. J Stat Softw 67(1):1–48. https://doi.org/10.18637/jss.v067.i01

Baxendale C, Orwin KH, Poly F, Pommier T, Bardgett RD (2014) Are plant–soil feedback responses explained by plant traits? New Phytol 204(2):408–423. https://doi.org/10.1111/nph.12915

Bezemer TM, Jing J, Bakx-Schotman JT, Bijleveld EJ (2018) Plant competition alters the temporal dynamics of plant-soil feedbacks. J Ecol 106(6):2287–2300. https://doi.org/10.1111/1365-2745.12999

Bowen JL, Kearns PJ, Byrnes JE, Wigginton S, Allen WJ, Greenwood M, Tran K, Yu J, Cronin JT, Meyerson LA (2017) Lineage overwhelms environmental conditions in determining rhizosphere bacterial community structure in a cosmopolitan invasive plant. Nat Commun 8(1):433. https://doi.org/10.1038/s41467-017-00626-0

Callaway RM, Cipollini D, Barto K, Thelen GC, Hallett SG, Prati D, Stinson K, Klironomos J (2008) Novel weapons: Invasive plant suppresses fungal mutualists in America but not in its native Europe. Ecology 89:1043–1055. https://doi.org/10.1890/07-0370.1

Calviño-Cancela M, van Etten EJ (2018) Invasive potential of Eucalyptus globulus and Pinus radiata into native eucalypt forests in Western Australia. For Ecol Manage 424:246–258

Corbin JD, D'Antonio CM (2012) Gone but not forgotten? Invasive plants' legacies on community and ecosystem properties. Invasive Plant Science and Management 5(1):117–124. https://doi.org/10.1614/IPSM-D-11-00005.1

Corbin JD (2025) The influence of exotic species' legacies on pathways of restoration. Restor Ecol: e14381. https://doi.org/10.1111/rec.14381

Crawley M (2007) The R book. 2007. Imperial College London at Silwood Park. UK: 527–528. https://doi.org/10.1007/s00362-008-0118-3

Day NJ, Dunfield KE, Antunes PM (2016) Fungi from a non-native invasive plant increase its growth but have different growth effects on native plants. Biol Invasions 18:231–243. https://doi.org/10.1007/s10530-015-1004-2

de Abreu RC, Durigan G (2011) Changes in the plant community of a Brazilian grassland savannah after 22 years of invasion by *Pinus elliottii* Engelm. Plant Ecolog Divers 4(2–3):269–278. https://doi.org/10.1080/17550874.2011.594101

Dickie IA, St John MG, Yeates GW, Morse CW, Bonner KI, Orwin K, Peltzer DA (2014) Belowground legacies of Pinus contorta invasion and removal result in multiple mechanisms of invasional meltdown. AoB Plants 6:plu056. https://doi.org/10.1093/aobpla/plu056

Dickie IA, Bufford JL, Cobb RC, Desprez-Loustau ML, Grelet G, Hulme PE, Klironomos J, Makiola A, Nuñez MA, Pringle A (2017) The emerging science of linked plant–fungal invasions. New Phytol 215(4):1314–1332. https://doi.org/10.1111/nph.14657

Dickie IA, Sprague R, Green J, Peltzer DA, Orwin K, Sapsford S (2022) Applying ecological research to improve long-term outcomes of wilding conifer management. New Zealand J Ecol 46(2):1–16. https://doi.org/10.20417/nzjecol.46.23

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26(19):2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Edwards P, Stahlmann-Brown P, Thomas S (2020) Pernicious pests and public perceptions: Wilding conifers in Aotearoa New Zealand. Land Use Policy 97:104759. https://doi.org/10.1016/j.landusepol.2020.104759

Flory SL, Kleczewski N, Clay K (2011) Ecological consequences of pathogen accumulation on an invasive grass. Ecosphere 2(10):1–12. https://doi.org/10.1890/ES11-00191.1

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol Ecol 2(2):113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol 84(3):489–500. https://doi.org/10.1111/j.1469-8137.1980.tb04556.x

Hayward J, Horton TR, Pauchard A, Nuñez MA (2015) A single ectomycorrhizal fungal species can enable a Pinus invasion. Ecology 96(5):1438–1444. https://doi.org/10.1890/14-1100.1

Heinen R, Hannula SE, De Long JR, Huberty M, Jongen R, Kielak A, Steinauer K, Zhu F, Bezemer TM (2020) Plant community composition steers grassland vegetation via soil legacy effects. Ecol Lett 23(6):973–982. https://doi.org/10.1111/ele.13497

Holmes PM, Esler KJ, van Wilgen BW, Richardson DM (2020) Ecological restoration of ecosystems degraded by invasive alien plants in South African fynbos. Is spontaneous succession a viable strategy? Transactions of the Royal Society of South Africa 75:111–139. https://doi.org/10.1080/0035919X.2020.1781291

Jordan NR, Larson DL, Huerd SC (2008) Soil modification by invasive plants: effects on native and invasive species of mixed-grass prairies. Biol Invasions 10(2):177–190. https://doi.org/10.1007/s10530-007-9121-1

Ke PJ, Zee PC, Fukami T (2021) Dynamic plant–soil microbe interactions: the neglected effect of soil conditioning time. New Phytol 231(4):1546–1558. https://doi.org/10.1111/nph.17420

Kostenko O, Bezemer TM (2020) Abiotic and biotic soil legacy effects of plant diversity on plant performance.

Front Ecol Evol 8:87. https://doi.org/10.3389/fevo.2020.00087
Kulmatiski A, Beard KH, Stevens JR, Cobbold SM (2008) Plant–soil feedbacks: a meta-analytical review. Ecol Lett 11(9):980–992. https://doi.org/10.1111/j.1461-0248.2008.01209.x
Kuznetsova A, Brockhoff PB, Christensen RH (2017) lmerTest package: tests in linear mixed effects models. J Stat Softw 82:1–26. https://doi.org/10.18637/jss.v082.i13
Langdon B, Pauchard A, Aguayo M (2010) Pinus contorta invasion in the Chilean Patagonia: local patterns in a global context. Biol Invasions 12:3961–3971. https://doi.org/10.1007/s10530-010-9817-5
Legendre P, Legendre L (2012) Numerical ecology. 3(24). Elsevier
McAlpine KG, Howell CJ (2024) List of environmental weeds in New Zealand 2024. Sci Conserv 340:1-37
McLeod ML, Cleveland CC, Lekberg Y, Maron JL, Philippot L, Bru D, Callaway RM (2016) Exotic invasive plants increase productivity, abundance of ammonia-oxidizing bacteria and nitrogen availability in intermountain grasslands. J Ecol 104(4):994–1002. https://doi.org/10.1111/1365-2745.12584
Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG (2016) FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol 20:241–248. https://doi.org/10.1016/j.funeco.2015.06.006
Nilsson RH, Anslan S, Bahram M, Wurzbacher C, Baldrian P, Tedersoo L (2019) Mycobiome diversity: high-throughput sequencing and identification of fungi. Nat Rev Microbiol 17(2):95–109. https://doi.org/10.1038/s41579-018-0116-y
Nsikani MM, van Wilgen BW, Gaertner M (2018) Barriers to ecosystem restoration presented by soil legacy effects of invasive alien N_2-fixing woody species: implications for ecological restoration. Restor Ecol 26(2):235–244. https://doi.org/10.1111/rec.12669
Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'hara R, Simpson GL, Solymos P, Stevens MHH, Wagner H (2013) Package 'vegan. Community Ecology Package, Version 2(9):1–295
Paradis E, Schliep K (2019) ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35(3):526–528. https://doi.org/10.1093/bioinformatics/bty633
Peck C, Williamson M, Rohani M (2022) Benefits and costs of additional investment in wilding conifer control. Prepared for the Ministry for Primary Industries on behalf of the National Wilding Conifer Control Programme
Peltzer DA (2018) Ecology and consequences of invasion by non-native (wilding) conifers in New Zealand. J New Zealand Grasslands 39–46. https://doi.org/10.33584/jnzg.2018.80.359
Policelli N, Bruns TD, Vilgalys R, Nuñez MA (2019) Suilloid fungi as global drivers of pine invasions. New Phytol 222(2):714–725. https://doi.org/10.1111/nph.15660
Põlme S, Abarenkov K, Henrik Nilsson R, Lindahl BD, Clemmensen KE, Kauserud H, Nguyen N, Kjøller R, Bates ST, Baldrian P (2020) FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. Fungal Diversity 105:1–16. https://doi.org/10.1007/s13225-020-00466-2
Pyšek P, Richardson DM, Rejmánek M, Webster GL, Williamson M, Kirschner J (2004) Alien plants in checklists and floras: towards better communication between taxonomists and ecologists. Taxon 53(1):131–143. https://doi.org/10.2307/4135498
Ren H, Xie J, Li K, Shi S, Li X, Guo H, Liu M, Yu Q (2023) Diversity-conditioned soil strengthens plant diversity–productivity relationship. J Appl Ecol. https://doi.org/10.1111/1365-2664.14454
Reynolds PL, Glanz J, Yang S, Hann C, Couture J, Grosholz E (2017) Ghost of invasion past: legacy effects on community disassembly following eradication of an invasive ecosystem engineer. Ecosphere 8(3):e01711. https://doi.org/10.1002/ecs2.1711
Richardson DM, Pyšek P, Rejmánek M, Barbour MG, Panetta FD, West CJ (2000) Naturalization and invasion of alien plants: concepts and definitions. Divers Distrib 6(2):93–107. https://doi.org/10.1046/j.1472-4642.2000.00083.x
Rognes T, Flouri T, Nichols B, Quince C, Mahé F (2016) VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584. https://doi.org/10.7717/peerj.2584
Sapsford SJ, Brandt AJ, Davis KT, Peralta G, Dickie IA, Gibson RD, Green JL, Hulme PE, Nuñez MA, Orwin KH (2020) Towards a framework for understanding the context dependence of impacts of non-native tree species. Funct Ecol 34(5):944–955. https://doi.org/10.1111/1365-2435.13544
Sapsford SJ, Dickie IA (2023) Slow soil enzyme recovery following invasive tree removal through gradual changes in bacterial and fungal communities. J Ecol 111(12):2614–2626. https://doi.org/10.1111/1365-2745.14186
Shannon S, Flory SL, Reynolds H (2012) Competitive context alters plant–soil feedback in an experimental woodland community. Oecologia 169:235–243. https://doi.org/10.1007/s00442-011-2195-6
Simberloff D, Von Holle B (1999) Positive interactions of nonindigenous species: invasional meltdown? Biol Invasions 1(1):21–32. https://doi.org/10.1023/A:1010086329619
Sullivan JJ, Williams PA, Timmins SM (2007) Secondary forest succession differs through naturalised gorse and native kānuka near Wellington and Nelson. N Z J Ecol 31:22–38
Tedersoo L, May TW, Smith ME (2010) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20(4):217–263. https://doi.org/10.1007/s00572-009-0274-x
Tedersoo L, Smith ME (2013) Lineages of ectomycorrhizal fungi revisited: foraging strategies and novel lineages revealed by sequences from belowground. Fungal Biol Rev 27(3–4):83–99. https://doi.org/10.1016/j.fbr.2013.09.001
Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R, Ruiz LV, Vasco-Palacios AM, Thu PQ, Suija A (2014) Global diversity and geography of soil fungi. Science 346(6213):1256688. https://doi.org/10.1126/science.1256688
Teste FP, Kardol P, Turner BL, Wardle DA, Zemunik G, Renton M, Laliberté E (2017) Plant-soil feedback and the maintenance of diversity in Mediterranean-climate

shrublands. Science 355(6321):173–176. https://doi.org/10.1126/science.aai8291

Torres A, Alarcón PA, Rodríguez-Cabal MA, Nuñez MA (2018) Secondary invasions hinder the recovery of native communities after the removal of nonnative pines along a precipitation gradient in Patagonia. Forests 9(7):394. https://doi.org/10.3390/f9070394

Van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, Kardol P, Klironomos JN, Kulmatiski A, Schweitzer JA (2013) Plant–soil feedbacks: the past, the present and future challenges. J Ecol 101(2):265–276. https://doi.org/10.1111/1365-2745.12054

Walbert K, Ramsfield TD, Ridgway HJ, Jones EE (2010) Ectomycorrhizal species associated with Pinus radiata in New Zealand including novel associations determined by molecular analysis. Mycorrhiza 20:209–215. https://doi.org/10.1007/s00572-009-0277-7

Waller LP, Allen WJ, Barratt B, Condron L, França F, Hunt J, Koele N, Orwin KH, Steel G, Tylianakis JM, Dickie IA (2020) Biotic interactions drive ecosystem responses to exotic plant invaders. Science 368(6494):967–972. https://doi.org/10.1126/science.aba2225

Waller LP, Allen WJ, Black A, Condron L, Tonkin JD, Tylianakis JM, Wakelin A, Dickie IA (2024) Asymmetric sharing of generalist pathogens between exotic and native plants correlates with exotic impact in communities. J Ecol. https://doi.org/10.1111/1365-2745.14392

Wang X, Kou Y, Liu J, Zhao W, Liu Q (2023) Soil microbial legacy determines mycorrhizal colonization and root traits of conifer seedlings during subalpine forest succession. Plant Soil 485(1–2):361–375. https://doi.org/10.1007/s11104-022-05835-1

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: a Guide to Methods and Applications 18(1):315–322

Wilson HE, Carroll GC, Roy BA, Blaisdell GK (2014) Tall fescue is a potential spillover reservoir host for Alternaria species. Mycologia 106(1):22–31. https://doi.org/10.3852/12-330

Wood JR, Dickie IA, Moeller HV, Peltzer DA, Bonner KI, Rattray G, Wilmshurst JM (2015) Novel interactions between non-native mammals and fungi facilitate establishment of invasive pines. J Ecol 103(1):121–129. https://doi.org/10.1111/1365-2745.12345

Wubs EJ, van der Putten WH, Mortimer SR, Korthals GW, Duyts H, Wagenaar R, Bezemer TM (2019) Single introductions of soil biota and plants generate long-term legacies in soil and plant community assembly. Ecol Lett 22(7):1145–1151. https://doi.org/10.1111/ele.13271

Zobel M, Öpik M (2014) Plant and arbuscular mycorrhizal fungal (AMF) communities–which drives which? J Veg Sci 25(5):1133–1140. https://doi.org/10.1111/jvs.12191

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