



Plant species establishing beyond their introduced range are not always released from belowground enemies

Giancarlo M. Chiarenza · Angela T. Moles

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Abstract

Background and aims Interactions with soil biota can shape the success of plants, but we still have much to learn about the role of belowground interactions in plant invasions. In this study, we investigated how soil factors affect the growth of an introduced plant species.

Methods In a glasshouse experiment, we grew *Plantago lanceolata* L. seeds from Europe (native range) and Australia (introduced range) in soil samples collected from 21 sites within and beyond the established range of *P. lanceolata* in Australia.

Results *P. lanceolata* seedlings grown in unsterilized soil were 34% smaller than seedlings grown in sterilized soil. The effect of soil biota on plant growth was not correlated with latitude or whether the soil

was from the established range of *P. lanceolata*, just outside its range, or well beyond the species' distribution. Individuals growing in new sites richer in organic carbon than their initial range experienced stronger release from belowground enemies.

Conclusions Our study does not support the idea that plant species experience significant release from belowground enemy pressure when colonizing new regions. Rather, soil organisms have strong net negative impacts on plant growth regardless of soil origin.

Keywords Enemy release · Latitudinal biotic interaction hypothesis · Plant-soil relationships · Plant invasion · Soil pH · Organic carbon · Soil available nitrogen · *Plantago lanceolata*

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Introduction

The establishment of a plant in a new location depends on a range of factors, such as the number of seeds reaching the site and how closely the biotic and abiotic conditions at the new site climate match the conditions in the species' original range (Donaldson et al. 2014; Simberloff 2009; Taylor et al. 2016). Often, biotic interactions determine whether a plant species can successfully establish (Shan and Hou 2024; Taylor et al. 2016). The interactions between plants and the belowground are generally stronger than plant-aboveground interactions (Cahill 1999; Lewis and Tanner 2000; Schnitzer et al. 2005).

Nonetheless, soil microbes are less often considered in invasion ecology, biocontrol, or in plant distribution models than are aboveground herbivores, predators, or pollinators (Callaway et al. 2004; Coudun et al. 2006; Dawson 2015; Dawson et al. 2016). Microbial interactions can act as a facilitator or barrier to plant establishment (Agrawal et al. 2005; van der Putten et al. 2013), but we lack information on how this duality works over large scales. In this study, we employed *Plantago lanceolata* L. (Plantaginaceae) to quantify large-scale patterns in plant-soil interactions. We used *P. lanceolata* because it is globally widespread (Green 2021; WFO 2025), we have information on its populations in Australia (Belbin et al. 2021; Victorian Biodiversity Atlas 2024), and it can be imported in Australia without severe biocontrol measures (DAFF 2025). First, we investigated several factors that are hypothesized to influence the effect of soil microbes on plant growth: latitude, position in the plant's distribution, soil properties, and seed origin. Second, we tested whether the extent of belowground enemy release varies with any of the studied factors.

Our first goal was to quantify the relationship between latitude and the net effect of soil microbes on plant growth. Traditional ecological theory suggests that biotic interactions are strongest and most specialized at the equator (Schemske et al. 2009), but support for this hypothesis is mixed (Anstett et al. 2016; Moles and Ollerton 2016; Moles et al. 2011; Zvereva and Kozlov 2021). Most tests of the biotic interaction hypothesis focus on aboveground interactions, such as herbivory (Baskett and Schemske 2018; Lim et al. 2015; Moles et al. 2010; Zvereva and Kozlov 2021), pollination (Gorostiague et al. 2023; Schleuning et al. 2012), or predation (Roslin et al. 2017; Schemske et al. 2009; Wu et al. 2022). Studies of latitudinal gradients in the effects of soil microbes on plant growth to date have yielded mixed results. Neither of two studies (Ma et al. 2019; Van Grunsven et al. 2009) found a significant latitudinal gradient in the effect of microbial inoculation on plant biomass in temperate Europe, though the null results could be related to the fact that neither study spanned more than 10° of latitude. A study in continental China found a higher diversity of nematodes and pathogens at lower latitudes, and found that native (but not introduced) *Alternanthera* plants had higher growth when grown with soil from higher latitudes, but soil

microbe effects could not be fully isolated from the effects of soil physical and chemical properties as they did not include a control treatment with sterilized soil (Lu et al. 2018). We hypothesize that there will be a stronger effect of soil biota on plant biomass towards the tropics.

Our second goal was to determine whether plants colonizing areas outside their established distribution experience less pressure from soil microbes than do plants colonizing within their established distribution. The core parts of a species' range, where plants have long been established, often host a suite of coevolved organisms (Baltrus 2017; Inderjit and Cahill 2015). However, the diversity and abundance of coevolved organisms is thought to decrease gradually towards the edges of a species' range (Van der Putten et al. 2012). Species expanding into completely new ranges (e.g., during invasions or shifting in response to climate change) are hypothesized to escape from some of their coevolved predators, pathogens, and competitors (Enemy Release Hypothesis; in Keane 2002). For instance, plants at the edge of their range can experience less foliar disease (Katz and Ibáñez 2016) and lower herbivory (Urli et al. 2016) than plants in the range core. However, other studies have found no general difference between biotic interactions in range cores and range edges (von Holle et al. 2020; Wan and Bonser 2016), and some have even found more leaf herbivory at the range edge than at the range core (Katz and Ibáñez 2016). The only study we are aware of on soil biota effects between range edges and range cores examined *Clarkia xantiana* (Onagraceae) at five points along a single transect and did not find a strong directional pattern (Benning and Moeller 2021). Here, we quantified the gradient in belowground enemy release on *P. lanceolata* from locations within its established range with occurrences dating back at least 30 years, to just outside its range, to well beyond its current range along seven transects (Fig. 1).

Next, we asked to what extent soil fertility can alter the effects of soil biota on plant growth. In sites with lower nutrients, microorganisms can exert strong selective pressure on plants (Jones et al. 2005; Lodge et al. 1994; Schimel et al. 1989; Schmidt et al. 1997). On the other hand, when fertility is high, the competition for resources decreases, making nutrient-rich locations more easily invadable because the lower competition allows for the

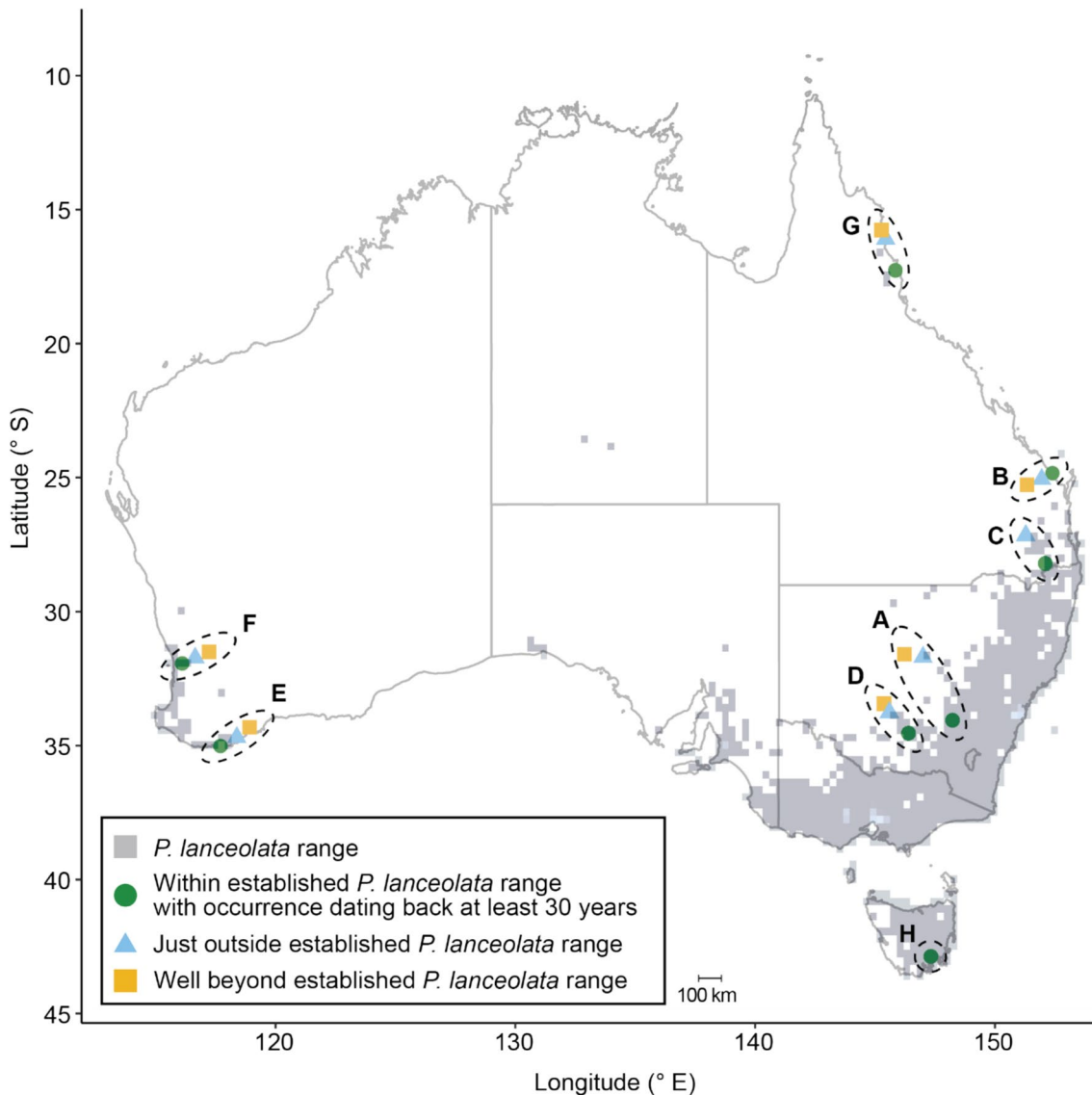


Fig. 1 Sampling points of the study. Each point represents sites where *Plantago lanceolata* was present at least 30 years ago (●), just outside (at least 5 km) the current *P. lanceolata* range (▲), or well beyond (at least 50 km) the

current *P. lanceolata* range (■). Each transect is defined by a letter (A–H). The gray area represents a simplified *P. lanceolata* distribution estimated from the Atlas of Living Australia data (ala.org.au)

establishment of more species (Davis et al. 2001). However, two glasshouse experiments observed that nutrient addition did not have strong influence on the effects of soil microbes on the growth of introduced species (Gundale et al. 2014; Van Grunsven et al. 2007). Here, we complemented these controlled studies by quantifying how natural fertility influences the interaction between soil biota and plant growth. We hypothesized that soil organisms

in more fertile locations will have a weaker effect on plant growth.

Our fourth goal was to test if soil microorganisms from established Australian *P. lanceolata* sites affect the growth of *P. lanceolata* of Australian origin more than European origin. Plants can evolve rapidly in new environments (Brandenburger et al. 2020; Buswell et al. 2010; Sekor and Franks 2018a; b), with local microbes adapting in response to better exploit

their plant hosts (Carpenter and Cappuccino 2005; Flory and Clay 2013; Hawkes 2007). Over time, this reciprocal adaptation can result in tightly coevolved plant–microbe pairs (Bever et al. 2010). For example, sugar maple (*Acer saccharum*, Sapindaceae) has shown decreased performance when establishing in soils without mutualistic mycorrhizae (Carteron et al. 2020). Experiments on *Phragmites australis* (Poaceae) showed that some exotic lineages reintroduced to the native range experienced less foliar pathogen damage than the native lineage (Allen et al. 2020). A study on an old-field site showed that a local and an exotic source of the same plant species might associate differently to the same locally adapted mycorrhizae species (Klironomos 2003). We need more data on how soil biota may affect different lineages of the same species, as it might improve our efforts for restoration (Klironomos 2003) and invasions control (Reinhart et al. 2003).

Our fifth goal was to test if belowground enemy release may be weaker at lower latitudes. This prediction arose from the idea that biotic interactions are more intense toward the equator (Schemske et al. 2009). Thus, moving away from the tropics could provide a stronger release from enemy pressure. Most studies of enemy release across latitudinal gradients are on aboveground herbivory, and their outcomes have been variable. For example, two studies on *Phragmites australis* in North America found that the largest difference in herbivory between native and invasive lineages was at lower latitudes (Allen et al. 2016; Cronin et al. 2015). A meta-analysis bringing together data for almost 1100 species (Xu et al. 2020), and a study of 26 species (ten invasive, 16 native congeners) in Norway (Iverson et al. 2022), showed no significant latitudinal patterns in enemy release. Finally, Allen et al. (2016) found a larger difference in herbivory between native and introduced lineages at high latitudes. Belowground effects in native and invasive *Tragopogon* (Asteraceae) species in temperate Europe did not show a significant latitudinal pattern (Van Grunsven et al. 2009). Other studies of belowground enemy release lacked a control in sterile soil (Lu et al. 2018), and therefore could not disentangle the effects of latitude and pathogens. Most field-based research has been done in temperate locations and span less than 15° of latitude, which might not be sufficient to show significant

latitudinal patterns. Understanding where species experience weak or strong belowground biotic interactions could help ecologists predict where range expansions are most likely to occur and could inform biocontrol strategies.

Our sixth goal was to quantify how belowground enemy release changed with soil fertility. While many studies consider the effect of soil fertility of invaded or potentially invadable sites (Brooks 2003; Gelbard and Belnap 2003; Stohlgren et al. 1999; Wagner et al. 2017), there is little data on how differences in soil fertility may affect belowground enemy release. One study on the invasive fern *Lygodium microphyllum* suggested that higher nutrients at the invaded site (USA), compared to the home range (Australia), might have allowed *L. microphyllum* to reallocate resources towards growth and reproduction (Soti et al. 2020). However, the researchers neither measured plant biomass nor investigated the role of soil microbes in plant growth. Our hypothesis was that belowground enemy release would be stronger when *P. lanceolata* grew in soils outside of its established range with higher fertility than sites from its established range.

Finally, we hypothesized that *P. lanceolata* of European origin would experience less enemy release than *P. lanceolata* of Australian origin. If belowground enemies are not strongly affecting newly arrived populations of their host in the established sites, we would expect the levels of enemy release within and outside the range of *P. lanceolata* in Australia to be similar. Populations of *P. lanceolata* that have been established for longer have likely developed a higher enemy load and thus might experience stronger release from enemies when invading new sites outside of their distribution. Few studies have compared the level of enemy release between populations of the same species. A group of studies on *Phragmites australis* observed invasive populations experienced less aboveground enemy pressure than native populations (Allen et al. 2016; Cronin et al. 2015), but they did not test how enemy release levels changed outside the species' distribution.

We hope that improving understanding of the impact of soil microbes on the growth of introduced plants will help invasion biologists to identify sites at particular risk of plant invasions, and to improve their understanding of the factors underpinning the success of introduced species in new environments.

Materials and methods

Study species

P. lanceolata is native to temperate Eurasia and naturalized in every continent except Antarctica (Green 2021; WFO 2025). In Australia, records of *P. lanceolata* start from the late eighteenth century (Victorian Biodiversity Atlas 2024). The success of *P. lanceolata* and its long-recorded history make it an ideal study species to test for plant-soil interactions.

Site locations

We downloaded occurrence records for *P. lanceolata* from the Atlas of Living Australia (Belbin et al. 2021) on December 12, 2021 to quantify the distribution of *P. lanceolata* via QGIS 3.22.3 (QGIS Development Team 2021). The established distribution is based on the occurrence data and represents the area where the species is most likely to occur. Details are available in Supplementary information.

We selected seven transects (Fig. 1) with the aim of sampling widely across the full breadth of the *P. lanceolata* distribution in Australia. Transects were at least 100 km from each other, with each location within the transect sharing relatively similar climates (sensu Beck 2018). Each transect was composed of three points: a) a site within the established range of *P. lanceolata*, with occurrence dating back 30+ years (i.e., presence recorded in or before 1990); b) a site just outside the established range of *P. lanceolata*, located at least 5 km but less than 35 km from the border of the estimated *P. lanceolata* range; c) a site well beyond the established range, at least 30 km from the site just outside the established range, and 50 km from the site within the established range of *P. lanceolata*. Sites within the established range were always situated in locations where *P. lanceolata* was recorded at least 30 years ago (Atlas of Living Australia 2025). Distance thresholds were set on the assumption that it would be unrealistic for *P. lanceolata* to establish a self-sustaining population beyond 30 km from the edge of the established range within the timeframe between data compilation and fieldwork.

When more than one suitable site within the established range of *P. lanceolata* was available for sampling, we randomly chose one but kept the others as

backup in case of unsuccessful sampling (e.g. a failure to locate sufficient plants at the first location). We sampled one additional site within the established range in Hobart (Tasmania) to include a colder location and extend the latitudinal span of the study (see Supplementary Information 2 for site details).

Sampling of each transect started from the site within the established range, according to coordinates and notes provided by records from the Atlas of Living Australia. For each location, we investigated plant communities and habitats with potentially suitable conditions for *P. lanceolata*, e.g., roadsides, pastures, parks (Cavers et al. 1980). When necessary, we extended our investigation area a further 3 km, moving in a random direction and sampling the first site encountered with suitable conditions that could support *P. lanceolata*. In case of points within the established range, if we still could not find a population of *P. lanceolata*, we would select a different suitable site from the backup list. Otherwise, we removed the transect from the study.

Soil samples collection

At the points within the established *P. lanceolata* range of each transect, we located a dense patch of *P. lanceolata* and started sampling from the rhizosphere of the largest individual. Following the first sample, we randomly selected a compass bearing between 1° and 360° and a number of steps between 5 and 30, sampling from the next closest individual of *P. lanceolata*. In the presence of clustered individuals, we selected the largest ones. When possible, we collected the rhizosphere soil by delicately removing the soil stuck to the roots using a brush or a garden trowel. Otherwise, we dug the roots up and collected the rhizosphere by gently shaking or using a brush or a garden trowel.

To collect soil from sites without *P. lanceolata*, we looked for a habitat that would be potentially suitable for *P. lanceolata*. Then, we located the nearest path within this suitable habitat (to provide an unbiased starting point), and used a random number generator to determine the direction and number of steps to the first sampling spot. We then repeated the procedure with a new combination of random direction and number of steps, starting from the point just sampled. If a combination placed the sampling spot outside the

suitable habitat, we repeated the draw until a valid location within the habitat was selected.

For all sites, we continued the sampling procedure until we had collected 15 L of soil, with subsamples pooled together to create a representative site sample. Pooling was needed to get enough material to generate the sterilized controls and inocula for the glasshouse experiment. Soil samples were dug from within the top 10 cm of the soil profile using a trowel, excluding bigger roots and stones when possible. All equipment was washed with warm soapy water and disinfected with 70% ethanol after each use to reduce cross-contamination, with boot soles additionally sprayed with 20% commercial bleach solution.

Seeds collection

We obtained seeds of *P. lanceolata* from two European (native range) and two Australian (introduced range) sources. We purchased European seeds from retailers in Udine (Italy, <https://www.semenostrum.it/>) and Ladendorf (Austria, <https://www.weltpflanz.en.at/>), ensuring they were not hybrids or genetically modified, and delivered via flight courier to UNSW Sydney (Australia). Seeds from Australia were collected in Warwick (Queensland) and Hobart (Tasmania), matching two of our soil sampling sites.

Soil samples treatment

We ensured that all soil samples adhered as closely as possible to ISO 18400–206 (International Organisation for Standardisation 2018). Upon reaching lab facilities after each sampling occasion, we sifted a portion of the fresh soil through a sterilized 2 mm-mesh sieve and stored it inside a double-layered resealable bag at 4 °C until the inoculation phase. To avoid creating an anaerobic environment, bags with soil were regularly opened, gently shaken, and resealed carefully. For each site, the remaining soil was air-dried for a week, sieved at 2 mm, and oven-dried for 72 h at 30 °C. Larger roots and other coarse organic material were removed during the sieving procedure.

At the conclusion of the sampling campaign, we started the inoculation process (Fig. 2). Dried samples were sterilized via gamma irradiation at 50 kGy at Steritech (Sydney, NSW, Australia). Inoculation into sterilized soil was intended to control for the

flush of nutrients that occurs post-sterilization (Brinkman et al. 2010; Gundale et al. 2019). Unsterilized samples were removed from refrigeration and left to gradually reach room temperature over 12 h (the slow temperature changes were intended to reduce heat stress). The sterilized soil from each site was divided into two portions: a sterilized “control” treatment and a substrate designated for the “live” treatment. The “live” treatment was prepared by inoculating the sterilized substrate (95% of substrate volume) with its corresponding unsterilized soil (5% inoculant v:v) in a sterilized glass bottle (Fig. 2). Soil content was homogenized by vigorously shaking each glass bottle.

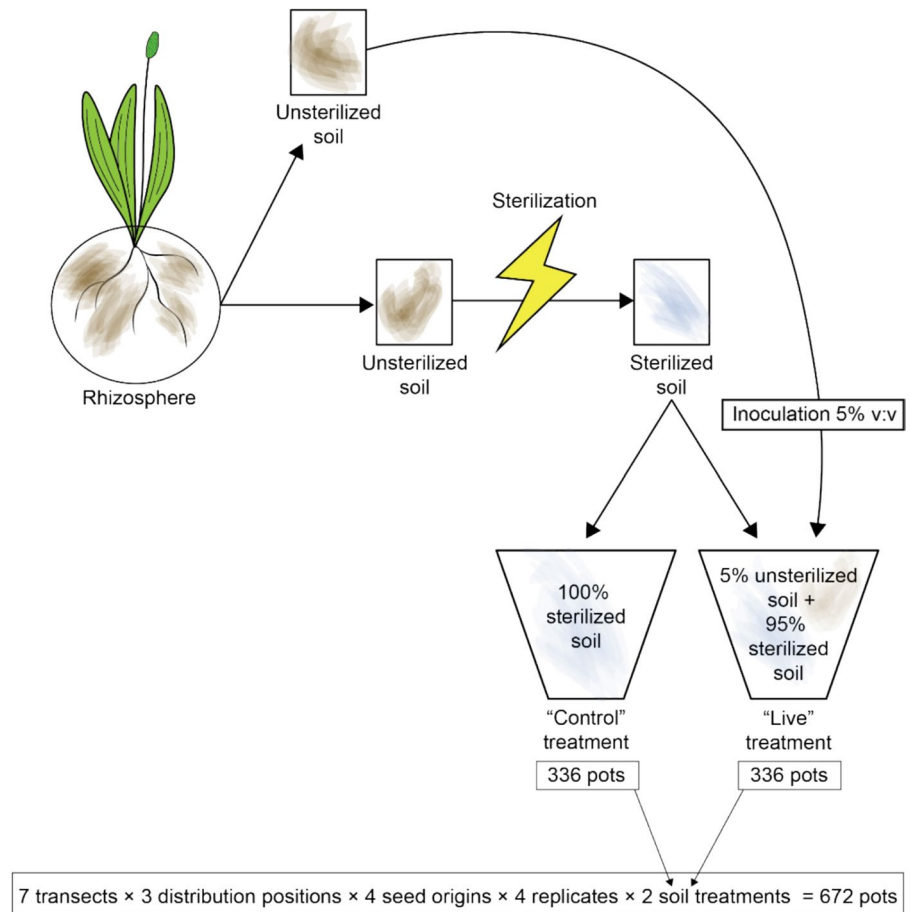
We used a portion of sterilized soil samples to assess chemical properties. Samples were sent to Southern Cross University Environmental Analysis Laboratory to analyze organic carbon using LECO Trumac analyzer and available nitrogen as KCl extractable nitrate and ammonium. Soil pH in 1:5 H₂O was measured at UNSW Sydney following ISO10390:2021 (International Organisation for Standardisation 2021) using a benchtop pH-meter (SmartCHEM-LAB, TPS, Australia).

Glasshouse experiment

The experiment occurred within a limited-access acclimatized chamber at the UNSW Sydney glasshouse facility. Autoclaved germination pots (4 cm wide, 8 cm deep, 110 ml) were prefilled with 30 ml of autoclaved perlite and a square of paper towel at the bottom to avoid losses of substrate. Each pot was then filled with 60 ml of soil from the designated site and treatment. To reduce the risk of contamination, we filled the pots on different sides of the room and at different times. The glasshouse experiment was fully factorial and included a combination of 2 treatments (sterilized vs. unsterilized) × 7 transects (see Fig. 1) × 3 distribution positions (within, just outside, and well beyond the established range) × 4 seed origins (2 seed populations for Australia and 2 for Europe) × 4 replicates = 672 pots, each sown with 3 seeds from the same population, except for Warwick where we planted 2 seeds (due to limited seeds available). Pots were placed at randomized locations on the glasshouse bench from October 2nd, 2022.

Pots were watered once a day for 3 min using the automated sprinkling system, and the room temperature set to 23° C for day and night. The position of

Fig. 2 Simplified representation of fieldwork from soil collection to inoculation



pots was randomized every three weeks. To reduce the risk of cross-contamination, we disinfected any tool that came in contact with soil with 70% ethanol between uses. From five days after planting, pots were inspected every two days for germination. A seed was considered as germinated when we could see the emerging hypocotyl. A colored toothpick was placed next to each germinated seed, color-coded based on the germination date. One month after the start of the glasshouse experiment, we re-sowed the pots without seedlings following the same procedure as before. When the oldest seedling in each pot reached 30 days after germination, we randomly selected one of the surviving individuals to keep and removed the others.

We harvested and weighed the aboveground biomass of each plant 2.5 months after germination. This timeframe allowed *P. lanceolata* to grow well enough to yield robust biomass data and keep a uniform rhizosphere community (Blakney et al. 2024; Chaparro et al. 2014), but not too long for individuals to

reach full flowering, which might alter the soil bacterial composition (Chaparro et al. 2014).

We noticed an effect of germination date on seedling growth (potentially related to seed vitality, day-length, or imperfect climate control within the glasshouse). To avoid confounding, and because most of our seeds germinated in the first week after being planted, we only included data from seeds germinated within a week from sowing in our analysis. Excluding later-germinated seeds left us with data for 539 of the 652 plants harvested, and did not have a qualitative effect on the results of the analyses (Supplementary Information).

Data analysis

We ran statistical analyses with R version 4.3 and RStudio version 2023.3 (Posit Team 2023; R Core Team 2023). Mixed models were run using “lme4” 1.1–34 (Bates et al. 2015). Confidence intervals

were calculated according to Wald t-distribution approximation using “easystats” 0.6 (Lüdtke et al. 2022). To account for differences in covariates between each replicate and grouping variables, we followed up the regression analyses with estimated marginal means or trends and estimated marginal contrasts, computed via “emmeans” 1.8.8 (Lenth 2021). Unless stated otherwise, the contrasts were calculated between the paired sites within and well beyond the established *P. lanceolata* distribution. Results including sites just outside the *P. lanceolata* range can be found in Supplementary Information. Adjustment of p-values for multiple comparisons, when necessary, was conducted with the “false discovery rate” method (Benjamini and Hochberg 1995). Effect sizes reported are bias-reduced partial omega-squared (ω^2) calculated using “effectsize” v 0.8.6 (Ben-Shachar et al. 2020).

We tested if soil biota had an effect on plant growth with a linear mixed model with fresh aboveground biomass ($\log_{10}$ -transformed) as the response variable; seed origin (Australian vs. European), soil treatment (unsterilized vs. sterilized), distribution position, and site latitude as fixed effects; and site and transect as random effects. We then calculated the contrast of fresh aboveground biomass between sterilized and unsterilized soil.

We calculated the total effect of soil biota on plant biomass as the $\log_{10}$ ratio between the fresh aboveground biomass of plants grown in unsterilized vs. sterilized soil (Eq. 1), averaged by seed population and site. We excluded the “Hobart” site from this analysis, as it only had one level for distribution position.

$$\text{effect of soil biota on plant growth} = \log_{10} \left(\frac{\text{fresh aboveground biomass in unsterilized soil}}{\text{fresh aboveground biomass in sterilized soil}} \right) \quad (1)$$

A value of 0 in Eq. 1 means plants growing in unsterilized and sterilized soil weighed the same (i.e., there was no net effect of soil biota on plant growth). Positive values indicate plants grew bigger in unsterilized soil than in sterilized soil (i.e., positive effect of soil biota). Negative values mean plants grew bigger in sterilized soil than in unsterilized soil (i.e., negative effect of soil biota). In our study, a stronger effect of soil biota on plant growth means that plants growing in unsterilized soil were bigger than in sterilized

We quantified how soil biotic effects on plant growth changed with distribution positions using a linear mixed model with soil biotic effects on plant growth as response variable, and latitude, seed origin, distribution position, and transect as fixed effects, and site as a random effect. We then ran a contrast analysis of soil biotic effects on plant growth vs. the three levels of distribution position. We tested how soil biotic effects on plant growth related to soil variables using the previous model and adding the target soil variable. Soil variables studied were organic carbon ($\log_{10}$ -transformed), available nitrogen ($\log_{10}$ -transformed), and pH (quadratic transformation, as optimum pH tends to be at intermediate values (Jones 2012)). We also ran a regression with all soil variables included and calculated the variance inflation factor (see Supplementary Information). We tested how belowground enemy release changed with latitude by running a linear mixed regression with soil biotic effects on plant growth as response variable, seed origin, distribution position interacting with latitude, and transect as a fixed effect, and site as a random effect. We followed up with a contrast analysis of the latitudinal trend of soil biotic effect on plant growth vs. the two levels of distribution position (see Supplementary Information for analysis including all three levels).

We calculated the release from belowground enemies as the effect of soil inocula derived from established sites vs. just outside or well beyond *P. lanceolata* established range (Eq. 2), averaged by seed origin and site. Similarly, we calculated the relative difference in soil variables between the established and the new range sites (Eq. 3).

soil. We would say that the stronger the effect of soil biota, the more it limits plant growth.

We investigated how the effect of soil biota on plant growth correlates with latitude using a linear mixed model with plant weight ($\log_{10}$ -transformed) as response variable and seed origin, distribution position, soil treatment interacting with latitude as fixed effects, and site nested within transect as random effects. We then compared the latitudinal trend of plant weight against the levels of soil treatment.

$$\text{Difference in belowground enemy pressure} = \log_{10} \left[\frac{\log_{10} \left(\frac{\text{fresh aboveground biomass in unsterilized soil}}{\text{fresh aboveground biomass in sterilized soil}} \right) \text{ in just outside or well beyond established range}}{\log_{10} \left(\frac{\text{fresh aboveground biomass in unsterilized soil}}{\text{fresh aboveground biomass in sterilized soil}} \right) \text{ within established range}} \right] \quad (2)$$

$$\text{Relative difference in soil variable} = \log_{10} \left(\frac{\text{soil variable value in "just outside" or "well beyond" established range}}{\text{soil variable value within established range}} \right) \quad (3)$$

We quantified how the difference in belowground enemy pressure correlated to the relative difference in soil variables by using a linear mixed effects regression with seed origin, site latitude, transect, and distribution position included as fixed effects, and site as a random effect. The analysis was run separately for each soil variable with the same transformations described above. Finally, to understand whether belowground enemy release correlated with seed origin (Europe vs. Australia), we ran a linear mixed regression with soil biotic effect on plant growth as response variable, seed origin, site latitude, and distribution position interacting, and transect included as

fixed effects, and site as a random effect. We followed up with a contrast analysis between the two levels of seed origin vs. the two levels distribution position (see Supplementary Information for analysis including all three levels).

Results

On average, plants growing in unsterilized soil weighed 34.1% less than those in the sterilized treatment (marginal ratio=0.66, 95% CI [0.59, 0.73], $p < 0.001$; treatment $\omega^2 = 0.29$, Fig. 3a). However,

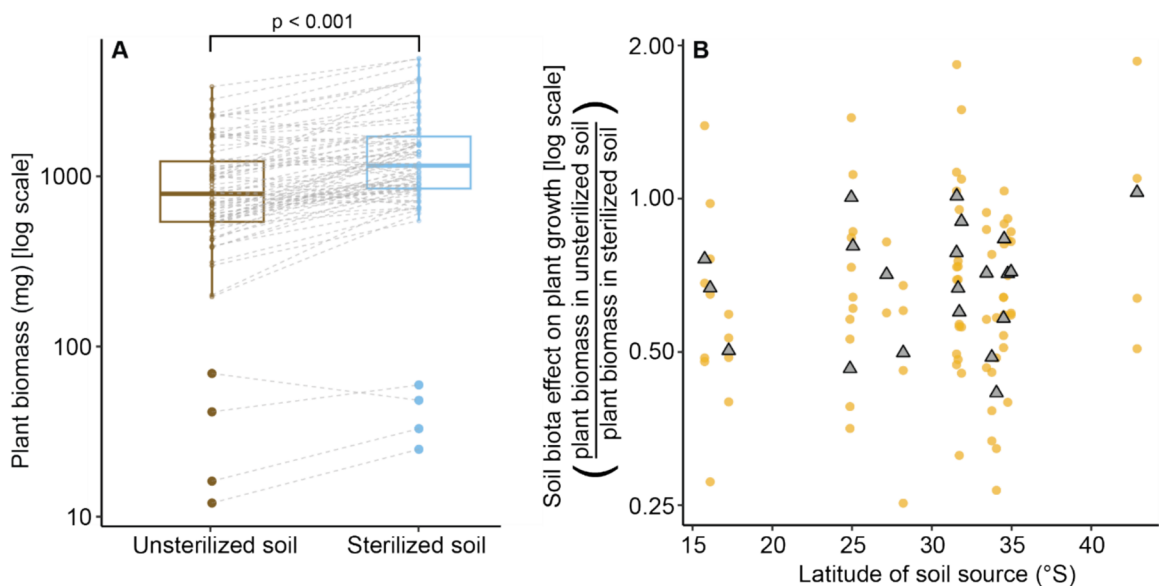


Fig. 3 **A** Difference in fresh aboveground biomass of plants growing in unsterilized soil (i.e., “live” treatment, in green) vs. sterilized soil (i.e., “control” treatment, in light blue). Dashed lines link plants treated with the same inoculum. **B** Effects of soil biota on plant growth for soil sourced

from sites along a latitudinal gradient. Each orange dot (●) represents the mean soil biota effects for each site x seed origin combination, and each gray triangle (▲) represents the site mean soil biota effects

there was no evidence that the effect of soil biota on plant growth followed a latitudinal pattern (marginal contrast of treatment $\times$ latitude = -0.003 , 95% CI $[-0.01, 0.00]$, $p=0.39$; Fig. 3b).

We found no evidence for differences in the effect of soil biota on *P. lanceolata* growing in sites within (mean = 0.63) vs. just outside (mean = 0.61) the established *P. lanceolata* range ($p=0.92$), nor between sites just outside and well beyond (mean = 0.60) the established range ($p=0.92$; Fig. 4). That is, plants establishing beyond the present distribution of *P. lanceolata* do not seem to experience reduced soil enemy pressure.

We predicted that the effect of soil biota on plant growth would be lower (i.e., less deleterious) at high levels of fertility. Consistent with this prediction, we found that plants grown in sites richer in organic carbon experienced less severe soil microbial effects ($\log_{10}$ slope = 0.49, 95% CI $[0.17, 0.81]$, $p=0.024$, organic carbon $\omega^2=0.29$). The magnitude of this effect is substantial: on average, plants growing in soil with 2% organic carbon suffered 1.41 times lower

soil microbial effects on their biomass than did plants growing in soil with 1% organic carbon (Fig. 5a). Contrary to our hypothesis, available nitrogen was not correlated with soil microbial effects ($\log_{10}$ slope = -0.12 , 95% CI $[-0.23, 0.00]$, $p=0.16$; Fig. 5b). Finally, there was a marginal indication that soil pH is associated with the effects of soil microbes on plant growth (slope = -0.71 , 95% CI $[-1.34, -0.08]$, $p=0.028$ for linear term, slope = -0.38 , 95% CI $[-0.97, 0.22]$, $p=0.21$ for quadratic term; soil pH $\omega^2=0.25$, Fig. 5c). When all soil variables were considered, we found that none were statistically correlated with soil biota effect on plant growth, and that soil variables were not statistically correlated with each other (each Variance Inflation Factor <3 ; Supplementary Information).

Individuals of *P. lanceolata* from Australian seeds grew 1.3 times bigger than individuals from European seeds in sterilized soil (fresh biomass of European vs. Australian origin = 1.3, 95% CI $[1.14, 1.49]$, $p<0.001$). However, soil biota affected individuals growing from Australian seeds roughly 20% more

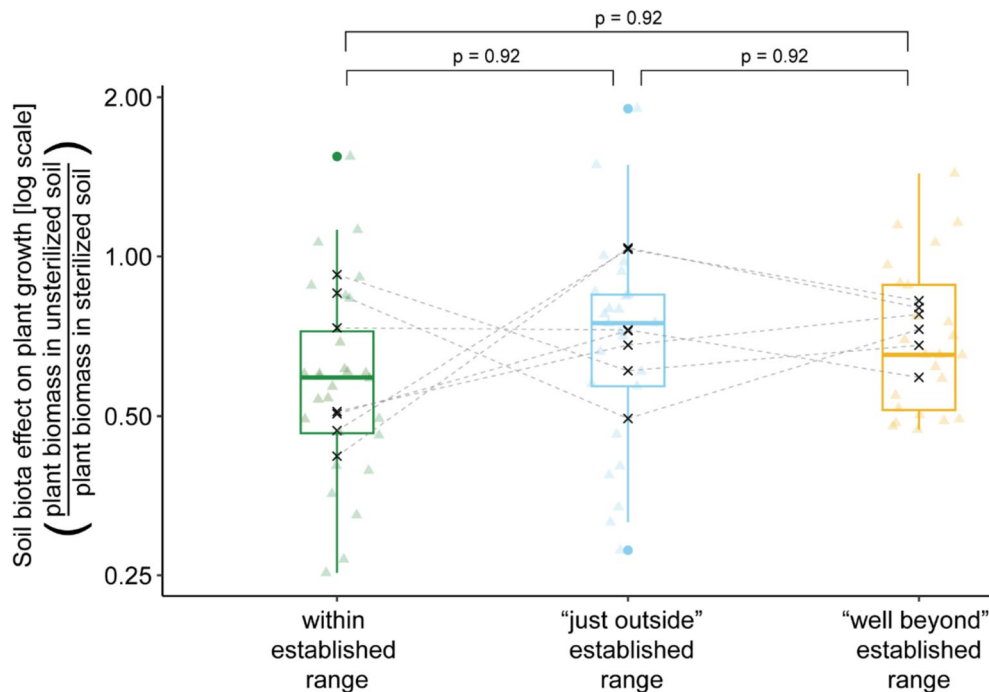


Fig. 4 Belowground effects at different positions of *P. lanceolata* distribution. Each triangle ($\blacktriangle$) represents the mean soil biota effects on plant growth for each site $\times$ seed popula-

tion combination. Each cross ($\times$) represents the mean soil biota effects per site across all four populations, and the dashed lines link sites within the same transect

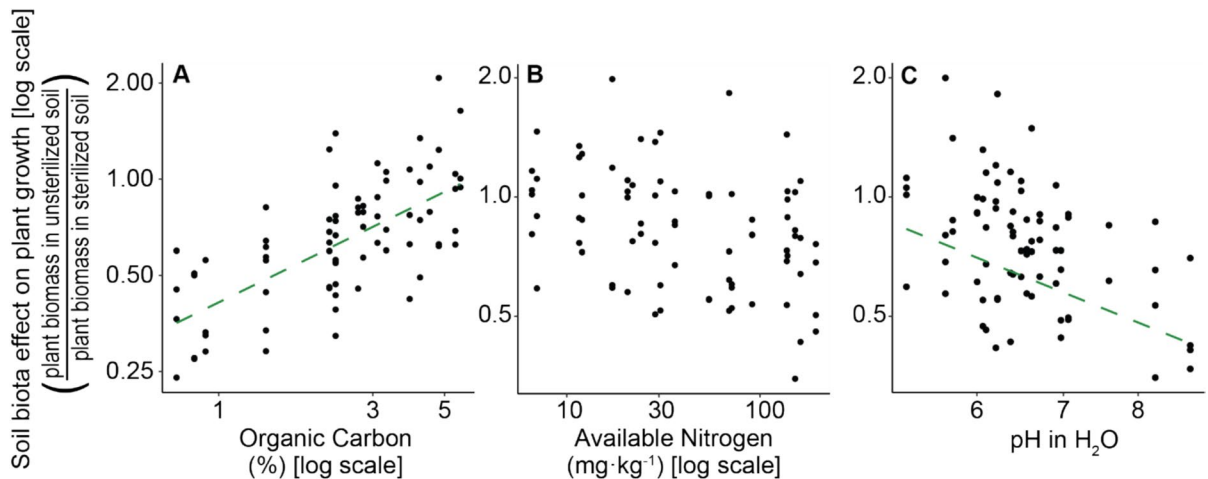


Fig. 5 Conditional relationships between soil biota effects on plant growth and soil fertility variables (**A**: organic carbon, **B**: available nitrogen, **C**: pH in water). The other covariates are set to median and seed origin to Australia,

see Supplementary Information). Dashed lines represent significant relationships ($p < 0.05$). Each dot (●) represents the mean soil biota effect for each site $\times$ seed population combination

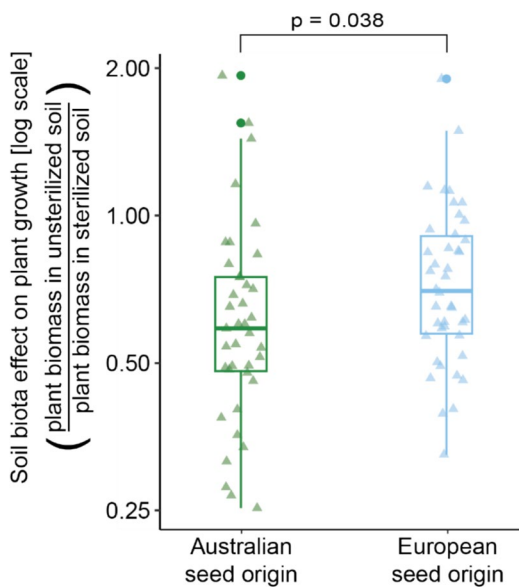


Fig. 6 Differences in soil microbial effects on *P. lanceolata* growth from seeds of different origins. Each triangle (▲) represents the mean soil biota effects for each site $\times$ seed population combination

than from European seeds (soil biotic effects on plant growth of Australian vs. European origin = 0.83, 95% CI [0.70, 0.99], $p = 0.038$; Fig. 6).

Contrary to what we predicted, there was no evidence that the belowground enemy release experienced

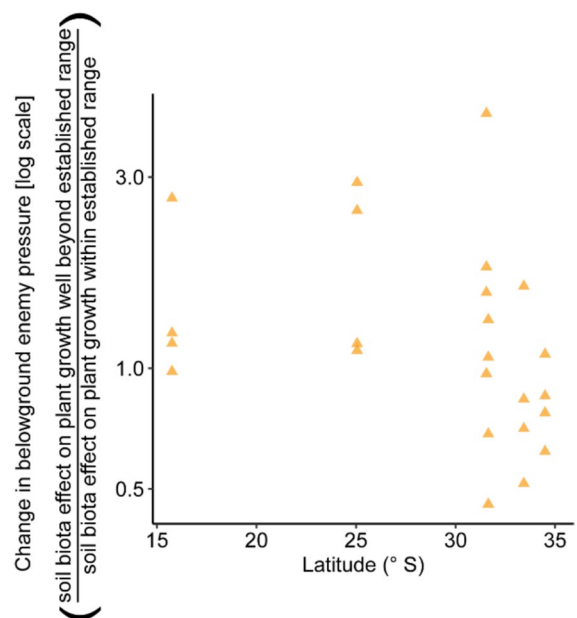


Fig. 7 Patterns in belowground enemy release along a latitudinal gradient. Each triangle (▲) represents the mean for each transect $\times$ seed population combination

by *P. lanceolata* was related to site latitude (marginal contrast of distribution position $\times$ latitude = 0.01, 95% CI [−0.02, 0.05], $p = 0.37$; Fig. 7).

The strength of belowground enemy release outside *P. lanceolata*'s established range was positively

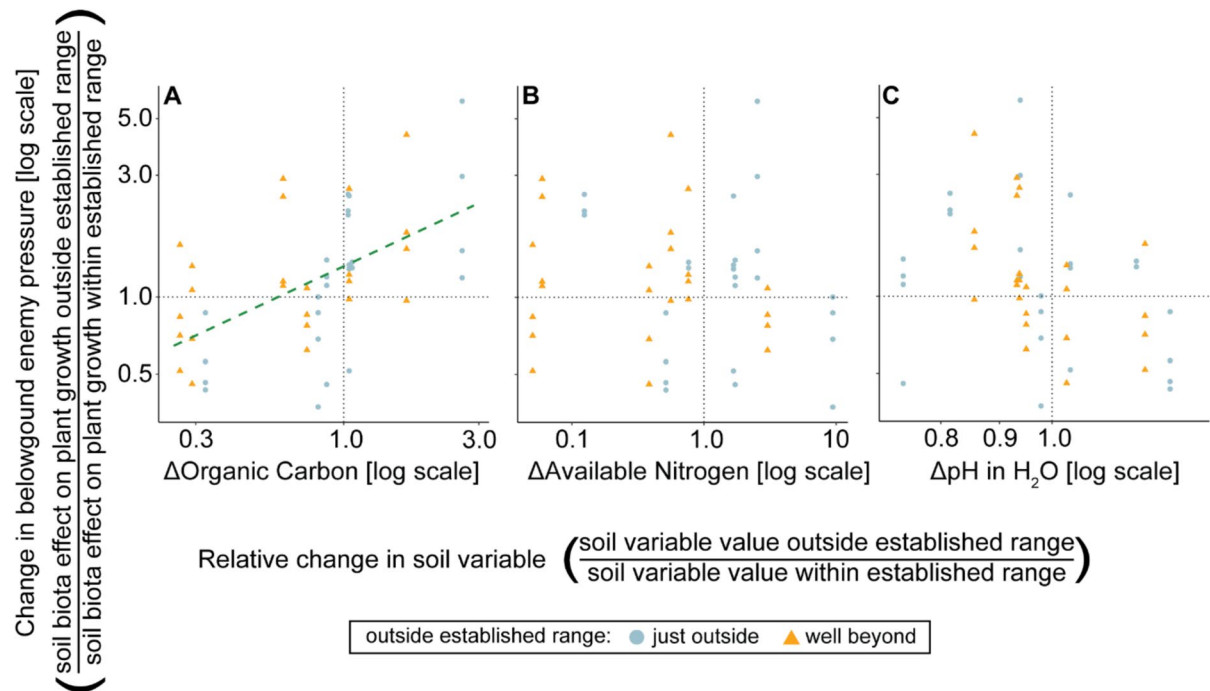


Fig. 8 Relationships between belowground enemy release and soil variables (**A**: organic carbon, **B**: available nitrogen, **C**: pH in water) in *P. lanceolata*. Dashed line represents significant relationships ($p < 0.05$). Dotted lines represent

no net change, values > 1 represent increase, values < 1 represent decrease. Each point represents the mean for each transect $\times$ seed population combination

correlated with the difference in soil organic carbon between the new location outside the plant's range and the location within its established range (Fig. 8a). Since previous analyses found no significant differences in soil biotic pressure between sites just outside and well beyond *P. lanceolata*'s distribution (Fig. 4), we combined these categories for a more robust inference. For example, *P. lanceolata* growing at a new site 1% richer in organic carbon than its previous location would experience 1.35 times less severe soil biotic effects ($\log_{10}$ slope = 0.52, 95% CI [0.26, 0.78], $p = 0.001$; organic carbon $\omega^2 = 0.52$). However, we found no evidence that this effect applied to available nitrogen ($\log_{10}$ slope = -0.07 , 95% CI [-0.24 , 0.11], $p = 0.51$; Fig. 8b) or pH ($\log_{10}$ slope = -0.54 , 95% CI [-2.49 , 1.41], $p = 0.63$; Fig. 8c).

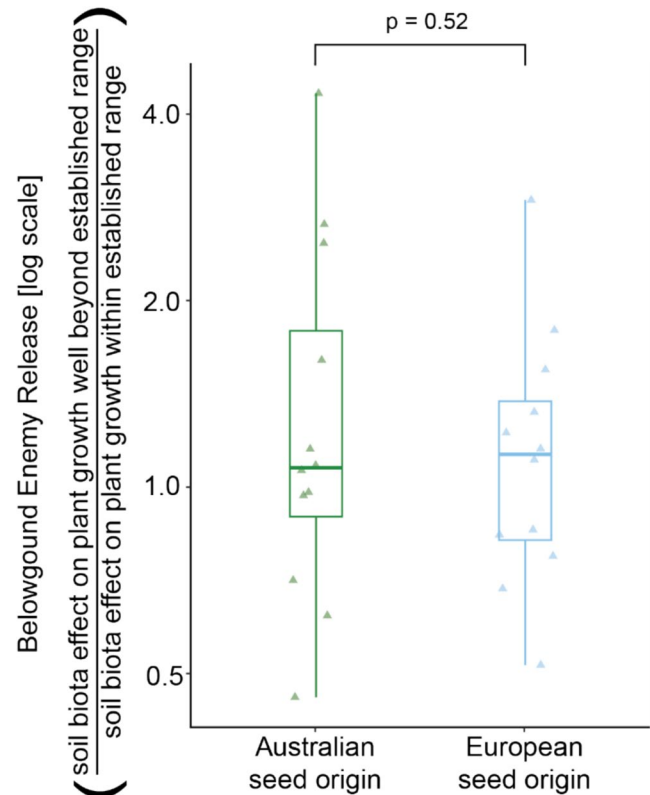
Contrary to our hypothesis, *P. lanceolata* grown from European seeds did not suffer lower belowground enemy release than Australian seeds when moving beyond the Australian established range (marginal ratio = 1.14, 95% CI [0.76, 1.70]; $p = 0.52$; Fig. 9).

Discussion

Our study showed strong evidence that soil biota reduced *P. lanceolata* biomass (Fig. 3a), supporting the net antagonistic role soil microbes play in plant growth (Agrawal et al. 2005; von Holle et al. 2020). We observed some cases in which *P. lanceolata* biomass was lower in the sterilized soil treatment pair, which could be related to the loss of mycorrhizal fungi or other beneficial organisms present in the unsterilized soil sample. However, this was the exception, not the rule. There is currently no standardized protocol for inoculation experiments, for example how much soil should be used, whether pooling samples is acceptable, or in which circumstances it is better to use the whole soils or a liquid suspension method (van de Voorde et al. 2012). The soil inoculation method, like the one used here, seems to maintain the most biodiversity from the original soil (van de Voorde et al. 2012), and therefore may better mirror the effects that would occur in nature.

The antagonistic role of soil microbes on plant biomass was not stronger towards the tropics

Fig. 9 Differences in belowground enemy release between *P. lanceolata* seeds of different origins. Each triangle (▲) represents the mean soil biota effects for each site × seed population combination



(Fig. 3b), contrary to the latitudinal biotic interaction hypothesis (Schemske et al. 2009). Our research adds to a substantial body of evidence showing mixed patterns in biotic interactions with latitude (Moles and Ollerton 2016; Zvereva and Kozlov 2021), and is consistent with the idea that plant-soil relationships seem to be only weakly affected by latitudinal gradients (Crawford et al. 2019; Van Grunsven et al. 2009). Thus, it seems that, while soil communities change with latitude (Lu et al. 2018), the total negative effects exerted by micro and meso-organisms are independent of latitude. The soil system has limiting effects and it could act as a significant barrier to plant establishment even at seemingly suitable locations. Our experiment focused on the initial growth stages of *P. lanceolata*, but the microbial composition of the rhizosphere may change with time (Blakney et al. 2024; Chaparro et al. 2014). Long-term studies on how microbial biodiversity changes across growth stages and generations would provide a more complete understanding of how plant-soil relationships can affect the establishment of new plant populations.

The negative effects of soil biota on *P. lanceolata* were similar in sites within vs. outside the Australian established distribution (Fig. 4). That is, we found no support for the enemy release hypothesis. Our results seem to not align with previous literature which has shown an escape from soilborne enemies in plants' expansion range (Callaway et al. 2004; Van Grunsven et al. 2009). The degree of enemy release was also not associated with latitude (Fig. 7). This finding aligns with Xirocostas et al. (2023), who found that climatic variables were not correlated with the level of aboveground enemy release experienced by 16 plant species. The lack of consistent biogeographic patterns in explaining soil biotic effects on plant species aligns with the general difficulty of predicting which ecosystems are most vulnerable to invasions (Eschtruth and Battles 2011; Fridley et al. 2007).

Among soil variables, the strongest correlate of belowground enemy release was soil organic carbon (Fig. 8a). That is, the increase in organic matter of the new location compared to the previously colonized site can be used to predict invasion success. Most studies support the idea that more fertile sites,

or sites with added nutrients, are more at risk of invasion (Lake and Leishman 2004; Li and Norland 2001; Soti et al. 2020). Counter to our predictions, neither available nitrogen (Fig. 8b) nor pH (Fig. 8c) showed a significant relationship with belowground enemy release, rejecting our hypothesis, although pH was correlated with soil biotic effects overall. These findings suggest the role of soil nitrogen might be secondary and regulated by organic carbon and other soil factors.

Consistent with our hypothesis, *P. lanceolata* individuals grown from European seeds were less affected by soil microbes than were plants grown from Australian seeds, although marginally (Fig. 6, $p=0.038$). It could be that different lineages of the same species might have different invasion potentials (Watermann et al. 2022). While we do not know how long the populations have been separated, the isolation between European and Australian *P. lanceolata* might have been enough for this species to undergo evolution to some extent, as suggested by the literature on rapid evolution (Allen et al. 2016; Brandenburger et al. 2020; Buswell et al. 2010; Maron et al. 2004). In this study, we tested the effects of four populations of *P. lanceolata*, two from the native European distribution and two from the colonized Australian distribution. Future studies including a broad array of populations would strengthen understanding of the role of intraspecific variation and the establishment potential of plant species. Further, genetic studies will be useful to better understand the extent and rate of adaptation of invasive plants to new environments, and incorporating seeds from ex-situ seed banks could help track the history of propagules and their adaptation and evolutionary trajectories (León-Lobos et al. 2012; Smith et al. 2011). Understanding the regional adaptation of plants and their interaction partners is paramount, as globalization and anthropogenic pressures are causing more biotic invasion than ever before (Lee and Chown 2009; Lenoir and Svenning 2014; Pyšek et al. 2017).

Globally, herbivory reduces plant biomass by approximately 15% (Cebrian and Lartigue 2004; Cyr and Pace 1993), while the soil biota effects in our study showed a decrease of over 30% (Fig. 3a). Previous research has also indicated significant belowground impacts, with biomass reductions of up to 70% (De Deyn et al. 2004; Hendriks et al. 2013). Although this work cannot extend to all species with

invasion potential, it highlights we should aim to consider both aboveground and belowground interactions for a better understanding of invasions, range expansions, and translocation success.

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Author contributions GMC and ATM conceived the idea and designed the methodology; GMC collected and analysed the data; GMC and ATM wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data availability Supplementary material, datasets, and R code is available via OSF online repository: <https://tinyurl.com/PlantagoLatitude>

Declarations

Competing interests The authors have no relevant interests to disclose.

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