

Growth of invasive *Lupinus polyphyllus* (Fabaceae) is not affected by site invasion history

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For invasive plant species that associate with mutualists, the presence of suitable partners or a lack thereof can be important to invasion success. We studied the effects of site invasion history and soil microbiota on the performance, folivore damage, and resistance to a fungal disease of the invasive legume *Lupinus polyphyllus* (Fabaceae). We discovered that the plant invader benefited from soil microbes: plants treated with an intact soil inoculum nodulated more than those grown in autoclaved soils, and tended to have higher biomass and a smaller relative investment in roots as compared with that in shoots. However, the origin of soil inoculum (previously invaded, uninvaded) had no effect on the invader's performance, the occurrence of folivory or resistance to fungal disease. Our results indicate that mutualistic associations in soils outweigh potential antagonistic interactions between plants and soil pathogens. Nevertheless, these mutualistic associations are not unique to previously invaded sites in the study area.

Introduction

Soil microbiota can play an essential role in the success of invasive plants as soil microbes may either facilitate or hinder plant establishment and colonisation, depending on the nature of plant–microbe interactions (Kliromonos 2002, Nijjer *et al.* 2007, Dawson & Schrama 2016, Fahey & Flory 2022). Interactions between plants and soil microbes can be expected to be particularly important for plant species that are at least partially relying on mutualistic partners, such as nitrogen-fixing bacteria (rhizobia) or mycorrhizal fungi. For example, nitrogen-fixing plants can change the soil bacterial community composition (e.g., Le Roux *et al.* 2018, Keet *et al.* 2021), with mutualistic rhizobia becoming more

abundant over time, and enhancing the establishment and growth of the host plant (Le Roux *et al.* 2018). As plants obtain rhizobia from soil after germination, the lack of suitable mutualistic partners in new habitats may retard early stages of plant colonisation (e.g., Parker 2001, Simonsen *et al.* 2017). Such limitation of invasion spread may occur at a small spatial scale (even a few metres) if mutualist abundance declines with increasing distance from established invasions (Lopez *et al.* 2021). However, previous studies reported that introduced plants tend to be less dependent on their mutualistic partners than their native conspecifics (e.g., Seifert *et al.* 2009, terHorst *et al.* 2016, Kalske *et al.* 2022a), which may call into question the importance of mutualists in plant invasions. On the other hand, the

accumulation of soil pathogens on invasive plant species with time may slow down their establishment and colonisation of new areas (Flory & Clay 2013, Dostal *et al.* 2013).

In addition to direct effects of soil microbiota on plant performance, soil microbes can affect the success of plant invasions through species interactions (e.g. with herbivores) and diseases (e.g., Kempel *et al.* 2009, Schädler & Ballhorn 2017, Grunseich *et al.* 2020). As an example, high nitrogen availability to plants with rhizobia may result in an increase in N-based chemical defences and, consequently, a reduction in herbivory (Ochieno *et al.* 2021). Indeed, in annual *Arabidopsis thaliana*, soil microbiota altered leaf metabolome and, consequently, plant resistance to herbivores, with larvae consuming a smaller proportion of leaves from plants grown on soil with than without microbes (Badri *et al.* 2013). Alternatively, plants with rhizobia might be expected to be more attractive to herbivores due to their higher leaf nitrogen content (Kempel *et al.* 2009). Rhizobia may also confer antimicrobial properties to the plant in terms of pathogen protection, including fungal diseases (Smigielski *et al.* 2019, reviewed in Kebede 2021).

We studied the effects of site invasion history and soil microbiota on the performance of the invasive perennial legume *Lupinus polyphyllus* (Fabaceae) by conducting a common garden experiment. The species is considered invasive in many European countries (Fremstad 2010), where it had spread rapidly during the 20th century (Fremstad 2010), negatively affecting local species across several plant and animal taxa (e.g., Valtonen *et al.* 2006, Ramula & Sorvari 2017, Hansen *et al.* 2021). It forms mutualistic associations with nitrogen-fixing bacteria from the families Bradyrhizobiaceae and Rhizobiaceae (reviewed in Stępkowski *et al.* 2018). In Finland, only associations with bacteria from Bradyrhizobiaceae have been observed (first author's unpubl. data). *Lupinus polyphyllus* is consumed by generalist herbivores, mostly the land snail *Arianta arbustorum*, but also by some lepidopteran larvae (authors' pers. obs.). It is also commonly infected by powdery mildew (*Erysiphe* spp.), an airborne fungal disease (Bradshaw *et al.* 2021), which can overwinter in leaf litter.

In this study, we focused on the following two questions: (1) How does site invasion history affect the interaction between the plant invader and soil microbes? (2) How does site invasion history affect folivore damage and the invader's resistance to powdery mildew? We assumed that soils from previously invaded sites already contain rhizobia and predicted that a soil inoculum originating from such sites would thus enhance the invader's performance as compared with a soil inoculum originating from uninvaded sites. We also predicted that plants growing on soils inoculated with soil microbiota from previously invaded sites, putatively containing rhizobia, would experience less folivory and would be more resistant to powdery mildew than those growing on soils inoculated with microbiota from uninvaded soils.

Material and methods

Study species and populations

Lupinus polyphyllus is a short-lived perennial herb, 50–100 cm high, native to North America and invasive in many continents including Europe (Fremstad 2010). In the introduced range, the species inhabits different habitat types from road verges to forest understoreys and meadows (Fremstad 2010, Ramula & Pihlaja 2012, Tyler *et al.* 2015, Hansen *et al.* 2021). As a tall-growing legume, *L. polyphyllus* effectively competes with local plant species (Valtonen *et al.* 2006, Hansen *et al.* 2021), and it is classified as one of the most harmful plant invaders in Europe (Nentwig *et al.* 2018). In our study area in SW Finland, the species reproduces mainly by seed, and plants flower at earliest in their second year (authors' pers. obs.). However, vegetative propagation via underground adventitious shoots or root splitters might occur (Eckstein *et al.* 2023). Plant tissues contain nitrogen-based chemical compounds, quinolizidine alkaloids (Wink 1984, Kalske *et al.* 2022b), which might protect individuals against generalist herbivores (Wink 2019) and pathogens (Wink 1984, 1992).

In July 2018, we collected seeds from six Finnish populations of *L. polyphyllus* located 10 to 500 km apart (Table 1). These populations

grew on road verges, wastelands, former fields or forest understoreys, and contained thousands of individuals. They had been present at least since 2010, and the areas covered by the invader varied from 108 m² to 4800 m² (Table 1). In each population, seeds were gathered from about 15 randomly chosen plants that were at least two metres apart, and stored the seeds at room temperature until the next spring. The seeds from different mother plants within each population were mixed.

For the inoculation treatments, we collected 4 litres of soil from each of the six populations of *L. polyphyllus* and from six uninvaded sites located in their vicinity. The uninvaded sites were at least 20 metres away from the invaded ones, and represented similar habitat types. At each site, we collected the soil samples from five locations from a depth of 10 cm either from the rhizosphere of *L. polyphyllus* (invaded sites), or from the rhizosphere of a plant species dominant at uninvaded sites. The spade was sterilised with commercial bleach and rinsed after each site to prevent cross-contamination. As the exact age of the invasions was unknown, we pooled the soil samples across the populations within each soil origin after removing stones and visible organic material. The soil collection took place in mid-May in 2019, and the soils were stored in black plastic bags outdoors until use (for about two weeks). Based on 16S rRNA amplicon sequencing of soil bacterial communities for three out of the six paired sampling sites performed in 2021, both invaded and uninvaded site soils contained bacteria belonging to Bradyrhizobiaceae (Table 1), while bacteria belonging to Rhizobiaceae were rare (S.A. Mousavi & S. Ramula unpubl. data).

Soil inoculation experiment

To study the effect of site invasion history through soil microbiota on the invader's performance, we conducted a soil inoculation experiment in a common garden (Ruissalo Botanical Garden, University of Turku). We chose to use this approach because it explicitly evaluates whether site invasion history (previously invaded vs. uninvaded) is critical to a given plant invader in terms of plant growth in a natural setting with the presence of resident microbial community. According to the results of our earlier study (Ramula *et al.* 2022), changes in the soil bacterial community composition do not necessarily translate into changes in *L. polyphyllus* performance in the common garden.

For the experiment, at the end of May 2019 we chose 50 fully developed seeds from each of the six populations. To remove epiphytic microbes, we surface-sterilised the seeds in 0.5% commercial bleach (NaOCl) solution for 15 min rinsing them afterwards three times with deionised water following Ryan-Salter *et al.* (2014). Then we hand-scarified the seeds, placed them on a moist paper towel in foil containers, covered the containers with a plastic film, and left them at room temperature for four days. The seedlings that emerged were planted in 30 × 60 cm plastic trays filled with a nutrient-poor (low concentrations of NPK, pH = 5.5), autoclaved (at 120 °C, 1 bar, for 20 min) potting mix for garden plants (*Kekkilä karkea ruukutusseos*). We grew the seedlings in an unheated greenhouse for about a week before assigning them to specific treatments.

At the beginning of June, we planted the seedlings individually into 384 plastic 1-l

Table 1. Sampling site details. Coordinates point to the site invaded by *Lupinus polyphyllus*. Mean abundance of Bradyrhizobiaceae in soil bacterial communities was estimated for invaded (I) and uninvaded (U) sites using 16S rRNA amplicon sequencing ($n = 4$ samples per location).

Lat. (°N), long. (°E)	Habitat type	Invaded site area (m ²)	Bradyrhizobiaceae abundance in 2021
60.414, 22.740	forest understorey	1000	11.6% (I), 7.6% (U)
60.357, 22.272	road verge	357	6.1% (I), 5.8% (U)
60.425, 22.389	former field	2400	6.3% (I), 18.7% (U)
63.133, 27.995	road verge	108	not estimated
62.621, 27.124	wasteland	300	not estimated
61.850, 25.166	former field	4800	not estimated

pots (64 seedlings per population) filled with a nutrient-poor, autoclaved (at 120 °C, 1 bar, for 20 min) growth substrate consisting of 50% commercial (Kekkilä) sand and 50% potting mix (*Kekkilä karkea ruukutusseos*). The commercial growth substrate was used to ensure homogeneous low-nutrient growth conditions across treatments, and possible increase in nutrient availability caused by soil autoclaving (Trevors 1996, Hu *et al.* 2019) was mitigated by sand addition. To inoculate the pots, onto the substrate surface in a pot we added 0.5 dl (~4.8% of the total substrate volume; Howard *et al.* 2017) of either intact or autoclaved soil originating from either previously invaded or uninvaded sites, amounting to 96 pots per treatment.

At the beginning of the experiment, we checked microbial activity in autoclaved and intact soils following Trevors (1996) i.e., by observing microbial growth (total cover of microbial colonies per plate) on tryptone-yeast agar plates (10 plates per soil type) after 10-day incubation. We found that the total cover of microbial colonies in plates primed with autoclaved soil inoculum was about $94\% \pm 3\%$ (SD) lower than that in the intact soil. It is to be noted that we were interested primarily in the comparison between invaded and uninvaded sites regardless of soil inoculum treatment (autoclaved or intact), because should differences in the invader's performance, the occurrence of folivory or resistance to a fungal disease be found, they would indicate that site invasion history can affect the outcome of plant invasions.

To prevent the soil in pots from drying in the sun, the pots were buried in sandy soil (about 20 cm apart and at 8 cm depth, leaving 3 cm aboveground) in 8 rows separated by 70 cm gaps. Each row contained 48 randomly distributed pots (two sets of each 'treatment \times inoculum origin' combinations for all 6 populations). The sand field was fenced with a metal net to exclude mammalian herbivores and a sprinkler was placed outside the field for watering. No fertiliser was given during the experiment. The field and pots were weeded twice during the growing season to avoid weed proliferation.

Four days after planting, we measured the initial height of each plant with a ruler to the nearest 0.5 cm, and recorded leaf numbers.

These measurements were repeated at the end of June and in early August (after four and nine weeks since planting, respectively). After about 11 weeks (at the end of August), we recorded leaf herbivory as a categorical variable with three levels (no damage = a plant was intact, mild damage = a plant had a few signs of chewing or grazing herbivory with 1–3 leaflets damaged, severe damage = more than 3 leaflets had been damaged by chewing or grazing herbivores), and the presence or absence of powdery mildew, and subsequently harvested the plants. None of the plants flowered during the experiment and mortality was low (of 384 plants only 4 died). We washed the roots in tap water and recorded the number of nodules as a proxy for colonisation by mutualists. According to Kalske *et al.* (2022a), nodule activity is generally high, even more than 90% are usually active (fixing nitrogen). To obtain total dry biomass of each plant as well as calculate root-to-shoot ratio describing a plant's investment into belowground and aboveground parts, we separated shoots and roots, dried them at +65 °C for 48 h, and weighed individually on a scale (Mettler Toledo) to the nearest 1 g.

Statistical analyses

To study the effects of site invasion history on plant growth, we constructed a linear mixed model (LMM) for plant height, total biomass, number of nodules, and root-to-shoot ratio (using `lme4::lmer()`; Bates *et al.* 2015) in R software (R 3.5.3; <https://www.R-project.org/>). Height was log-transformed, biomass and root-to-shoot ratio were square-root-transformed, and number of nodules was square-root-($x+1$)-transformed to improve normality of residuals. We used soil inoculum origin (invaded, uninvaded) and its treatment (intact, autoclaved) and their interaction as fixed categorical explanatory variables. Initial plant height at the beginning of the experiment (day 4 after planting) was included as a fixed continuous covariate, while population of plant origin and row in which a pot with plant was located were included as random factors. In the model of plant growth (height), we also included time (two levels: June, August) as a categorical explanatory variable, and all pos-

sible interactions with soil inoculum origin and its treatment. Plant ID was considered a random factor to take into account three sets of repeated measurements taken from each plant.

For all models, we also explored whether the effect of soil inoculum origin or its treatment differed across the six populations by fitting different slopes by population. However, such models were not supported, resulting in AIC values higher than those based on the common slopes ($\Delta\text{AIC} > 2$).

Similarly, we used a generalised linear mixed model (GLMM) with a binomial distribution and log-link function to investigate the effect of site invasion history (inoculum origin: invaded vs. uninvaded) on the occurrence of folivory and resistance to powdery mildew in the studied *Lupinus polyphyllus* (glmmTMB; Brooks *et al.* 2017). Due to a small number of severely damaged plants, we pooled the mild and severe leaf damage categories, and included herbivory as a binary variable (plant damaged by chewing or grazing by herbivores, or intact). The origin of soil inoculum, inoculum treatment, and their interaction were used as fixed categorical explanatory variables, with initial plant height as a covariate. Population of plant origin and row in which a pot with plant was located were included as random factors.

For all LMMs, we verified models visually from residual plots and when necessary transformed the response variable to improve normality of the residuals (*see above* for details). For GLMMs, we checked the residual plots for

potential overdispersion and zero inflation using the DHARMA package (Hartig 2018) and found none. The significance of the fixed variables was evaluated with an *F*-test based on the Kenward-Roger method for LMMs (lmerTest::anova(); Kuznetsova *et al.* 2017) and with Wald's χ^2 -test for GLMMs (car::anova(); Fox & Weisberg 2019).

Results

Inoculum origin (from site previously invaded vs. uninvaded) or its treatment (intact vs. autoclaved) had no effect on growth during the experiment (Table 2). As expected, plant height increased over time (mean = 9.39 cm [95%CL = 8.85–9.87] in June and 13.87 cm [95%CL = 13.07–14.73] in August), and was positively associated with plant initial height measured on day 4 after planting (intercept = 1.495, slope = 0.086, SE = 0.006; Table 2).

Soil inoculum origin on its own or in combination with inoculum treatment had no effect on biomass, number of nodules or root-to-shoot ratio (Table 3). Soil inoculum treatment affected nodulation (Table 3): plants inoculated with intact soil produced 17% more nodules than those inoculated with autoclaved soil (Fig. 1b). Moreover, plants grown in the intact soil inoculum tended to produce 14% more biomass and allocated 5% less of total biomass into roots as compared with plants grown in autoclaved soils (Table 3, Fig. 1a and c). Initial plant height at

Table 2. Effects of soil inoculum origin (invaded, uninvaded), its treatment (intact, autoclaved), time, and other factors and their combinations on growth (height) of *Lupinus polyphyllus* during the experiment as evaluated by a linear mixed model (LMM). Population of plant origin, row in which a pot with plant was located, and plant ID were used as random factors; df and ddf denote the degrees of freedom in the numerator and denominator, respectively. Significant effects ($p < 0.05$) are set in boldface.

Explanatory variables	$F_{df, ddf}$	p
Inoculum origin (invaded, uninvaded)	0.445 _{1,365}	0.505
Inoculum treatment (intact, autoclaved)	0.872 _{1,365}	0.351
Time (June, August)	669.375 _{1,378}	< 0.001
Initial plant height	229.041 _{1,370}	< 0.001
Inoculum origin × inoculum treatment	0.316 _{1,365}	0.574
Inoculum origin × time (June, August)	0.525 _{1,378}	0.469
Inoculum treatment × time (June, August)	2.427 _{1,378}	0.120
Inoculum origin × inoculum treatment × time (June, August)	0.024 _{1,378}	0.877

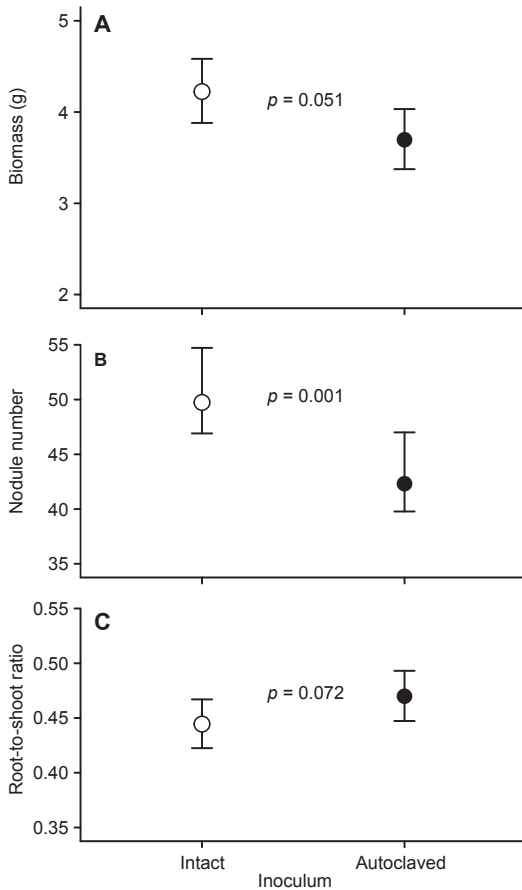


Fig. 1. The effects of inoculum treatment on *Lupinus polyphyllus* biomass, number of root nodules, and root-to-shoot ratio back-transformed least-square mean \pm SE) as evaluated by LMM.

the beginning of the experiment predicted individuals performance 11 weeks later at harvest (Table 3), being positively associated with total biomass (intercept = 0.966, slope = 0.122, SE =

0.021) and number of nodules (intercept = 5.195, slope = 0.240, SE = 0.047), and negatively associated with root-to-shoot ratio (intercept = 0.790, slope = -0.015, SE = 0.003).

Occurrence of folivory or resistance to fungal infection was not affected inoculum origin, its treatment, or their interaction (Table 3), with on average $63.4\% \pm 2.5\%$ (SE) of the plants experiencing leaf herbivory and $86.0\% \pm 1.7\%$ (SE) being infected by powdery mildew. Resistance to fungal infection was negatively associated with initial plant height, with shorter plants being more resistant than the taller ones (Table 3, intercept = -0.749, slope = -0.174, SE = 0.090).

Discussion

We found partial support for our first hypothesis that *L. polyphyllus* would benefit from soil microbes as plants grown in pots with the intact soil inoculum produced more biomass and root nodules than those grown in pots with the autoclaved inoculum. However, the overall invader's performance (quantified as four traits: height, biomass, number of root nodules, root-to-shoot ratio) was not affected by site invasion history, with plants growing equally well in soil originating from previously invaded and uninvaded sites. Our second hypothesis regarding the effects of soil microbes on plant resistance to herbivory and powdery mildew was not supported; occurrence of folivory or resistance to the fungal disease did not differ between plants grown in pots inoculated with soil from invaded and uninvaded sites.

Lupinus polyphyllus grown in pots with the intact inoculum nodulated more than those

Table 3. Effects of soil inoculum origin and its treatment on selected characteristics of *Lupinus polyphyllus* as evaluated by LMM or GLMM. Population of plant origin, row in which a pot with plant was located were used as random factors in all models, df and ddf denote the degrees of freedom in the numerator and denominator in LMMs, respectively (for GLMMs df is one); values indicating significant ($p < 0.05$) effects are set in boldface.

Explanatory variables	Biomass		Nodules no.		Root-to-shoot ratio		Folivory		Fungal disease	
	$F_{df,ddf}$	p	$F_{df,ddf}$	p	$F_{df,ddf}$	p	χ^2	p	χ^2	p
Inoculum										
origin (invaded, uninvaded)	0.10 _{1,361}	0.750	0.91 _{1,364}	0.340	0.06 _{1,360}	0.799	1.446	0.229	1.283	0.257
treatment (intact, autoclaved)	3.82 _{1,361}	0.051	11.80 _{1,364}	0.001	3.25 _{1,360}	0.072	0.147	0.701	0.018	0.895
Initial plant height	34.08 _{1,363}	< 0.001	25.02 _{1,212}	< 0.001	20.93 _{1,357}	< 0.001	2.138	0.144	3.747	0.053
Inoculum origin \times its treatment	0.24 _{1,361}	0.623	1.01 _{1,364}	0.316	0.39 _{1,360}	0.533	0.120	0.729	1.339	0.247

grown in pots with the autoclaved inoculum, and tended to have higher biomass and smaller root-to-shoot ratio. These findings indicate the importance of mutualistic soil microbes for plant performance in nutrient-poor growth substrates, with more abundant nodulation probably reducing relative investment in belowground biomass. In *L. polyphyllus*, mutualistic microbes may primarily consist of rhizobia because the benefits of potential mycorrhizal associations are poorly known (Shi *et al.* 2017). However, this species can associate with arbuscular mycorrhizal fungi (Oba *et al.* 2001, Shi *et al.* 2017), and such associations might have a positive effect on the host plant particularly under low soil phosphorus supply (Shi *et al.* 2017). Mutualistic rhizobia, in turn, are beneficial particularly in harsh environments and likely to become less important in favourable environments (Thrall *et al.* 2007). In Fennoscandia, *L. polyphyllus* inhabits nutrient-poor habitats, such as sandy road verges, wastelands, and meadows in semi-urban environments (Fremstad 2010, Ramula & Pihlaja 2012, Tyler *et al.* 2015), where the presence of suitable rhizobia might indeed facilitate plant establishment and colonisation.

In contrast to our prediction, inoculum origin (previously invaded *vs.* uninvaded) had no effect on growth and nodulation of *L. polyphyllus*. The negligible effect of site invasion history may indicate that mutualistic rhizobia could have been present also in the soil inoculum originating from uninvaded sites, and that there was no major difference in the abundance of soil pathogens between the two origins of soil inocula that would have reduced plant performance. Our preliminary results on soil bacteria based on 16S rRNA amplicon sequencing support this view of minor differences in the soil microbial communities between sites invaded and uninvaded by *L. polyphyllus* in SW Finland, with the members of Bradyrhizobiaceae being present at both (Mousavi S.A. & Ramula S. unpubl. data). Similar to our findings here, Wandrag *et al.* (2103) reported a minor effect of rhizobia on the invasion success of three *Acacia* species in New Zealand. However, the opposite was true for the annual legume *Medicago polymorpha*, in which the lack of suitable rhizobia reduced plant biomass and had the potential to limit population

spread even at a small spatial scale (50 m) in California (Lopez *et al.* 2021). These results collectively suggest that the distribution of suitable rhizobia in soil can greatly vary across different legume species.

There are at least two explanations for the negligible effect of site invasion history on *L. polyphyllus* performance in the present study. First, for plants growing in pots inoculated with soil from previously invaded sites, soil pathogens may have counteracted the positive effects of mutualistic partners on the invader. Pathogens have been suggested to accumulate over the course of plant invasions (Flory & Clay 2013), potentially reducing the invader's growth (e.g., Dostal *et al.* 2013). Second, it may be due to the degradation of mutualistic associations in the introduced range over time. As an example, Seifert *et al.* (2009) found that the introduced populations of the perennial herb *Hypericum perforatum* were less dependent on mycorrhizal fungi than native populations. Similarly, previous findings from a greenhouse experiment revealed that invasive (Finnish) populations of *L. polyphyllus* were less dependent on their mutualistic partners in soil than native (North American) populations (Kalske *et al.* 2022a). Such changes in plant–mutualist interactions in the introduced range can occur rapidly. For the short-lived invasive herb *Vicia villosa*, the positive effects of soil microbes on plant performance were already weaker in 10-year-old invasions than in more recent invasions (Lau & Suwa 2016). Residence time of *L. polyphyllus* in Finland is more than 100 years (Fremstad 2010) and although the exact age of the six study populations is not known, all of them have been present at least since 2010 and contain thousands of *L. polyphyllus* individuals. Local residence times of the populations are thus probably long enough for plant–microbe interactions (mutualistic and/or antagonistic) to evolve.

Contrary to our prediction, the occurrence of leaf herbivory was not associated with our soil inoculum manipulations, indicating that microbial associations did not affect plant attractiveness to folivores. Also Kalske *et al.* (2022a) in a greenhouse study found no difference in resistance to a generalist mollusc herbivore between individuals of *L. polyphyllus* grown on substrates

inoculated with either intact or autoclaved soil from invaded sites. However, as we used herbivory as a binary variable in the present study, we cannot rule out the possibility that there might have been differences in leaf palatability between plants grown in pots with two types of soil inocula. Similar to folivory, soil microbes had a negligible effect on plant resistance to powdery mildew. The majority of plants (86%) were infected by this airborne fungal disease in late summer, confirming its prevalence in the study area. In two other legume species (*Medicago truncatula* and *Pisum sativum*), rhizobia increased plant resistance to powdery mildew caused by the fungus *Erysiphe pisi* (Smigielski et al. 2019).

Overall, our results indicate that soil mutualists can be important to the host plant at least in competition-free environments (e.g., in small open microhabitats during plant establishment). In this study, all but one plant grown in pots with autoclaved soils produced nodules. Such nodulation suggests that the pots located in the common garden have obtained microbes mostly from the local environment during the experiments as autoclaving eliminated about 94% of microbial activity in soils in the beginning of the experiment. Therefore, soil mutualists might play a more prominent role in plant performance in soils with minimal microbial densities. The current results have important implication regarding the colonisation by plant species that host soil mutualists. For *L. polyphyllus*, soil microbes have the potential to improve plant growth, suggesting that mutualistic associations mostly outweigh antagonistic interactions (if any) in soil. However, these mutualistic associations are not unique to previously invaded sites at least in Finland, indicating that the spread of *L. polyphyllus* may not be limited by soil microbiota.

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