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Susan J. Nuske<sup>1</sup> , Alex Fajardo<sup>2</sup> , Martin A. Nuñez<sup>3,4</sup> , Aníbal Pauchard<sup>5,6</sup> , David A. Wardle<sup>7</sup> , Marie-Charlotte Nilsson<sup>1</sup> , Paul Kardol<sup>1</sup> , Jane E. Smith<sup>8</sup>, Duane A. Peltzer<sup>9</sup> , Jaime Moyano<sup>3</sup>  and Michael J. Gundale<sup>1</sup> 

<sup>1</sup>Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, Umeå 90183, Sweden; <sup>2</sup>Instituto de Investigación Interdisciplinario (I<sup>3</sup>), Universidad de Talca, Campus Lircay, Talca 3460000, Chile; <sup>3</sup>Grupo de Ecología de Invasiones, INIBIOMA-UNComa, CONICET, Bariloche 8400, Argentina; <sup>4</sup>Department of Biology and Biochemistry, University of Houston, Houston, TX 77204, USA; <sup>5</sup>Laboratorio de Invasiones Biológicas (LIB), Facultad de Ciencias Forestales, Universidad de Concepción, Concepción, Chile; <sup>6</sup>Institute of Ecology and Biodiversity (IEB), Santiago, Chile; <sup>7</sup>Asian School of the Environment, College of Science, Nanyang Technological University, Singapore 639798, Singapore; <sup>8</sup>US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Corvallis, OR 97331, USA; <sup>9</sup>Manaaki Whenua Landcare Research, Lincoln 7608, New Zealand

## Summary

Authors for correspondence:

Susan J. Nuske

Email: susan.nuske@slu.se

Michael J. Gundale

Email: michael.gundale@slu.se

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**Key words:** abiotic, context-dependent, ectomycorrhizas, enhanced mutualism hypothesis, enemy release hypothesis, invasive, missed mutualism hypothesis, plant–soil feedback.

- The success of invasive plants is influenced by many interacting factors, but evaluating multiple possible mechanisms of invasion success and elucidating the relative importance of abiotic and biotic drivers is challenging, and therefore rarely achieved.
- We used live, sterile or inoculated soil from different soil origins (native range and introduced range plantation; and invaded plots spanning three different countries) in a fully factorial design to simultaneously examine the influence of soil origin and soil abiotic and biotic factors on the growth of invasive *Pinus contorta*.
- Our results displayed significant context dependency in that certain soil abiotic conditions in the introduced ranges (soil nitrogen, phosphorus or carbon content) influenced responses to inoculation treatments.
- Our findings do not support the enemy release hypothesis or the enhanced mutualism hypothesis, as biota from native and plantation ranges promoted growth similarly. Instead, our results support the missed mutualism hypothesis, as biota from invasive ranges were the least beneficial for seedling growth. Our study provides a novel perspective on how variation in soil abiotic factors can influence plant–soil feedbacks for an invasive tree across broad biogeographical contexts.

## Introduction

Studying how plants interact and respond in new environments provides insights into community assembly processes (David *et al.*, 2017) and controls on plant fitness (Kuebbing & Nuñez, 2016). Many hypotheses have been proposed to explain invasion success in introduced environments, including those that are based on population ecology, community ecology, environmental effects, evolution and genetics (Blackburn *et al.*, 2011; Gundale *et al.*, 2014b). For instance, at larger scales, climatic factors such as temperature and precipitation can affect the abundance of a plant species in its introduced and native ranges (Taylor *et al.*, 2016). At local scales, growth and abundance of plant species can be strongly influenced by biotic interactions (e.g. with mutualists, pathogens and herbivores; Klironomos, 2002; Van Der Putten *et al.*, 2005; Zamora Nasca *et al.*, 2018) and abiotic conditions

(e.g. nutrient, light and moisture availability; Castle *et al.*, 2016; Smith-Ramesh & Reynolds, 2017). Additionally, genetic effects of founding populations and frequency of introduction events can also determine the invasion success of plants in new environments (Dlugosch & Parker, 2008). Because many potential factors affect the success of plant invasions, it is necessary to evaluate multiple mechanisms simultaneously in order to disentangle their relative importance (Moles *et al.*, 2012).

The interactions between plants and their soil environment (i.e. plant–soil feedbacks, PSFs) comprise one local-scale factor that can involve multiple mechanisms and is known to affect plant invasions (Stricker *et al.*, 2015; Gundale & Kardol, 2021). Several studies have found evidence that plants respond differently to native and introduced range soils (Maron *et al.*, 2013; Yang *et al.*, 2013; Dostálek *et al.*, 2016) and that differences in soil biota can help to explain these patterns (Felker-Quinn *et al.*,

2011; Maron *et al.*, 2014; Crawford & Knight, 2017). However, the mechanisms underlying these biotic effects are less clear (Callaway *et al.*, 2011). For example, plants in new ranges have been shown to interact with soil biota more positively (e.g. higher biomass, lower mortality) compared with their native ranges (Reinhart & Callaway, 2006; Zuppinger-Dingley *et al.*, 2011; Gundale *et al.*, 2014a; Dostálek *et al.*, 2016) and have more positive interactions with soil biota than native plants in their new range (positive PSF; Klironomos, 2002; Van Grunsven *et al.*, 2007; Engelkes *et al.*, 2008; Hawkes *et al.*, 2009). This suggests that plants in new environments are either escaping negative interactions (enemy release hypothesis (ERH); Shea & Chesson, 2002) from the native environment or are able to establish more positive interactions (enhanced mutualism hypothesis (EMH); Richardson *et al.*, 2000; Reinhart & Callaway, 2004, 2006) in their new environment by encountering novel communities or associating with cointroduced mutualists (Dickie *et al.*, 2010; Nuñez & Dickie, 2014). However, if essential mutualists are missing from their new range, this could inhibit the invasion of introduced species (missed mutualism hypothesis (MMH); Mitchell *et al.*, 2006; Catford *et al.*, 2009).

Abiotic soil properties such as nutrient availability can also affect PSFs of invasive species and may override or interact with biotic effects (Van Grunsven *et al.*, 2007; Castle *et al.*, 2016; Png *et al.*, 2019). For instance, in a nutrient-rich soil, mutualists may be less beneficial (i.e. less necessary) for plants (Johnson, 2010; Remke *et al.*, 2020), and plant investment in defenses can change to positively or negatively affect interactions with soil pathogens (Blumenthal, 2006; Sampedro *et al.*, 2011; Smith-Ramesh & Reynolds, 2017). Additionally, intraspecific differences in plant genotype and phenotypic expression can alter the soil microbial community and composition by altering the quantity and quality of litter inputs and root exudates (Schweitzer *et al.*, 2008, 2013; Lu-Irving *et al.*, 2019). For example, different genotypes within the same plant species may exhibit differences in their requirement for mutualists (Smith & Goodman, 1999; Keller & Lau, 2018) or tolerance of pathogens (Mazzola & Gu, 2002). Disentangling the effects of soil biota, the abiotic soil environment (e.g. element concentrations) and genotypic effects on plant performance in new environments can help to elucidate why some plants become invasive only in certain contexts (Van Nuland *et al.*, 2016; Smith-Ramesh & Reynolds, 2017; De Long *et al.*, 2019).

In this study, we examined PSFs for a globally invasive tree, *Pinus contorta*, in its native and introduced ranges. Specifically, we sought to disentangle how soil abiotic and biotic factors interacted with each other to influence seedling growth across a broad range of environmental contexts, including its native range and three countries where it has become invasive (New Zealand, Argentina and Chile). To our knowledge no previous study has addressed how PSFs of an introduced species vary across the broad array of environmental contexts we consider in this study. We addressed three main objectives: to test how the growth of *P. contorta* seedlings differs between soil originating from the native and introduced ranges, and between introduced range plantations and invasion fronts stemming from those

plantations; to test how soil biotic and abiotic factors interact to impact seedling growth among these different soil origins; and to test whether contrasting tree provenances interacted with these soil factors. This study complements a previous study by Gundale *et al.* (2014a) focused on PSFs of *P. contorta* at native Canadian sites compared with introduced sites in Sweden where closely related Pinaceae exist (i.e. *P. sylvestris*). Our current study differs from this previous study because no native Pinaceae exist in the southern hemisphere countries we evaluated which could have important consequences for the strength or direction of PSFs; and further, we employed a sampling approach that allowed us to examine how PSFs were influenced by local environmental context factors, specifically soil elemental concentrations. We focused on *P. contorta* because it is one of the most invasive pine species (Richardson & Rejmánek, 2004; Rejmánek & Richardson, 2013) and as all pine species are ectomycorrhizal, the soil microbial communities in introduced ranges can have a major influence in their success as invaders (Nuñez *et al.*, 2009; Gundale *et al.*, 2014a).

The study was performed using two parallel growth chamber experiments to address our objectives. In Expt 1, we compared seedling growth across three types of living soil (native, introduced plantation, and invasive front) to evaluate plant response to broad soil differences. In Expt 2, to disentangle the effects of soil abiotic and biotic components, we sterilized each soil type and then cross-inoculated microbial communities in a full factorial combination. This allowed us to directly compare plant responses to sterile controls (no biota) and inoculated soils (with biota), while controlling for variation in abiotic conditions. For both experiments, we additionally tested for interactive effects of seed provenance with soil treatments using two provenances of *P. contorta* seeds in all experimental units.

We used these experiments to test several major hypotheses in invasion ecology. First, we expected *P. contorta* seedlings to grow larger with biota from introduced plantation soil compared with native soil, and that sterilizing native soil would enhance seedling growth while sterilizing plantation soil would inhibit seedling growth relative to inoculated soil (Hypothesis 1). This is based on predictions from the ERH (Shea & Chesson, 2002) and the EMH (Reinhart & Callaway, 2006). Specifically for pines, more positive associations with mutualists may result from plantations in introduced ranges harboring ectomycorrhizal taxa from the native range that were intentionally or unintentionally cointroduced, which may in turn provide positive benefits to plants without the pathogen load found in the native range (Dickie *et al.*, 2010; Walbert *et al.*, 2010; Nuñez & Dickie, 2014; Pickles *et al.*, 2015; Gundale *et al.*, 2016). Second, we expect lower growth from seedlings in response to biota in soil from the invasion plots compared with plantation plots (Hypothesis 2). This is consistent with the MMH (Mitchell *et al.*, 2006; Catford *et al.*, 2009). Within pine invasions, suitable ectomycorrhizal species have been found to be less abundant or absent relative to plantation soil (Nuñez *et al.*, 2009; Salgado Salomón *et al.*, 2011; Gundale *et al.*, 2016). Additionally, as previous studies have suggested that plant interactions with soil biota can differ substantially among genotypes

(Schweitzer *et al.*, 2008; Lu-Irving *et al.*, 2019) or in response to environmental context (Johnson, 2010; Remke *et al.*, 2020), we predicted that seedling responses to soil biota treatments would differ between two selected seedling provenances or in response to soil element concentrations of individual soils (Hypothesis 3). Regarding soil element concentrations, we specifically expected microbial inoculation to provide the greatest benefit to seedlings when grown in soils with low N or P concentrations, high C concentrations, or high C:N ratios, because ectomycorrhizal fungi are known to benefit their hosts more when nutrient element concentrations are low, or when soils are rich in organic matter (Becquer *et al.*, 2019). Testing these hypotheses in combination will provide new insights into how multiple site-level factors that vary across regions can impact the growth of introduced species.

## Materials and Methods

### Field sites

Soil was collected from three types of *P. contorta* plots that served as the units of replication in this study: naturally occurring forest plots within the native range in Oregon, USA; plantation plots in the introduced range; and under invading tree plots at the invasive front of each introduced range site. *Pinus contorta* has a relatively large native range that spans from the Yukon in Canada to southern California in the USA, and from the Rocky Mountains front to the west coast of North America. In the native range, we sampled one plot at each of 15 independent native range sites in central Oregon where *P. contorta* ssp. *murrayana* (Grev. & Balf.) Critchfield occurs, because the latitude and climate are most similar to southern hemisphere regions where *P. contorta* has become invasive, and because there is some evidence that this subspecies is introduced to the southern hemisphere (Gundale *et al.*, 2014b). For introduced range southern hemisphere sampling, we selected five sites in each of three countries, New Zealand (NZ), Chile and Argentina, where *P. contorta* is invasive (three countries  $\times$  five sites = 15 introduced range sites in total; Fig. 1). Sites within each introduced range country were selected to provide a geographic spread of areas that had a similar climate to that of the native range of *P. contorta* ssp. *murrayana*. Within each southern hemisphere site, we sampled two paired plots, including a plantation plot that was established in the 1970s, and an adjacent invasion plot that stemmed from that plantation. We determined the source and direction of invading individuals based on visual spatial patterns of invading trees from the edge of plantations (i.e. the invasion kernel), which followed the prevailing wind direction (Fig. 2). Invasion plots were selected by walking from the edge of the plantation into the surrounding vegetation until no more *P. contorta* were observed, and were located at least 200 m from the plantation edge. Because the invading trees in each case originated from the plantations, invading tree and plantation plots were paired within a site. Within each invasion plot, we focused our soil sampling on soils beneath individual trees at the leading edge of the invasive front (i.e. furthest from the plantation source; Fig. 2).

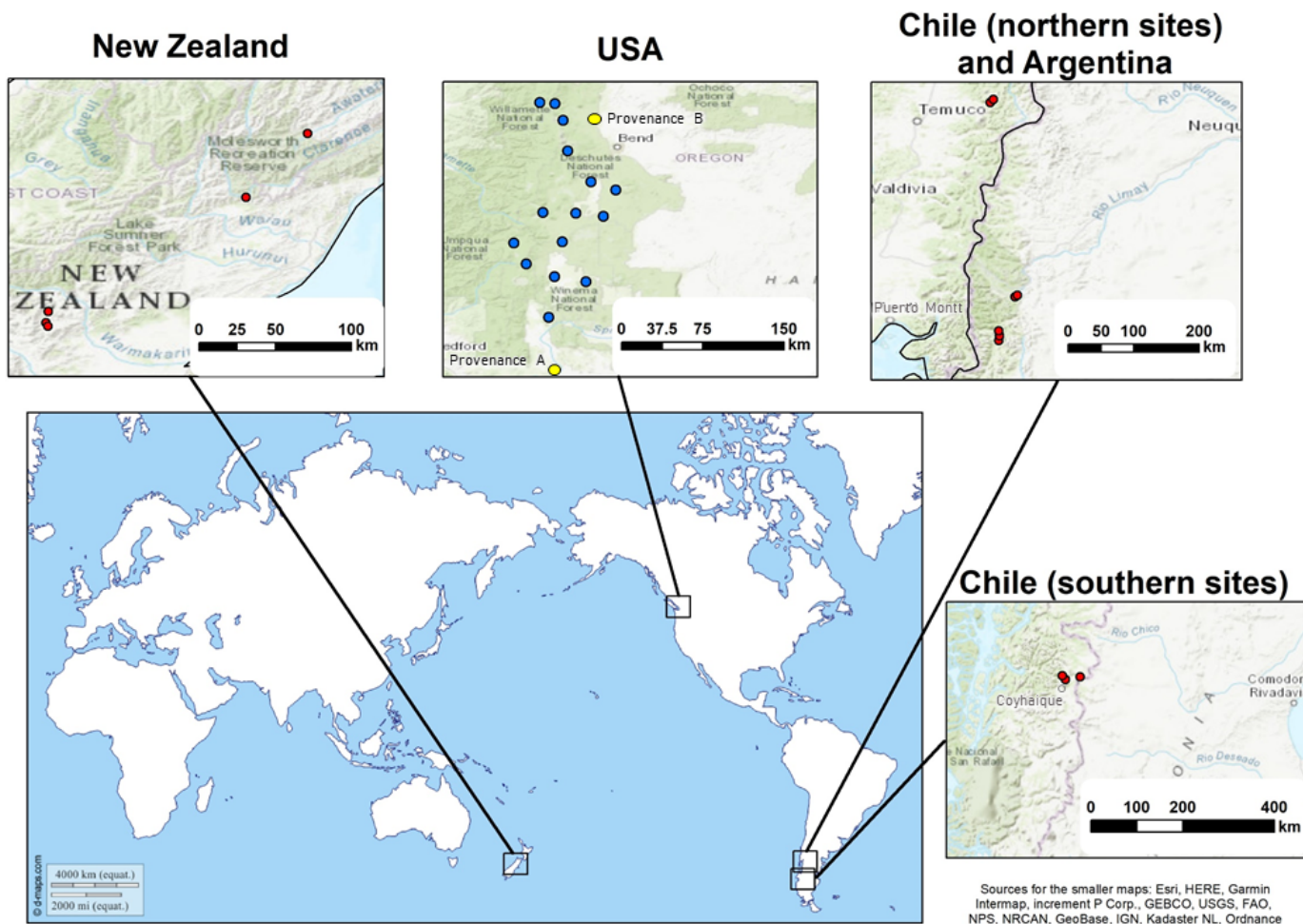
### Soil collection

Soil was collected during early summer within each range (June 2017 for USA and November and December 2017 for introduced ranges). Within each plot, a 3 l sieved sample of bulk soil was obtained by combining multiple soil cores within a plot (10 cm depth including both humus and mineral soil but excluding fresh litter) from beneath 10 individual trees within a 100 m<sup>2</sup> area. The soil was then sieved to 1 cm to remove large stones and large root fragments and coarse organic debris. Fine roots with their associated biota were retained. From this point onwards, composite soils from each plot were kept separate from one another (following experiment type D described by Gundale *et al.*, 2017, 2019). After collection, soils were refrigerated and then shipped in a cooler to Umeå, Sweden, arriving within 5–7 d of collection. All soils were then frozen upon arrival and kept frozen for a minimum of 2 wk (southern hemisphere soils) and up to 6 months (USA soils). We prioritized sampling soil in the same season for each hemisphere as this would introduce less variation in soil conditions than freezing soils for differing lengths of time. Soils were defrosted immediately before the experimental setup.

Before the start of the experiment, we measured extractable mineral nitrogen ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ), pH and mass fraction of carbon (C), nitrogen (N) and phosphorus (P) were analyzed from a portion of the sterilized composite soil samples from each plot ( $n=45$ ), using standard protocols (Gundale *et al.*, 2011). As sterilized soil constituted the majority of field-collected soil added to our experimental pots (i.e. 90%; see later), these measurements represented the soil abiotic environment that the seedlings encountered during the experiment. We also performed extractable mineral N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) analysis on nonsterilized soil from each plot to determine the impact of sterilization on these extractable nutrient pools (McNamara *et al.*, 2003).

### Growth chamber experiments

Our overall objective was to test for differences among native, introduced and invasive ranges in the influence of soil biotic and abiotic effects on the biomass of *P. contorta* seedlings, and whether these factors had different effects between the two seedling provenances. Two provenances of *P. contorta* ssp. *murrayana* seeds were obtained from the US Forest Service, Bend, Oregon: a southern provenance (originating near Klamath Falls, Oregon, hereafter referred to as provenance A); and a northern provenance (originating near Sisters, Oregon, hereafter referred to as provenance B; Fig. 1). The provenances were selected because they spanned the northern to southern extents of the native range soil sampling area (Fig. 1), and were sufficiently far apart (> 100 km) to potentially allow some degree of local adaptation difference between them, which is known to occur over relatively large spatial scales for *P. contorta* (Parchman *et al.*, 2011). Both provenances were grown for all experimental units, using the 15 native range plots, 15 introduced range plantation plots, and 15 invading front plots as replicates. Soils derived from these plots were used to conduct two growth chamber



**Fig. 1** Map of field sites used for soil collection. Panel 1 (top left): five sites in New Zealand, South Island (red circles). Panel 2 (middle top): 15 native range sites (blue circles) in Oregon, USA, and the location of the *Pinus contorta* ssp. *murrayana* seed sources (southern provenance originating near Klamath Falls, Oregon (provenance A) and northern provenance originating near Sisters, Oregon (provenance B); yellow circles). Soil was collected beneath *P. contorta* ssp. *murrayana* in similar latitude, climate and soils to regions in the southern hemisphere where *P. contorta* has been introduced and become invasive (Gundale *et al.*, 2014b). Panel 3 (top right): five sites in Argentina, near San Carlos de Bariloche and two of the Chilean sites near Malalcahuello. Panel 4 (bottom right): three of the Chilean sites near Coyhaique. Panels 1, 3 and 4 represent the introduced range sites, where each site (red circles) contains a matched plantation and invading front plot. Circles may overlap for sites that are close to each other.

experiments simultaneously. Expt 1 focused on comparing live soil (i.e. not sterilized) from three plot types (hereafter referred to as 'origins'); native, introduced plantation, and invasion front. This experiment tested all three of our hypotheses where we expected differences in the growth of seedlings in soil from introduced plantation vs native plots; introduced plantation vs invasive front plots; and between provenances. This experiment had a total of 90 pots (three soil origins  $\times$  15 replicates  $\times$  two provenances).

For Expt 2, where we had the goal to disentangle the effects of soil biota from abiotic soil properties, we sterilized a subsample of the same soils utilized in the first experiment, using gamma-irradiation (*c.* 40 kGy; Synergy Health Ede BV, Etten-Leur, the Netherlands), and applied soil community inoculation treatments (see later for details) in a full factorial design, as done in previous studies (Van Grunsven *et al.*, 2007; Gundale *et al.*, 2014a, 2019). The experiment followed experimental design type D described by Gundale *et al.* (2017, 2019), where both the

inoculum added to soil and soils receiving inoculum were kept separated (vs homogenized) so that sample sites corresponded to experimental units within the experiment. This experimental design was chosen because unlike our previous study comparing *P. contorta* in Canadian and Swedish soils (Gundale *et al.*, 2014a), in the current study we did not know the exact native range origin location for each introduced plantation, which required us to randomly sample each region of focus (Gundale *et al.*, 2019); and further, in the current study we had the explicit goal of investigating how environmental context influenced PSFs. For the inoculation treatment, each of the 15 native range sites were randomly matched to a site from the introduced range (with each site consisting of both an introduced plantation plot and an invasive front plot) for comparison. Matched sites for each origin (native, introduced plantation and invasive front) were subsequently arranged into blocks with all possible combinations of sterile soil origin (representing the abiotic component of the soil; hereafter 'abiotic soil origin') and inoculum origin treatments.



**Fig. 2** Illustrative diagram of soil collection within each introduced range site in paired plantation (top left) and invasive front plots (bottom right). The invading front follows the prevailing wind direction at a site as *Pinus contorta* seeds are wind-dispersed. Blue circles represent the relative positions of soil collection within the plantation plot and under invasive front trees furthest from their source plantation. Within each plot a 3-l sieved sample of bulk soil was obtained by combining multiple soil cores within a plot (10 cm depth including both humus and mineral soil but excluding fresh litter) from beneath 10 individual trees within a 100 m<sup>2</sup> area.

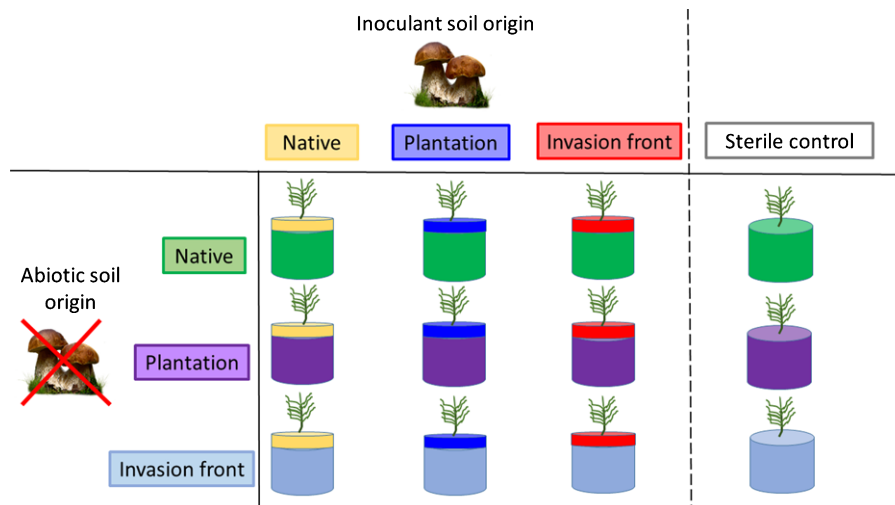
Thus, each block was treated as a statistically independent unit and contained 12 soil mixtures: three irradiated abiotic soil origins (native, introduced plantation and invasive front)  $\times$  three inoculant origins (native, introduced plantation and invasive front) and three fully sterile control soils for each abiotic soil origin (Fig. 3). This resulted in a total of 360 pots, consisting of 12 soil treatment combinations  $\times$  to provenances  $\times$  15 replicates (i.e. blocks).

Before adding our soil mixture to the pots for these two experiments and to avoid cross-contamination during setup and watering, we first placed a filter paper at the bottom of each pot. Then in each pot we placed a clean plastic sheet that sat flush with the perimeter and extended *c.* 10 cm above the pot as an additional barrier between pots (Supporting Information Fig. S1). For Expt 1, sterilized sand was mixed with equal volumes of soil (totaling 300 ml) to lower soil bulk density and ensure adequate drainage. This mixture was then transferred to pots. For Expt 2, we used this same sand mixing approach; however, the soil component comprised a 9 : 1 mixture of gamma-irradiated soil with either live soil to reinoculate with soil biota from each site, or an equivalent volume of sterile soil for controls (Gundale *et al.*, 2014a). The inoculum soil was completely mixed with the sand–soil mixture. Finally, as additional measures to avoid cross-contamination during watering, we added 100 ml of sand to the top of each pot (corresponding to *c.* 1 cm), suspended the pots (arranged in trays of 15 pots) over boxes, and periodically discarded runoff water (Fig. S1).

Before planting, seeds were stored in a freezer for 1 month, and were then brought up to room temperature, and surface-sterilized by soaking and periodically shaking them in 30% hydrogen peroxide with a few drops of Tween 20 for 20 min (Ángeles-Argáiz *et al.*, 2016). Seeds were then rinsed several times in distilled water and germinated in sterile sand. Germinated seedlings were then transplanted into the experimental pots. Two seedlings were planted per pot, and after 2 wk the less vigorous of the two seedlings was removed. Seedlings were watered daily and grown for 12 wk in a growth chamber under 18 h : 6 h, light : dark conditions, and a light intensity of *c.* 440  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , as previously done by Gundale *et al.* (2014a). The day and night-time temperatures were set at 21 and 15°C, respectively, which are normal temperatures encountered during the growing season in the focal regions. Throughout the experiment, we randomly repositioned each box of pots within the growth chamber to evenly distribute spatial variation of conditions in the growth chamber among the blocks. At harvest, the shoot (stem and needle) was separated from the roots of each plant, so that roots could be washed with milliQ water to remove soil. Shoots and roots were placed in separate paper bags, freeze-dried and weighed to the nearest microgram.

### Statistical analyses

For all statistical analyses total biomass was used as the response variable. For Expt 1, which consisted of plants growing in



**Fig. 3** Diagram representing the full factorial design of Expt 2. Pictures of pots represent the 12 soil treatments included within each experimental block and the color combination represents the different treatments. Soil treatments were either sterile bulk soil from each abiotic origin (native, green; plantation, purple; and invasive front, light blue) mixed with each inoculant origin in a 9 : 1 ratio (native, yellow; plantation, blue; invasive front, red) or left as a sterile control (gray box). We used one sterile control for each abiotic soil origin (native, green; plantation, purple and invasive front, light blue). Plantation and invasive front plots were paired within each introduced range site. This design was replicated 15 times for each field site (in the southern hemisphere introduced range and their matched native range site) to then represent 15 experimental blocks each with 12 soil treatments. Two seed provenances of *Pinus contorta* were grown in each experimental block. Expt 2 had a total of 360 pots (12 soil treatment combinations  $\times$  two provenances  $\times$  15 blocks).

unsterilized live soil, we split the data into two separate analyses that compared seedling biomass in either native vs introduced plantation soils or introduced plantation vs invasive front soils. This allowed us to explicitly test Hypotheses 1 and 2 separately – the differences in plant responses in soils between native and introduced plantation sites (Hypothesis 1) and between plantation and invading plots within the introduced sites (Hypothesis 2). Additionally, plantation and invading plot types were paired geographically (which was accounted for in the analysis), whereas the comparison of native and introduced range plots was not analyzed as a paired analysis. For the first analysis, we used linear mixed-effects models (LMMs; ‘lmer’ function in R (R Core Team, 2019) package LME4; Bates *et al.*, 2015) to test for effects of provenance and soil origin (fixed factors) on the biomass of seedlings. For the second analysis (Hypothesis 2), we also added country as a fixed factor to test for variation between the southern hemisphere countries (NZ, Argentina and Chile) and tested for all possible interactions among provenance, country and soil origin. For both analyses, site was specified as a random factor so that inclusion of provenance as a factor in the analysis did not artificially elevate the degree of replication for the region comparison, which should be constrained by the number of sites we sampled. For the second analysis, when comparing plantation and invasion plot types, the random factor was soil origin nested within site to specify the pairing of sites. When needed, Box-Cox power transformations (R package ENVSTATS; Millard, 2013) were used to meet model assumptions of heteroscedasticity and normality.

For Expt 2, where soils were sterilized and then inoculated, we lost three data points because of seedling death during the experiment. For the remaining data, we used LMMs to test for the

effects of provenance, abiotic soil origin, inoculant soil origin, and introduced country (including any interactions) as fixed factors on the biomass of seedlings. As soils were randomly paired from USA sites and southern hemisphere sites within each experimental block, we combined country and experimental block variables, resulting in a variable with three factor levels representing each southern hemisphere country (NZ, Chile and Argentina) and their randomly paired USA sites (hereafter, ‘country-block’). Experimental block was treated as a random factor. Biomass was log-transformed to meet model assumptions. Complex models using all possible interactions were examined first, and then models were selected for output by examining their Akaike information criterion (AIC) and Bayesian information criterion (BIC) values (the model with the lowest AIC and BIC was deemed the best model). Models were further compared using a  $\chi^2$  test (Table S2, see later). The final most parsimonious model included provenance, inoculant soil origin, abiotic soil origin, country-block and the two-way interactions between the latter three terms. Pairwise comparisons for main factors (inoculant soil origin, abiotic soil origin and country-block) were made using the output from a simple model without any interactions using the ‘glht’ function in the MULTCOMP package (Hothorn *et al.*, 2008) in R (with Tukey corrections). Pairwise comparisons for models with interacting factors were made using the PREDICT-MEANS package (Luo *et al.*, 2018) in R, using Tukey corrections.

We tested whether soil chemistry data differed between soils from different origins, analyzed separately for native vs introduced plantations, and for plantation vs invasive front plots. We split the analysis of soil chemical data into two separate analyses in the same manner as we did for Expt 1, to enable site-level pairing for comparisons of introduced plantation and invasive front.

We analyzed percentage and ratio data (%N, %C and %P and C:N, C:P and N:P ratios) using generalized linear models ('glm' function in the *STATS* package) and used gamma-distributed log link. We used linear models for  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and pH data, and data were log-transformed, square-root-transformed or Box-Cox-transformed ('boxcox' function in package *ENVSTATS*) to conform to model assumptions of normality and heteroscedasticity. We compared soil chemical data between invasion and plantation plots using LMMs with origin and country as fixed effects and site as a random effect, and for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  data we included sterilization as a fixed factor. Finally, to explore underlying relationships that may have contributed to context dependency in the introduced range, we explored the regression relationships between seedling biomass growth against soil %N, %P and %C content, and the C:N ratio, for sterile control and each inoculation treatment.

## Results

### Expt 1

Total biomass was similar between seedlings grown in native soil and introduced plantation soil and between plantation and invasive front soils (Fig. S2; Table S1). Seedling growth did not differ significantly among countries (NZ, Chile and Argentina) or between seed provenances, nor were there interactive effects between any pair of factors (Table S1).

The average biomass of seedlings more than doubled in sterile control soils in Expt 2 compared with live soils of Expt 1, and inoculation of sterile soil in Expt 2 further increased average seedling biomass by 68.5% (Fig. S3).

### Expt 2

Seedling biomass responded significantly to provenance, abiotic soil origin, inoculant soil origin, and country block, and there were two-way interactions between all possible pairs of the latter three factors (Table 1; Table S2; Fig. 4). Three-way interactions among these variables were not significant. In general, for all soil origins seedlings had a neutral to positive response to the inoculation treatment compared with completely sterile controls (Fig. 4). For the main inoculum origin effect, seedlings grew larger when inoculated with soil communities from native and plantation soils (mean  $\pm$  SE,  $27.7 \pm 2.77$  and  $24.7 \pm 2.17$  mg, respectively) than in invasive front soils ( $13.7 \pm 0.79$  mg) or completely sterile control soils ( $13.1 \pm 0.88$  mg; Fig. 4a; Table 1). For the main abiotic soil origin effect, seedlings on average had a higher biomass in soils from introduced plantation and invasive front soils ( $24. \pm 1.72$  and  $23.1 \pm 2.151$  mg, respectively) than from native range soils ( $12.2 \pm 0.691$  mg) or completely sterile controls (Fig. 4b; Table 1). In general, seedlings grown in soil from the NZ soil country-block (which includes the native soil paired with each site) had the largest seedlings, whereas seedlings in the Chilean soil were intermediate, and seedlings in the Argentinian soil country-blocks the smallest ( $27.8 \pm 2.53$ ,  $16.6 \pm 0.98$  and  $14.9 \pm 0.72$  mg, respectively; Fig. 4c; Table 1). Seedlings from

**Table 1** Summary of ANOVA output from a mixed effects model for Expt 2 showing the effects of abiotic soil origin, inoculant soil origin, seed provenance and country-block on the total biomass of *Pinus contorta* seedlings (experimental block was treated as a random effect).

Variable <sup>a</sup>	df	F	P
Abiotic soil origin	2, 320	54.98	< 0.0001
Inoculant soil origin	3, 320	36.56	< 0.0001
Provenance	1, 320	42.74	< 0.0001
Country-block <sup>b</sup>	2, 12	4.23	<b>0.0408</b>
Inoculant soil origin $\times$ abiotic soil origin	6, 320	3.92	<b>0.0008</b>
Country-block <sup>b</sup> $\times$ inoculant soil origin	6, 320	10.23	< 0.0001
Country-block <sup>b</sup> $\times$ abiotic soil origin	4, 320	12.80	< 0.0001

P-values in bold indicate significant differences at  $P < 0.05$ .

<sup>a</sup> Model:  $\log(\text{biomass}) \sim \text{provenance} + \text{abiotic soil origin} \times \text{inoculant soil origin} + \text{country-block} \times \text{inoculant soil origin} + \text{country-block} \times \text{abiotic soil origin}$  (random: block). Marginal pseudo- $R^2 = 0.514$  and conditional pseudo- $R^2 = 0.590$ .

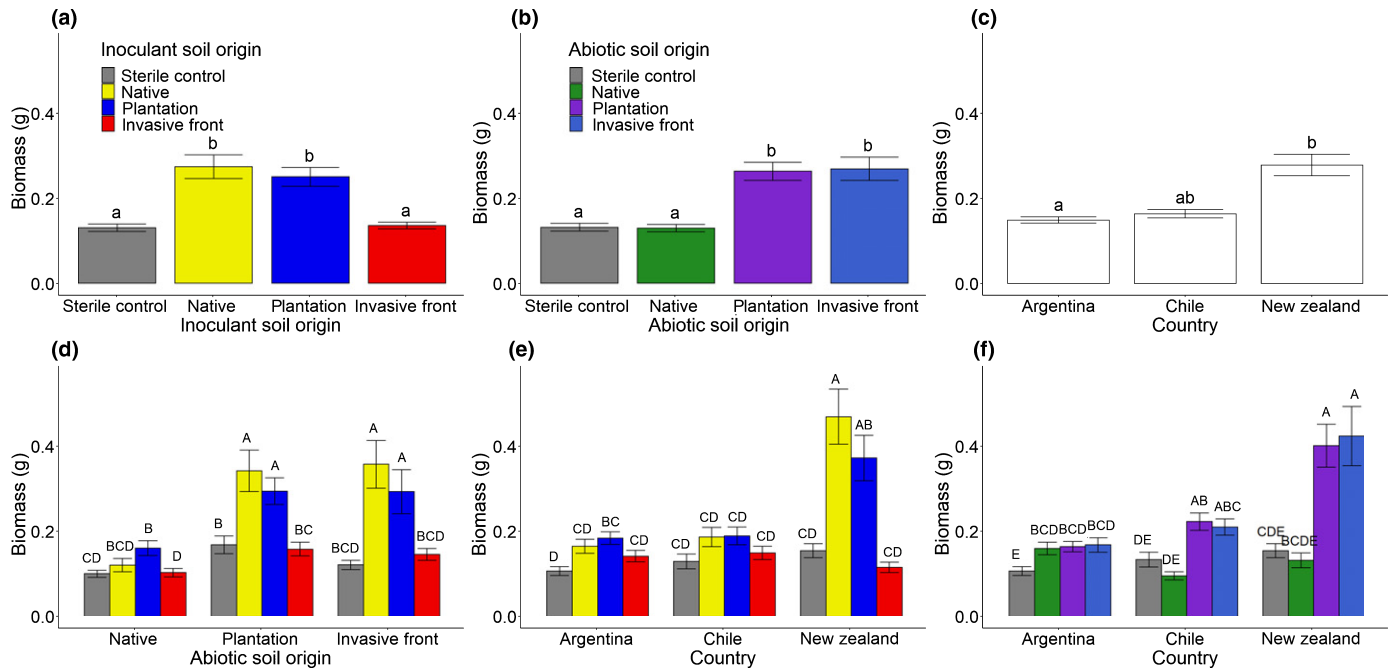
<sup>b</sup> The country-block factor is the combination of country with experimental block variables, resulting in three factor levels representing each southern hemisphere country (NZ, Chile and Argentina) and their randomly paired USA sites.

the northern provenance (provenance B) were, on average, 52.7% larger than those of the southern provenance (provenance A;  $F_{1,320,07} = 42.7411$ ,  $P < 0.001$ ; Table 1). However, provenance never showed interactive effects with the other factors (Table S2).

Seedlings grew larger in abiotic soil from the introduced range (plantation and invasive front) than in abiotic soil from the native range, and the size difference depended on the inoculant origin (Fig. 4d). Specifically, seedlings in abiotic soil from the introduced range (plantation and invasive front) mixed with inoculant from native and plantation soil had the highest growth (between 1.8-fold and 3.5-fold higher biomass than other seedlings in inoculated soils; Fig. 4d). By contrast, when only considering the soil inoculant origin effect, seedlings were never significantly larger in inoculant from introduced ranges compared with inoculant from native ranges (Fig. 4a,e). Comparing soils within the introduced range, we found that seedlings in soil with inoculant from the invasive front were significantly smaller than in soils with plantation inoculant only for NZ soils (3.2-fold higher biomass in NZ plantation soils compared with NZ invasive front soils; Fig. 4e). Additionally, the seedling growth difference between abiotic soil from introduced ranges (plantation and invasion) and native range soil was only apparent for soil from NZ and Chile (3.1-fold and 2.3-fold higher biomass in abiotic introduced range soil compared with abiotic native range soil for NZ and Chilean soil, respectively; Fig. 4f).

### Soil chemistry

Analysis of sterile soils revealed that native soils had a significantly higher C:N and lower N:P ratios and %N than all introduced range soils (Fig. 5a,b,d; Table S3). Plantation soils were significantly higher in %P and had a higher C:N ratio than soils from the invasive front (Fig. 5a,e; Table S4). On average, Argentinian soils had lower content of several nutrients (i.e. %P, %N and



**Fig. 4** Total biomass (mean  $\pm$  SE) of *Pinus contorta* seedlings grown in soil treatments for 3 months in Expt 2 (see Fig. 3). Soil treatments were either bulk sterile soil (abiotic origin) combined with live soil as inoculum in a 9 : 1 ratio (inoculant soil origin; colors) or sterile controls (gray). Panels show biomass responses across different treatments: (a) inoculant soil origin; (b) abiotic soil origin; (c) country-block (combination of country and experimental block variables, resulting in three factor levels representing each southern hemisphere country (Argentina, Chile, and New Zealand) and their randomly paired USA sites); (d) abiotic soil origin for each mixture of inoculant soil origin; (e) inoculant soil origin for each country-block; (f) abiotic soil origin for each country-block. Panels (a)–(c) show the significant main effects and different lower-case letters represent pairwise differences at  $P < 0.05$  with Tukey corrections from a mixed effects model without interactions. Panels (d)–(f) show the same data from a mixed effects model with interactions and significant pairwise differences represented with upper-case letters (see also Table 1). Bar colors in (a), (d) and (e) represent inoculant soil component of treatments (gray, no inoculant/sterile control soil; yellow, inoculant from native soil; blue, inoculant from introduced plantation soil; red, inoculant from invasive front soils). Bar colors in (b) and (f) represent the abiotic component of soil treatments (gray, no inoculant/sterile control soil; green, bulk soil from native sites; purple, bulk soil from introduced plantation stands; light blue, bulk soil from invasive front stands). For (e) and (f), native bars within country represent the USA soils paired with the southern hemisphere country (Argentina, Chile and NZ). Depicted means are averaged across two seed provenances.

$\text{NH}_4^+$   $\text{mg l}^{-1}$ ) and %C than did Chilean and NZ soils, and NZ soils had a significantly lower pH (Fig. 5h). There was considerable variation in nitrate concentrations between origins from the introduced range soil (plantation and invasive front); however, in general nitrate concentrations in NZ invasive front soils were lower than in Argentinian or Chilean invasive front soils (Fig. 5g). The soil C : P ratio did not differ across countries or plots of different origin (Tables S3, S4). Ammonium concentrations in sterile soil ( $8.6 \text{ mg l}^{-1} \pm 0.779$ ) were significantly higher than in live soil ( $3.39 \text{ mg l}^{-1} \pm 0.328$ ): an increase of 232–303% depending on country;  $P < 0.001$ , Tables S5, S6). Nitrate concentrations in sterile soil ( $0.286 \pm 0.0785 \text{ mg l}^{-1}$ ) were, on average, 53.2% lower than in live soil ( $0.537 \pm 0.1389 \text{ mg l}^{-1}$ ), but this difference was only marginally significant (Tables S5, S6). However, we found no significant interactions among sterilization, origin (native, plantation or invasive front) and southern hemisphere country on ammonium and nitrate concentrations (Tables S5, S6).

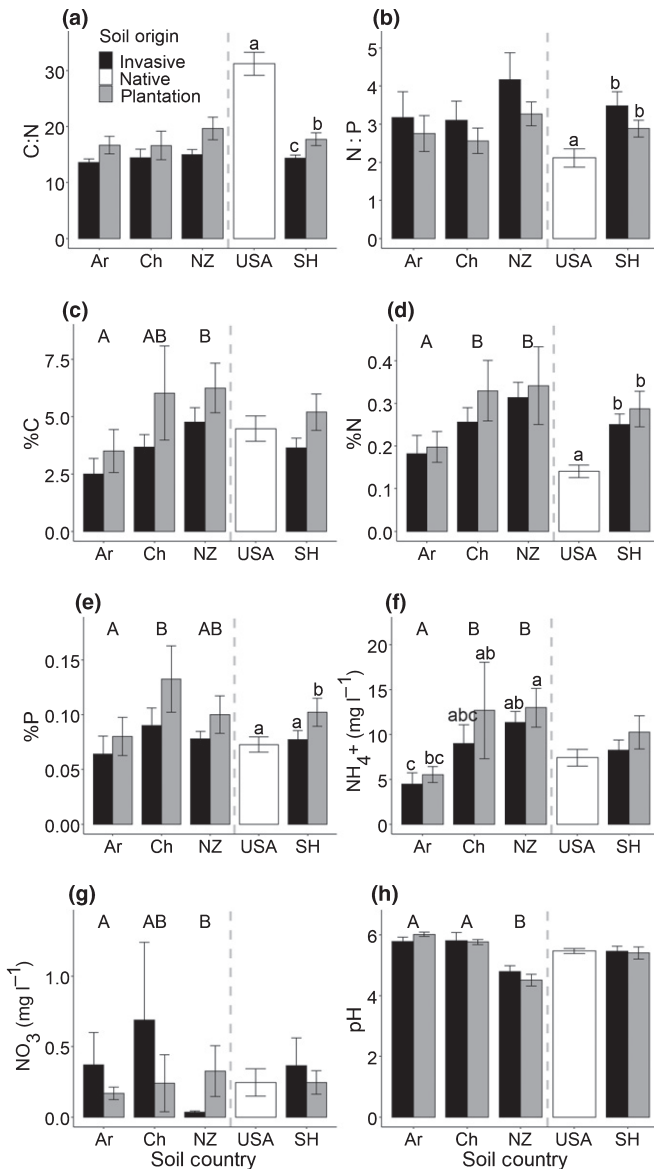
For the regressions of seedling growth response against soil element concentrations in introduced ranges, we found positive relationships with soil %N, %P and %C in sterile control and invasion inoculum-treated soil, and positive relationships with %

N and %C for native inoculum-treated soil (Fig. 6). No significant relationships between seedling growth and %P were found for native inoculum-treated soil, and no significant relationships were found for plantation inoculum-treated soil (Fig. 6). Further, no significant relationships between seedling growth and C : N ratio were found for any of the soil treatment types (data not shown).

## Discussion

Our study utilized an unprecedented home vs away study system to investigate the ecology of an invasive species, consisting of 15 native range sampling sites (in the USA) and 15 introduced range sites spanning three countries (New Zealand, Chile, and Argentina). The study system allowed us to investigate whether PSFs differed across native sites, introduced range plantations and invading fronts and to disentangle soil abiotic and biotic factors. Our study showed that the strength of PSFs were context-dependent; variation in abiotic soil components was a significant factor in determining whether soil biota explained growth patterns across site types. Here, we discuss each of our hypotheses.





**Fig. 5** The chemical properties of sterilized soil. Bars represent mean  $\pm$  SE for: (a) carbon : nitrogen ratio, (b) nitrogen : phosphorus ratio, (c) percentage carbon (%C), (d) percentage nitrogen (%N), (e) percentage phosphorus (%P), (f) extractable ammonium ( $\text{NH}_4^+$ ,  $\text{mg l}^{-1}$ ), (g) extractable nitrate ( $\text{NO}_3^-$ ,  $\text{mg l}^{-1}$ ), and (h) pH. Bars to the left of the dashed line represent invasive front (black) and introduced plantation (gray) soil origins for each country in the introduced range of *Pinus contorta* (Ar, Argentina; Ch, Chile; NZ, New Zealand). Lower-case letters represent significant pairwise differences (at  $P < 0.05$  with Tukey corrections; Supporting Information Table S3) between bars, and upper-case letters indicate significant differences between countries. Bars on the right of the dashed line represent native USA (white), invasive front (black) and introduced plantation (gray) stands averaged across all southern hemisphere countries (SH). Different letters represent significant pairwise differences at  $P < 0.05$  with Tukey corrections (Table S2).

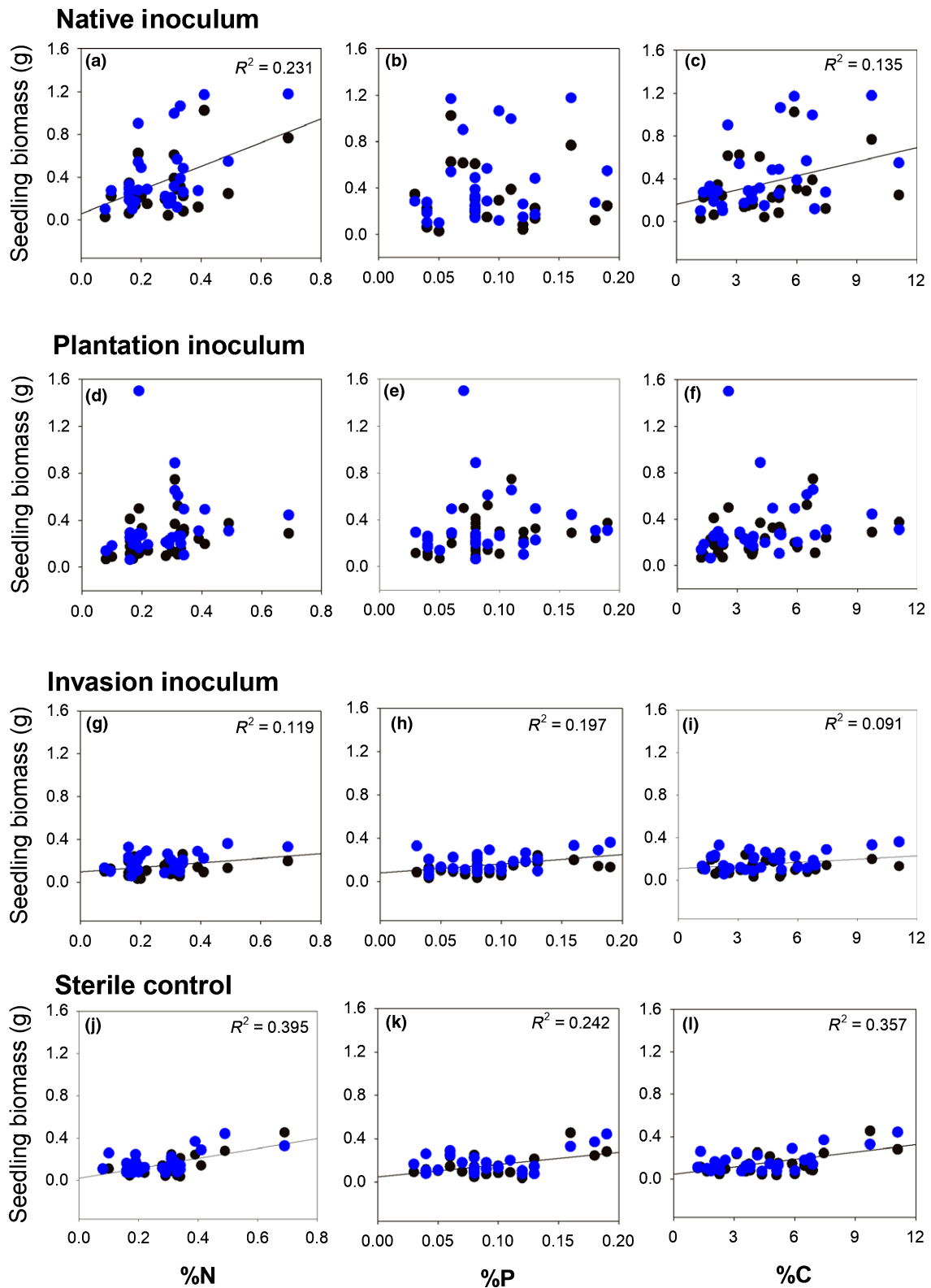
We hypothesized that seedlings would achieve higher growth in response to soil biota collected from introduced plantations compared with either the native range (Hypothesis 1) or invasive front soils (Hypothesis 2). Using live unsterilized soil in Expt 1, we found that seedling growth responses were similar irrespective

of soil origin, which did not support these hypotheses. By contrast, previous studies have found greater positive plant growth responses to soils from introduced than to those from native ranges (Hawkes, 2007; Callaway *et al.*, 2011; Gundale *et al.*, 2014a; Maron *et al.*, 2014; Dostálek *et al.*, 2016; Crawford & Knight, 2017; McGinn *et al.*, 2018), and that seedlings growing farther from plantations have lower biomass and survival than in plantations (Nuñez *et al.*, 2009). However, the biotic and abiotic drivers of these patterns are rarely disentangled, which was the objective of our second experiment.

In contrast to Expt 1, Expt 2 showed that seedling growth was significantly affected by both abiotic soil origin and inoculant soil origin, and these effects differed between southern hemisphere countries. Moreover, seedlings tended to respond in opposite directions to the abiotic and biotic components of the soil, depending on the origin of the soil. Specifically, the biotic effect from native range soils supported the highest seedling growth (Fig. 4a), whereas its abiotic properties supported low seedling growth (Fig. 4b). Thus, the different results of the native vs introduced range comparison in Expts 1 and 2 could be partially explained by soil abiotic and biotic components having opposing effects on seedling growth across native and introduced ranges that may have cancelled each other out in Expt 1, when these two factors were not isolated.

Experiment 2 further showed that, on average, seedlings responded neutrally or positively to inoculum treatments relative to sterile controls (Figs 4, S4), and this included inoculum from the native range. Additionally, sterilizing native soil did not promote seedling growth (Fig. 4d). This does not support our first hypothesis and the predictions of the ERH or EMH that biota from the native range would have more negative effects on plants compared with introduced ranges (Richardson *et al.*, 2000; Shea & Chesson, 2002; Reinhart & Callaway, 2004, 2006). Nevertheless, sterile soil from plantation plots promoted, on average, lower growth than inoculated plantation soils (Fig. 4d), indicating that mutualists are adequately present in introduced plantation soils (Dickie *et al.*, 2010). The finding that soil community interactions with seedlings ranged from positive to neutral fits with several ectomycorrhizal–tree interactions that have been described, including the greater benefit of ectomycorrhizal common networks compared with arbuscular networks (van der Heijden & Horton 2009), and enhanced growth of ectomycorrhizal seedlings grown in soils collected from under the same tree species.

Experiment 2 also provided evidence that the local-scale effects of plant–soil interactions were context-dependent. Seedlings in introduced plantation soils grew two- to three-fold larger than in native soil (Fig. 4b,d,f), especially where the soil N content was higher (NZ and Chile; Fig. 5d). This finding suggests that improved abiotic conditions, particularly N content in the introduced ranges, could explain the enhanced growth of *P. contorta* that has been shown in certain southern hemisphere locations compared with its native range (Taylor *et al.*, 2016). This illustrates the context dependency of the plant–soil interactions; only abiotic components of some plantation soils resulted in higher seedling growth and only in combination with soil biota from



**Fig. 6** Regression relationships between *Pinus contorta* seedling biomass against soil nitrogen (%N), phosphorus (%P), and carbon (%C) concentrations of sterile control soils (j–l) and soils inoculated with three different inoculant types: native inoculum originating from the native range (USA, a–c), or plantation (d–f) or invasion inoculum (g–i), originating from three southern hemisphere countries, New Zealand, Chile, and Argentina. Different color dots (blue and black) represent the two different provenances. Regression lines and  $R^2$  values are reported only for significant relationships ( $P < 0.05$ ).

plantation soil and native soil (presumably because key mutualists were present). This nuanced explanation for plant response might not be captured in previous studies that only attribute differences in growth between native and introduced ranges to soil biota (Callaway *et al.*, 2004, 2013; Felker-Quinn *et al.*, 2011; Gundale *et al.*, 2014a; Crawford & Knight, 2017). Studies of PSFs over wider environmental contexts often demonstrate that both soil biotic and abiotic effects influence invasive plant growth but vary predictably along soil nutritional gradients driven by succession or ecosystem development (De Deyn *et al.*, 2004; Castle *et al.*, 2016; Png *et al.*, 2019). Taken together, these findings demonstrate that biotic and abiotic soil effects are both important components that can influence invasion success of plants.

Interestingly, enhanced seedling growth was also observed in abiotic components of invasive front soils, but only in the presence of inoculum from native or plantation soils (Fig. 4d). One explanation for this pattern is that inoculant from native and plantation soils contains higher diversity and higher propagule density of ectomycorrhizal fungi species than soil from invasive fronts, as has been previously shown by Hayward *et al.* (2015a) and Gundale *et al.* (2016). Our prediction that seedlings would be larger in soils from plantations than from the invading front (Hypothesis 2) only occurred in response to soil biota (inoculant; Fig. 4a) and in NZ soil (Fig. 4e). This supports the suggestion that pine invasion can be limited by ectomycorrhizal partner availability for invasions distant from source plantations (Thiet & Boerner, 2007; Nuñez *et al.*, 2009; Urcelay *et al.*, 2017), thus supporting the MMH (Mitchell *et al.*, 2006); however, this appears to occur only in certain contexts. Differences in fungal communities and plant response between introduced ranges may account for some of this context dependency. Gundale *et al.*, (2016) previously found that root fungal endophytic communities associated with *P. contorta* also differ between the native and introduced ranges, and between NZ and Chilean introduced ranges (Fig. S4; Table S7). These different fungal communities and the higher N content in soils from NZ may interact to allow the high growth and recruitment of the next generation of *P. contorta*. Additionally, Suilloid fungi (*Suillus* spp.) have been reported as the sole fungal partner found in *P. contorta* far from the original plantations in Chile (Hayward *et al.*, 2015b) and has been suggested as a facilitator of pine invasion globally (Policelli *et al.*, 2019). Although this group of fungi disperse extremely well (Ashkannejhad & Horton, 2006), they may not serve as ideal partners as the more diverse community of fungi found in plantations or in the native range (Hayward *et al.*, 2015b; Gundale *et al.*, 2016). Thus differences in microbial community composition between the native and introduced ranges, between the introduced range plantations and invasion fronts, and between introduced range countries may contribute to the context dependency we observed.

Finally, in addition to the direct influence of soil abiotic and biotic factors on seedling growth, interactions between biota and abiotic components of the soil may interact to contribute to some of the context dependency we observed among soil treatments and introduced range countries. Specifically, we found seedlings in the introduced range often grew more when soil %N, %P, and

%C was higher (Fig. 6), which occurs when the soil organic matter content is higher. These abiotic properties varied across introduced range countries, and may partially explain why seedling growth was highest in New Zealand and lowest in Argentina. Interestingly, seedling growth in the introduced range soil was most strongly correlated with these soil element concentrations in the sterile control soils, where positive relationships with soil %N, %P and %C were found. Inoculum treatments sometimes dampened or interrupted these positive relationships, especially the inoculum types that promoted growth the most (e.g. plantation and native inoculum). This interpretation is consistent with our expectation that microbial inoculum may help to overcome stoichiometric constraints in the introduced range, and is also consistent with some previous work showing that soil mutualists can provide their greatest benefit when soil nutrients are low (Johnson, 2010; Remke *et al.*, 2020). However, it also highlights the fact that not all microbial community types appear to be equally effective in altering the plant growth relationships with soil stoichiometry (e.g. invasive inoculum; Fig. 6).

Our study may help to explain some of the context dependency and region-specific variation in invasion success observed for *P. contorta*. In a field study, Taylor *et al.* (2016) found that *P. contorta* grows the fastest and produces the most cones at a young age in NZ compared with other introduced regions (Chile and Argentina) or the native range (USA). In that study, variation in *P. contorta* invasion density between sites and countries was partially explained by distance from seed source, local vegetation, fire history, elevation, climate, plantation age and a demographic shift to faster growth and greater production in the introduced range. Our results suggest that variability in soil fertility and interactions of seedlings with soil biota also play a role in differences in *P. contorta* establishment and growth between native and introduced ranges and between sites within introduced ranges. Seedlings in NZ soil grew the largest in our experiment (Fig. 4c), and there was an overall benefit of the abiotic soil conditions of NZ soil compared with native and other introduced range countries.

Our third hypothesis predicted that the different plant provenances would interact with the other treatment factors (e.g. soil inoculum or sterile soil treatments) to impact plant growth, given that genetic variation in plant populations has been proposed as an important factor to explain plant biotic interactions and invasion success (Felker-Quinn *et al.*, 2011; Münzbergová & Šurinová, 2015; Van Nuland *et al.*, 2016; Keller & Lau, 2018). Although the two provenances differed modestly in seedling growth, we did not find any significant interactive effects between provenance and other experimental factors across our experiments. This finding is contrary to the literature showing that different plant genotypes can interact with soil biota differently (Smith & Goodman, 1999; Mazzola & Gu, 2002; Schweitzer *et al.*, 2008; Lu-Irving *et al.*, 2019), which may have occurred because *P. contorta* is known to exhibit spatial genetic structuring only over very broad spatial scales (e.g. > hundreds of km; Parchman *et al.*, 2011). At very broad spatial scales, *P. contorta* is a genetically diverse species, with potentially three different subspecies introduced widely to the nonnative ranges (Gundale

*et al.*, 2014b), and has well documented intraspecific variation in genotypic and phenotypic traits responding to environmental gradients and biotic communities (Hunt *et al.*, 1987; Ying & Hunt, 1987; Xie & Ying, 1995; Fries *et al.*, 2000; Chuine *et al.*, 2001; Eckert *et al.*, 2012; Feduck *et al.*, 2015). It is therefore possible that provenances or subspecies originating from much further apart than we considered would have shown significant interactions with soil inoculum or sterile soil treatments as a result of greater divergence in their local adaptation to these types of factors. It is further possible that these interactions might play out at some individual sites and not others, instead of at the across-site and -region scale that we evaluated; however, we note that our experimental design did not allow us to assess these types of interactions within individual sites.

## Conclusions

Our study provides the most comprehensive evaluation to date of the context dependency of PSFs for an invasive tree species both across and within a broad array of native, introduced and invasive sites. Our study design allowed us to disentangle the individual and interactive effects of soil biotic and abiotic factors, and to decipher how those interactions play out across biogeographic space. We found that seedlings generally responded positively to soil biota inoculation, including inoculum originating from native range sites. This finding does not support the ERH or the EMH, which has been shown for *P. contorta* introduced to environments where other *Pinus* species are present (Gundale *et al.*, 2014a), as well as some other plant taxa (e.g. Callaway *et al.*, 2011; Yang *et al.*, 2013; Maron *et al.*, 2014; McGinn *et al.*, 2018). Our results instead demonstrate beneficial effects of native range soil biota, which are also often present in nonnative plantation soils (Nuñez & Dickie, 2014). We further found that biota from invasive fronts were the least beneficial to seedling growth, which is consistent with the MMH, and suggests that lack of mutualists may constrain invasions. This finding points to a time-lag of coinvasion of soil mutualists at the invading front, and suggests that other factors may be the primary drivers of invasion success in these southern hemisphere environments (e.g. lack of competitors and herbivores and high propagule pressure; Nuñez *et al.*, 2017). These results also help to explain growth differences among introduced ranges of invasive Pinaceae that have been reported throughout the southern hemisphere, in particular the high growth rates in the relatively more fertile soils of NZ (Taylor *et al.*, 2016). Overall, our study highlights the importance of considering both the soil abiotic and biotic components when studying PSFs (Smith-Ramesh & Reynolds, 2017; Bennett & Klironomos, 2019; De Long *et al.*, 2019), particularly for plant invasions, and the high degree of context dependency in the factors that influence plant performance across broad biogeographical scales.

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





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## Author contributions

SJN conceptualized hypotheses, designed the experiment, carried out the experiment, analyzed data and wrote the manuscript; AF, MAN, AP, DAP and JM were involved in discussion and input for the research proposal, performed field work, and revised the manuscript; DAW, M-CN and PK were involved in discussion and input for the research proposal, conceptualized the hypotheses, designed the experiment, and revised the manuscript; JES was involved in discussion and input for the research proposal and revised the manuscript; MJG wrote the original research proposal and obtained funding for the project, conceptualized the hypotheses, designed the experiment, performed field work, supervised the experiment and data analysis, and revised the manuscript.

## ORCID

Alex Fajardo  <https://orcid.org/0000-0002-2202-6207>  
 Michael J. Gundale  <https://orcid.org/0000-0003-2447-609X>  
 Paul Kardol  <https://orcid.org/0000-0001-7065-3435>  
 Jaime Moyano  <https://orcid.org/0000-0002-7072-0527>  
 Marie-Charlotte Nilsson  <https://orcid.org/0000-0002-9254-2223>  
 Martin A. Nuñez  <https://orcid.org/0000-0003-0324-5479>  
 Susan J. Nuske  <https://orcid.org/0000-0002-5350-7425>  
 Aníbal Pauchard  <https://orcid.org/0000-0003-1284-3163>  
 Duane A. Peltzer  <https://orcid.org/0000-0001-7724-3738>  
 David A. Wardle  <https://orcid.org/0000-0002-0476-7335>

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## Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

**Fig. S1** Experimental setup showing seedlings growing in pots.

**Fig. S2** Mean total biomass of *Pinus contorta* seedlings in Expt 1.

**Fig. S3** Mean total biomass of *Pinus contorta* seedlings growing in live, sterile control and inoculated soil (Expts 1 and 2).

**Fig. S4** nMDS using Bray–Curtis distance metric of total fungal root endophytic communities observed in *Pinus contorta* from New Zealand and Chile using data collected from Gundale *et al.* (2016).

**Table S1** ANOVA output for Expt 1 showing the effects of soil origin and seed provenance of total *Pinus contorta* seedling biomass.

**Table S2** Mixed effects models fitted to data from Expt 2, their degrees of freedom (df), Akaike information criterion (AIC) and Bayesian information criterion (BIC) values and marginal and conditional pseudo- $R^2$  ( $R^2_m$  and  $R^2_c$ , respectively; Nakagawa & Schielzeth, 2013).

**Table S3** ANOVA output on chemical properties of sterile soil comparing soil origin (native vs plantation).

**Table S4** ANOVA output on chemical properties of sterile soil comparing soil origin (plantation vs invasive front) and country.

**Table S5** ANOVA output on soil ammonium and nitrate concentrations comparing soil origin (plantation vs native) and soil sterilization.

**Table S6** ANOVA output on soil ammonium and nitrate concentrations comparing soil origin (plantation vs invasive front), country and soil sterilization.

**Table S7** PERMANOVA output using Bray–Curtis distance metric on fungal root endophyte communities of *Pinus contorta* from New Zealand and Chile using data collected from Gundale *et al.* (2016).

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