Soil Biota in a Nonnative Range has a Net Positive Effect on the Perennial Herb *Lupinus polyphyllus*

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ABSTRACT. Invasive species can have detrimental effects on the health of local ecosystems. *Lupinus polyphyllus* is an herb native to western and northeast North America, but the species has become invasive worldwide. In northeastern Ohio, United States, the species is nonnative, but not invasive and not spreading rapidly. Because physical distance is not a barrier, there are other reasons behind the inability of the species to become locally invasive. Here, the net effect of the local soil biota on the range expansion of the species was tested to explore 2 alternative, non-mutually exclusive, hypotheses. First, if belowground enemies limit this species range, the net effect of the soil biota would be negative. Alternatively, soil mutualists might have a relatively greater net effect on plant fitness. A greenhouse experiment was conducted with 3 populations of seeds from the invasive range across 2 experimental treatments: a general fungicide (ZeroTol®) treatment and a water-control treatment. Fungicide treatment reduced total biomass in 2 out of 3 populations, consistent with limitations by the abundance of belowground mutualists. Fungicide treatment also changed root structure by reducing the number of nodules, root length, and diameter, while increasing root tissue density. Although the fungicide treatment hindered the growth of lupines overall, the changes in root structure indicate that the treated individuals were able to partially compensate by shifting to more of an outsourcing method of resource acquisition. The results suggest that in addition to belowground mutualistic interactions, phenotypic plasticity and intraspecific genetic variation may also contribute to the success of *L. polyphyllus* in its nonnative ranges.

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INTRODUCTION

Invasive species have become significant ecological issues worldwide. The prevalence of plant transport and introduction to nonnative ranges, both intentional and unintentional, have continued to increase over the last century. The early 1900s also gave rise to the distinction between the terms *native*, *introduced*, and *naturalized*, as the scientific community became increasingly aware of, and concerned with, biological invasions (Richardson et al. 2000b). Consequently, the number of plant species that have been identified as harmful to local ecosystems continued to increase as well. Invasive species are nonnative to a region and have undergone the naturalization process such that they are able to survive and successfully reproduce without human assistance. However, to qualify as *invasive*, the species must also be known to thrive and proliferate, which often leads to harmful effects on the ecosystem to which they are introduced. Invasive species have direct, negative effects on native species competing for the same spaces and resources. Invasive species often outcompete natives due to a lack of natural predators and other limiting enemies (e.g., pathogens and diseases) in the nonnative range. This phenomenon is known as the enemy release hypothesis (Keane and Crawley 2002), and there is considerable evidence consistent with enemy release (Wolfe 2002; Mitchell and Power 2003; Allen et al. 2015), including belowground enemies (Reinhart et al. 2003). In addition, mutualists belowground, including mycorrhizal fungi and nitrogen-fixing bacteria (rhizobia), might enhance invasive species success in some cases (Parker et al. 2006; Nuñez et al. 2009). For example, the facilitative effect of mutualisms may be stronger in the nonnative range than the negative effects of belowground pathogens (Reinhart and Callaway 2006).

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Lupinus polyphyllus Lindl. (Fabaceae; garden lupine), is a perennial herb native to western and northeast North America. The species has become highly invasive throughout parts of Europe, Australia, and South America following its introduction to these ranges. *L. polyphyllus* was initially introduced to new areas for ornamental purposes. The species is classified as nonnative to northeastern Ohio. Yet its native range includes the adjacent Michigan and Canadian land north of Lake Erie. Despite the ability of the species to spread into Cleveland habitats, it has not yet been established as an invasive species. Various reasons may explain the inability of this species to invade on the local level.

Consistent with the enemy release hypothesis, the relatively humid local climate may be one of the factors limiting the spread of the garden lupine (hereafter lupine) due to increased interactions with fungal pathogens in the soil. Lupine is especially known to be susceptible to fungal powdery mildew, caused by pathogen species of the genus *Erysiphe* (Bradshaw et al. 2022). Previous studies have investigated the use of a limited selection of pathogens as biocontrol agents. The parasites *Fusarium heterosporum* and *Didymella pisi* have been found to lethally cause crown and root rot in *L. polyphyllus* (Harvey et al. 1996). Although the 2 pathogens may effectively control invasive lupines, they may also spread to non-target plants. Investigations involving microbial communities require a comprehensive scope. In only experimenting with a single mutualist or pathogen, these studies lack representation of the full complexity of belowground communities and their interactions with plants (Callaway et al. 2011). A broader approach is necessary when placing the species in the context of various nonnative ranges.

Enhanced mutualisms belowground may also play a role in *L. polyphyllus* range expansion, as the species forms root nodules that provide hospitable environments for rhizobia. There is evidence that *L. polyphyllus* also relies on, and is affected by, mutualistic belowground interactions (Kalske et al. 2022). Many invasive species greatly depend on mutualisms in their nonnative ranges for the naturalization process (Richardson et al. 2000a). Mutualisms with soil biota are known to support plant invasions because they promote resource acquisition. A positive feedback loop often exists between the 2 symbionts. Invasive plants can alter the local environment to increase suitability for themselves and mutualists. In return, mutualistic microbes continue to support the growth and spread of invasive plants. These mutualisms often influence the overall effect of soil biota more in nonnative ranges than in the native range (Callaway et al. 2004). The resulting positive feedback loops increase the invasive potential of plant species.

The response of invaders to soil biota may be further explored using morphological traits related to the concept of the root economy, generating insights into mechanisms. Bergmann et al. (2020) highlights the concept of a plant's *root economy*, in which carbon is the main currency. The primary resource to be acquired aboveground is sunlight, which can only be achieved through the tissue of the plant. Plant species have shown the ability to shift their belowground mode of resource acquisition by establishing symbiotic relationships with soil biota, otherwise known as *outsourcing*. Outsourcing requires carbon investment into mutualisms and other expenditures toward growing specific roots. Roots with larger diameters or increased density are more efficient in mutualisms. The second technique for resource acquisition is coined as the faster, *do it yourself* (DIY) method. The plant may produce "cheaper" roots with shorter lifespans, but they are also more productive in absorbing nutrients directly from the soil due to an increase in surface area relative to root volume. Growing roots for the outsourcing approach tends to be more costly in terms of carbon investment than the fast DIY approach, characterized by thinner roots (Stock et al. 2021). Outsourcing is considered to be the slower and more conservative method of resource acquisition that utilizes roots with longer lifespans (Reich 2014). The concept of a root economy is defined by an inverse relationship between specific root length (SRL) and both root diameter and root tissue density (RTD) (Kong et al. 2019). An outsourcing plant will have shorter roots, but increased root diameter, or density. A DIY plant will have longer roots, but a smaller root diameter and reduced density. A plant's root characteristics may be indicative of the belowground conditions and presence of mutualists.

A greenhouse experiment was conducted to test the alternative, but not mutually exclusive, hypotheses of enemy release or enhanced mutualisms. A lack of knowledge exists on the relative importance between parasitic pathogens and mutualists as related to invasive success of the species. Therefore, the non-specific fungicide ZeroTol®, which kills both fungi and bacteria, was used to observe the general effect of a reduction in soil biota on *L. polyphyllus*. Total biomass has traditionally been used as a simple and quantifiable measurement that is generally indicative of fitness (Younginger et al. 2017). If belowground pathogens limit the range of *L. polyphyllus*, the fungicide treatment is predicted to result in greater total biomass. Alternatively, if soil mutualists have a larger overall influence on plant fitness, a lower total biomass in the fungicide treatment is expected. A change in the number of root nodules in treated lupines will indicate whether the interactions with mutualist rhizobia are also affected by the fungicide treatment, as well as if the species is generally dependent on soil mutualists in a nonnative range. If *L. polyphyllus* responds to mutualist loss with outsourcing, an increase in root density in the fungicide treatment is predicted (Comas et al. 2014; Kumar et al. 2019; McCormack and Iverson 2019). Alternatively, if *L. polyphyllus* is a DIY species, an increase in root length in the fungicide treatment should be observed.

METHODS AND MATERIALS

A greenhouse experiment was conducted with 3 seed populations of *L. polyphyllus* from the introduced range, and 2 experimental treatments: fungicide and control. The seeds were collected from 3 field populations of *L. polyphyllus* from southwestern and central Finland in July 2020 (Table 1). Each collection consisted of seeds from 12 maternal plants that were several meters apart. The populations grew in sandy soils on wastelands or road verges, with distances between them ranging from 16 to 500 km (9.94 to 310.69 mi). The exact age of the populations is unknown, but they have all existed since at least 2010 and consist of hundreds of lupine individuals. The population cover was between 96 to 420 m^2 (1,033.34 to 4,520.84 ft^2). In all populations, the study species was dominating (cover > 50%), being the only species in the genus *Lupinus*. Other common species in the populations were mostly tall grasses in the family of Poaceae.

Pots were filled with a potting mix of one-to-one peat moss to perlite. Using a razor blade, 20 seeds from each of the 3 source populations were lightly nicked to remove a part of the seed coat and encourage germination. Three petri dishes for each source population were covered by moistened filter paper. All scarified seeds were placed in a dish corresponding to the source population, with approximately the same space between each

Table 1
***L. polyphyllus* source population characteristics**

Population	Coordinates	Habitat type	Population size	Soil type
Population 1	60.370631 N 22.259363 E	Road verge	96 m^2	Sandy
Population 2	60.507882 N 22.39552 E	Wasteland	420 m^2	Sandy loam/ clay
Population 3	63.358673 N 27.750746 E	Wasteland	240 m^2	Sandy loam

seed. Each dish was watered once more before being placed into the growth chamber for germination for 1.5 weeks.

An A-2 design type block configuration was produced by randomizing individuals within 5 constrained blocks labeled A through E (Hurlbert 1984). Each block contained 12 individual pots split evenly between the 2 treatments: the control and experimental group. The study tested a total of 60 lupines with 30 replicates per each treatment. Following transplantation on June 22, 2021, the seedlings were watered every couple of days as needed so that the soil never dried out completely. The seedlings were monitored and failed ones were replaced by a new seedling of the same source population from the growth chamber for approximately 1-week after transplantation.

On July 27th, soils were collected from an existing population of *L. polyphyllus* in Cleveland, Ohio, for inoculation. *Matricaria chamomilla* and *Wisteria frutescens* individuals were also present within 1 m of the soil collection at the base of *L. polyphyllus* roots. The soil was very rich and dark, with little clay, but was still sieved to a fine texture. The purpose of the inoculum was to provide live soil biota to simulate a true outdoor population within the controlled greenhouse manipulation. One tablespoon of soil inoculum was placed around the base of each plant where the roots were located. All pots were then watered thoroughly following inoculation and were left for 2 weeks with occasional watering as needed.

Following the rest period of 2 weeks post-inoculation, the experimental group of lupines were drenched with a diluted solution of ZeroTol fungicide (36.97 mL ZeroTol per gallon of water). Lupines in the control group continued to only receive water. The fungicide drenches were reapplied weekly for 2 months.

The lupines were all harvested on October 4th and 5th. The only exception was pot 34, a control plant, which was left unharvested and was excluded from data collection because it was previously damaged from aphid herbivory. The roots of each plant were cleaned and separated from the shoots. The number of root nodules on each lupine were manually counted (Fig. 1). The program WinRHIZO™ was used to

FIGURE 1. Root nodules observed on a harvested lupine before drying

take measurements of root length, root diameter, and root volume. After the initial data collection, all parts of the lupines were stored and left to dry in a drying oven at 120 °C for a week. After the parts of the lupines thoroughly dried, the roots and shoots were individually weighed for their dry biomasses. *Root tissue density* (RTD) was then calculated as the quotient of root mass over root volume.

Specific root length (SRL): the ratio of root length over root mass is also reported as it is often thought to reflect the state of a root economy (Ostonen et al. 2007).

$$SRL = \frac{Root\ length}{Root\ dry\ mass} \tag{1}$$

Studying SRL helps to determine whether the species can compensate for lost mutualist services through belowground structural changes. For instance, a reduction in SRL suggests that *L. polyphyllus* redirects its energy, typically spent elongating the root system, to the outsourcing approach. Alternatively, an increase in SRL suggests that a DIY method of resource acquisition proves more productive.

Statistical Analyses

Statistical analyses were performed using R 4.1.1 (R Core Team 2021). Total biomass was calculated as the sum of shoot and root biomass, including the nodules. Root nodules were square root transformed and SRL and RTD were log-transformed to normalize the distribution. Multivariate mixed effects models were conducted for the response variables: total biomass, root mass, root nodules, root length, root diameter, RTD, and SRL using the "lmer function" (lme4 package). Treatment, source population, and their interaction were tested for as fixed effects. Block was included in the models as a random effect. Total mass was included as a covariate in the models for root nodules, root length, root diameter, RTD, and SRL to distinguish between the effects of reduced total mass versus reduced soil biota from the treatment. The function "stepAIC" (MASS package) was used to simplify the models (i.e., omit non-significant variables) for maximized statistical power. The random effect of block was also excluded if it did not contribute to any variation in the response variable. The removal of block for total mass, root mass, root length, RTD, and SRL converted them to simple linear (fixed effects) models with Gaussian error structure. Statistical significance for the fixed effects was determined with the "Anova" function (car package) (Table 2). For significant interactions between treatment and source population, pairwise differences among the means were tested with a Tukey's test using the "emmeans" function (emmeans package).

RESULTS

Although the source population did not have a significant effect on the total biomass of lupines, there was a significant interaction between the treatment and the source population (Table 2). Mean total biomass of lupines treated with fungicide tended to be smaller compared to those that were not drenched with ZeroTol in populations 2 and 3, with the difference being statistically significant in population 3. Population 1 did not respond to the fungicide treatment (Fig. 2a).

Lupines treated with ZeroTol fungicide had a marginally significant, lower mean root mass than the control group, with the source population having no effect on root mass (Table 2). However, there was again a significant interaction between treatment and source population on root mass (Table 2). Similar to total biomass, ZeroTol treatment tended to reduce mean root mass in populations 2 and 3, but tended to increase it in population 1. None of the multiple pairwise comparisons were significant at $p < 0.05$ (Fig. 2b), probably due to the small sample size.

The average number of root nodules found on ZeroTol-treated lupines was 29.62% less than that of the control group regardless of the source population (Table 2; Fig. 3). Source population significantly affected the number of root nodules, but no interaction was present between treatment and source population (Table 2). Population 2 produced the fewest root nodules overall, while population 3 produced the most. Population 3 grew 55% more root nodules than population 2. Total mass also had a significant, positive effect on the number of root nodules (Table 2; intercept = 2.64, slope = 4.75).

Treatment had an independent significant effect on root length (Table 2), with the average root length of the ZeroTol group being 30.13% less than that of the control group (Fig. 4). Total mass was positively associated with root length (Table 2; intercept = 198.95, slope = 206.18).

The treatment, source population, and total mass all had significant effects on root diameter (Table 2). The average root diameter in the ZeroTol group was 20.46% smaller than the control (Fig. 5). Source population 1 had the smallest average root diameter, while source population 3 had the largest average root diameter, which was 21.73% larger than that of source population 1. Total mass was positively related to root diameter (intercept = 0.49, slope = 0.33).

The mean root tissue density (RTD) of the ZeroTol group was 108% greater than that of the control group (Fig. 6; Table 2). The source population did not significantly affect the RTD, and there was no interaction present between treatment and source population (Table 2).

Lupines treated with the fungicide had a 1.75% lower mean specific root length (SRL) than lupines in the control group (Fig. 7). No source population effect was found, but the total mass was negatively related to SRL (Table 2; intercept = 7.81, slope = -1.048).

Table 2
Results from the simplified linear (mixed) models for the perennial herb *Lupinus polyphyllus*

Response	Predictor	DF^	F-ratio	*p*-value
Total biomass	Treatment	1	8.72	0.0048 **
	Source population	2	0.84	0.440
	P×T	2	4.17	0.021 *
Root mass	Treatment	1	3.54	0.066
	Source population	2	0.41	0.670
	P×T	2	4.80	0.013 *
Root nodules	Treatment	1	4.29	0.044 *
	Source population	2	4.98	0.012 *
	Total mass	1	46.89	7.31×10^{-9} ***
Root length	Treatment	1	5.23	0.026 *
	Total mass	1	40.35	4.05×10^{-8} ***
Root diameter	Treatment	1	7.76	0.0076 **
	Source population	2	3.46	0.039 *
	Total mass	1	60.96	2.83×10^{-10} ***
RTD	Treatment	1	14.86	0.00031 ***
	Source population	2	2.05	0.140
SRL	Treatment	1	7.10	0.010 *
	Source population	2	0.55	0.580
	Total mass	1	26.51	3.77×10^{-6} ***

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

^ DF = degrees of freedom

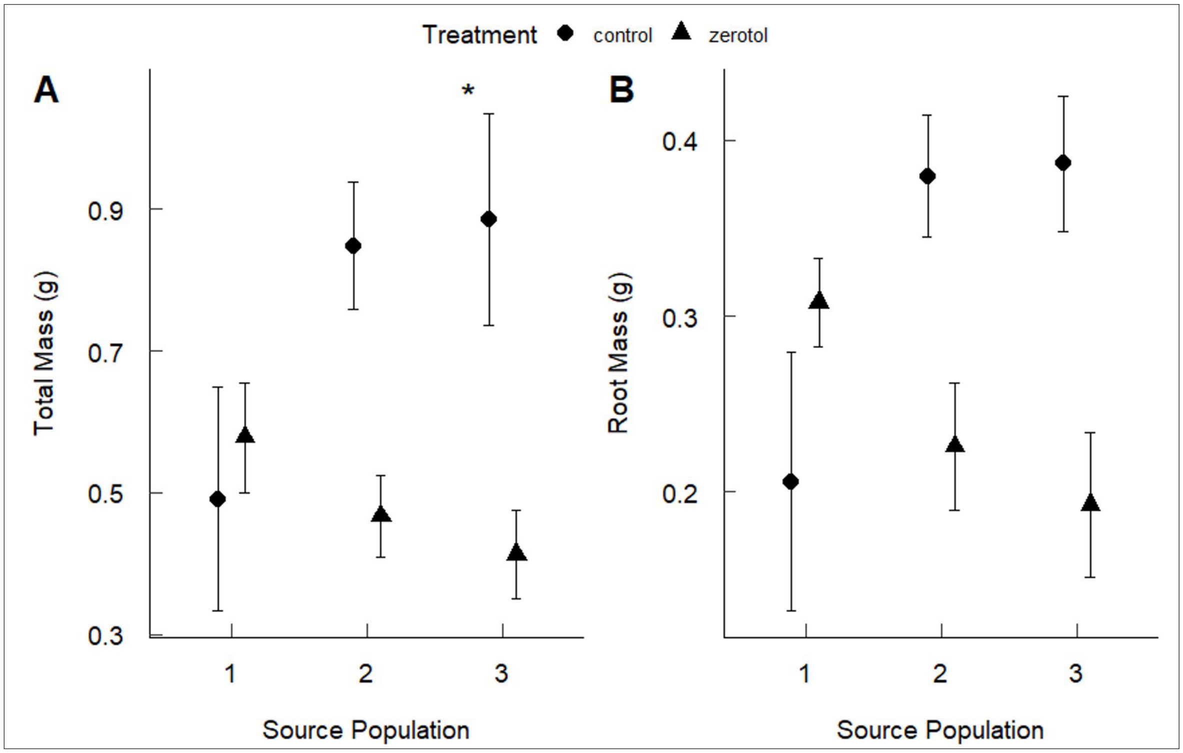

FIGURE 2. Estimated mean (A) total biomass and (B) root mass (± SE) between source populations of *Lupinus polyphyllus* within each treatment group. * Indicates statistical significance ($p < 0.05$, pairwise comparisons).

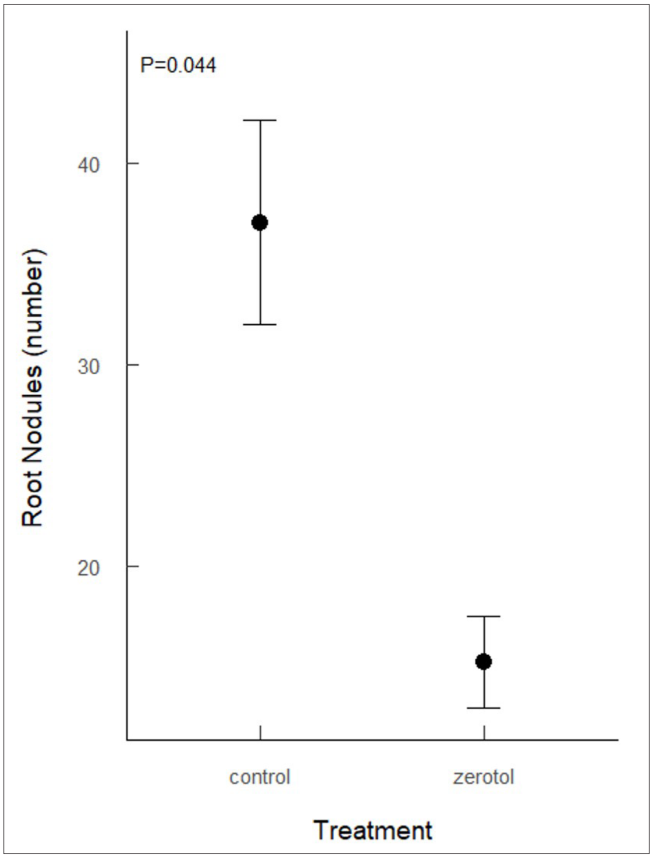

FIGURE 3. Estimated mean number of root nodules (± SE) for the control and ZeroTol groups of *Lupinus polyphyllus*

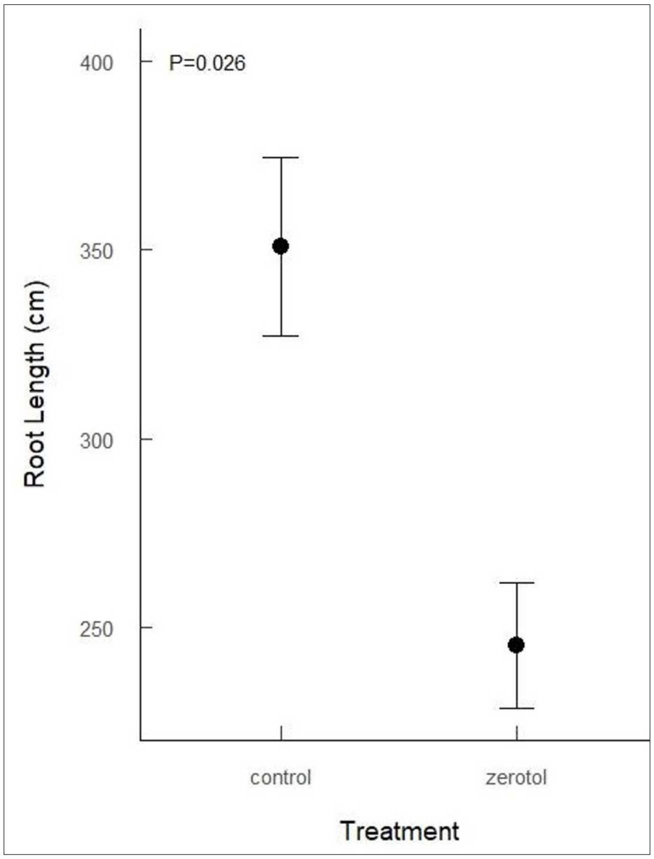

FIGURE 4. Estimated mean root length (± SE) for the control and ZeroTol groups of *Lupinus polyphyllus*

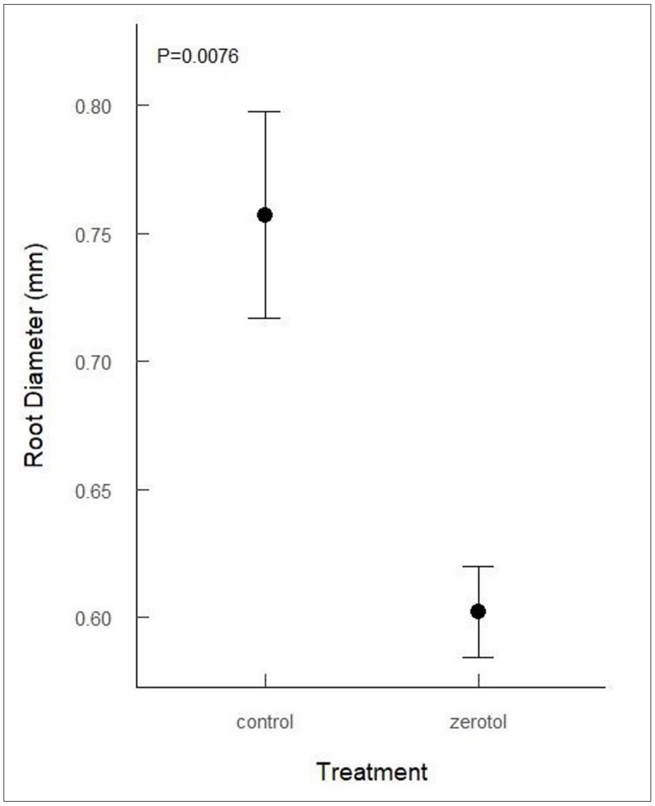

FIGURE 5. Estimated mean root diameter (± SE) for the control and ZeroTol groups of *Lupinus polyphyllus*

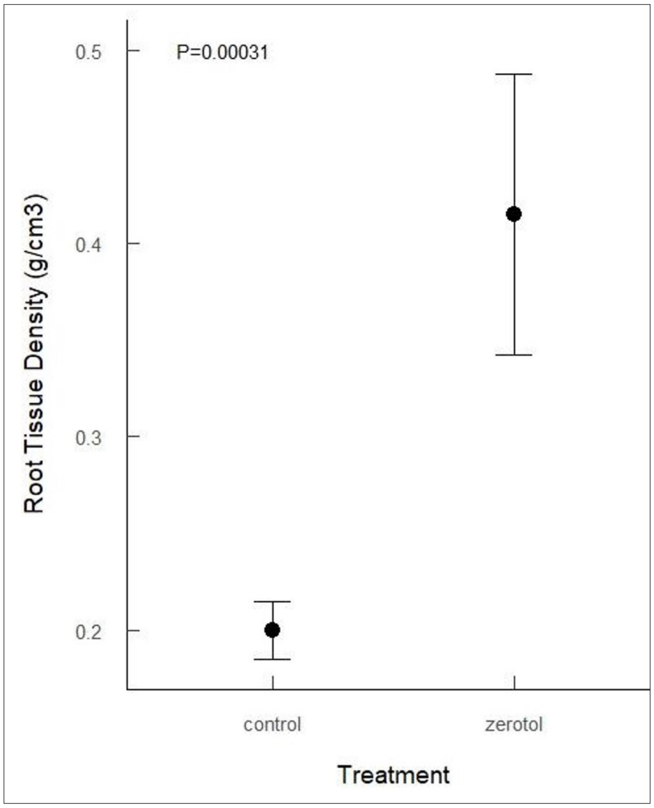

FIGURE 6. Estimated mean root tissue density (± SE) for the control and ZeroTol groups of *Lupinus polyphyllus*

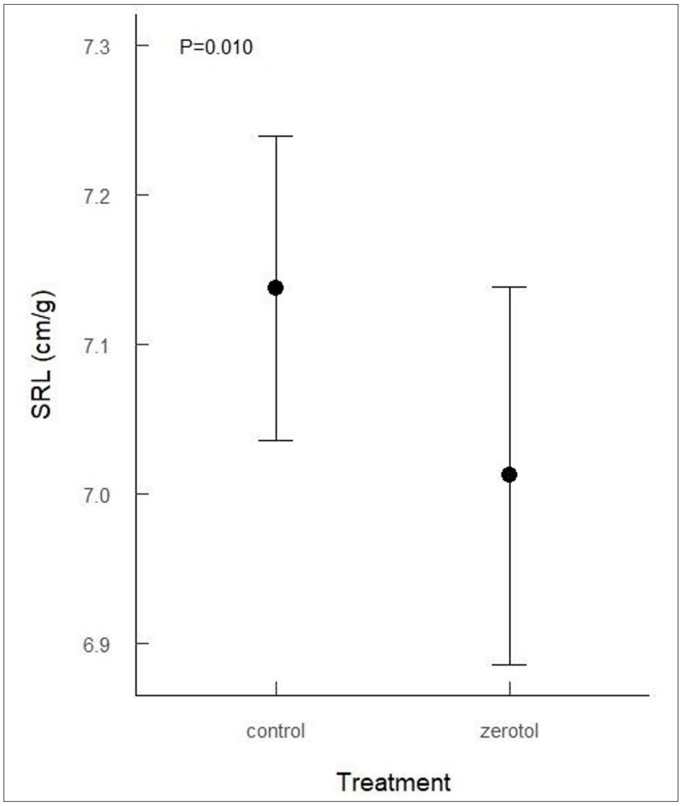

FIGURE 7. Estimated mean specific root length (SRL) (± SE) for the control and ZeroTol groups of *Lupinus polyphyllus*

DISCUSSION

The net negative effect of a general fungicide on local soil biota and *L. polyphyllus* suggested that *L. polyphyllus* benefits from mutualists in the soil. Belowground mutualists might thus be expected to influence invasiveness for this species outside its native range, though comparisons across range are needed. In the current study, the enemy release hypothesis was not supported because the fungicide did not exhibit a positive effect on the growth of the lupines (Maron et al. 2013). Additional work in the species invasive range, however, is still needed to rule out a role of enemy release overall. The enhanced mutualisms hypothesis may be better confirmed by quantifying a more positive effect of mutualisms on lupines than on native plants. Sun and He (2010) observed such a facilitative effect of mutualisms on the invasive herb *Solidago canadensis* compared to the native grass *Stipa bungeana*, and quantified similar support for the enhanced mutualisms hypothesis.

The presence of root nodules suggests that the species does rely on a mutualistic interaction with nitrogen-fixing bacteria because the formation of the structures requires energy expenditure and the input of other resource elements. Nodulation is a form of plant outsourcing, and species that typically invest in this method of resource acquisition are better suited to areas with nitrogen-deficient soil. Otherwise, in nitrogen-rich soils, the carbon and energy investments of the plant are better spent on fast methods of resource acquisition, characterized by lower root density (Leuschner et al. 2013). Nodulation is also known as a phosphorus sink in plants and the trade-off with rhizobia requires the input of many additional micronutrients, such as iron (Valentine et al. 2017; Kasper et al. 2019). Consequently, it is a costly process that only proves worthwhile when the plant is in need of increased nitrogen acquisition.

Lupines treated with fungicide drenches had a significantly lower number of root nodules compared to those from the control group. Although the ZeroTol is known as a general fungicide, it can be concluded that it specifically diminished the abundance of beneficial rhizobia in the soil as well. This loss in mutualists resulted in the lupines forming much fewer nodules. As a result, the lupines also lost their supply of nitrogen that is typically provided by the mutualism, and their productivity became stunted by this newly developed nutrient deficiency.

If the soil used had supplied sufficient nitrogen, extending and thinning out the roots could have been a more effective method of compensation regardless of the fungicide treatment. These alterations to root structure could potentially increase nutrient absorption directly from the soil. The lupines were planted in a soil mixture of one-to-one peat moss and perlite, which are nutrient-poor materials. Greater investment in outsourcing to find additional mutualisms was an effective method of compensation due to the poor nutritional quality of the soil. The reduction in available mutualisms resulted in increased competition for the remaining interactions with rhizobia, so this shift toward the conservative outsourcing acquisition method proved more productive.

The significant increase in lupine RTD corresponds to a conservative outsourcing strategy (Ryser 1996; Anderson et al. 2003; McCormack et al. 2012). The increased RTD also aligns with the decreased SRL, since increases in these traits indicate opposite methods of resource acquisition. The decrease in root length further supports a shift toward outsourcing in the ZeroTol-treated group. The reducing treatment effect on root diameter is an exception to the suggested shift. Smaller root diameter typically correlates with the DIY approach. However, total mass was also found to have a direct effect on root diameter, length, and SRL. The overall reduction in total mass may have played a role in the contradictory root diameter result. RTD was the only root trait response not found to be affected by total mass. Due to this lack of total mass effect, RTD may be the most indicative of the isolated treatment effect and state of the root economy. Previous literature has similarly suggested that RTD is an especially reliable predictor of root lifespan and method of resource acquisition in herbaceous perennials (Kramer-Walter et al. 2016; Sun et al. 2016). The roots were less dense in the control group, suggesting that *L. polyphyllus* traditionally relies more on a DIY approach when not subject to a ZeroTol treatment. The root economy of the species was never fully following the DIY method because of the presence of root nodules and its reliance on rhizobia in the control group. Lupines are known to readily associate with many varieties of rhizobia and typically depend on nitrogen fixation for the bulk of their nitrogen supply (Eckhardt et al. 1931; Stępkowski et al. 2018; Wysokiński and Kuziemska 2019). The fungicide treatment simply led to a shift in the root economy, to a greater reliance on the outsourcing approach than the typical reliance on DIY.

Phenotypic Plasticity

In addition to the mutualisms that likely aid *L. polyphyllus* in its invasive ranges, the species showed substantial plasticity in terms of phenotypic differences in response to the fungicide treatment. Phenotypic plasticity is strictly defined as the ability for multiple phenotypes to be expressed from a single genotype given different environmental conditions (Nürnberger 2013). Although this experimental setup was not designed to test for phenotypic plasticity, differing responses from the 3 populations support the presence of within-species variation. The findings are broadly suggestive of plasticity; the species can respond in different ways to new environmental conditions.

Considering that the treated lupines still managed to grow despite the fungicide treatment, the species displayed the ability to change and *partially* compensate for deficiencies and environmental stresses. Like the conclusions drawn on the synergistic relationship between mutualists and invaders, it has been found that plasticity in root morphology and physiology is essential to the success of an invasive plant because there is often competition belowground (Casper and Jackson 1997). Plant growth and success depend on the sum of root and shoot competitive abilities, so the observed ability of the lupine to alter their root systems in response to environmental change reveals its potential to thrive in different conditions. In addition to plasticity in root architecture, the ability to shift between methods of resource acquisition (that are indicated by root traits) is also suggested to play a role in the success of invasive species in low-resource environments (Funk 2013). The plasticity in root economy of this species may be an especially relevant factor to its invasibility because it has been suggested that the competitive abilities of invasive plants may be more relevant to their success over native species than is tolerance to environmental stresses (Schultheis and MacGuigan 2018). Therefore, the success of *L. polyphyllus* as an invader in many ecosystems may be attributed to this sort of plasticity in root architecture and functioning, as it allows for increased tolerance of new conditions through belowground mechanisms.

Future Investigations

For future investigation, it would be helpful to identify the microorganisms that were present in the soil inoculum and compare them to soils from native and invasive ranges. This additional study may provide insight into specific microbial constituents that facilitate the invasion of the species. Because a general fungicide was used for treatment, the effects it had on other, specific, belowground microorganisms cannot be determined. It can be concluded through quantitative measurement of the root nodules that the fungicide treatment reduced rhizobia, but no changes in other potential mutualists or harmful pathogens were directly observed. Along with fungal pathogens that thrive in humid environments, fungal mutualists like arbuscular mycorrhizal fungi were likely present as well.

The population-specific responses to fungicide treatment observed here could be indicative of the relevance of intraspecific genetic variation, which has been suggested to increase the success of plant invasions (Forsman 2013). As much as genetic variation can promote the stability of desired plant communities, it can also be an unwanted source of support for invasive species and their spread. It is possible that a genetically determined tolerance to different conditions may allow invasive species to thrive in their nonnative ranges. Further studies on the significance of intraspecific genetic variation within invasive lupine communities could be beneficial to future control methods. Additionally, considering that the species demonstrated plasticity, any future method of control may need to be especially pervasive to ensure that the lupines could not effectively mitigate its effects. Further investigation should supplement this study by comparing the interactions of the species between its native and invasive ranges. These comparisons may improve comprehension of invasive mechanisms, quantification of the enhanced mutualisms hypothesis, and identification of characteristics that allow for invasive success.

Conclusions

Overall, this study reveals the importance of belowground mutualisms in the success of *L. polyphyllus*. The significant decreases in nodulation and total biomass due to fungicidal treatment support the major mechanistic role of rhizobia in determining *L. polyphyllus* plant health and performance. Therefore, there is evidence consistent with the enhanced mutualisms hypothesis facilitating this particular biological invasion. Plasticity and intraspecific genetic variation may also contribute to the success of *L. polyphyllus* in its nonnative ranges, and further investigation is warranted to confirm the mechanistic role of these factors for this invasive species.

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LITERATURE CITED

Allen WJ, Young RE, Bhattarai GP, Croy JR, Lambert AM, Meyerson LA, Cronin JT. 2015. Multitrophic enemy escape of invasive *Phragmites australis* and its introduced herbivores in North America. Biol Invasions. 17:3419-3432. https://doi.org/10.1007/s10530-015-0968-2

Anderson LJ, Comas LH, Lakso AN, Eissenstat DM. 2003. Multiple risk factors in root survivorship: a 4-year study in Concord grape. New Phytol. 158(3):489-501. https://doi.org/10.1046/j.1469-8137.2003.00757.x

Bergmann J, Weigelt A, Van der Plas F, Laughlin DC, Kuyper TW, Guerrero-Ramirez N, Valverde-Barrantes OJ, Bruelheide H, Freschet GT, Iverson CM, Kattge J, et al. 2020. The fungal collaboration gradient dominates the root economics space in plants. Sci Adv. 6(27). https://doi.org/10.1126/sciadv.aba3756

Bradshaw M, Braun U, Götz M, Jurick W 2nd. 2022. Phylogeny and taxonomy of powdery mildew caused by *Erysiphe* species on *Lupinus* hosts. Mycologia. 114(1):76-88. https://doi.org/10.1080/00275514.2021.1973287

Callaway RM, Bedmar EJ, Reinhart KO, Gómez Silvan C, Klironomos J. 2011. Effects of soil biota from different ranges on *Robinia* invasion: acquiring mutualists and escaping pathogens. Ecology. 92(5):1027-1035. https://doi.org/10.1890/10-0089.1

Callaway RM, Thelen GC, Rodriguez A, Holben WE. 2004. Soil biota and exotic plant invasion. Nature. 427:731-733. https://doi.org/10.1038/nature02322

Casper BB, Jackson RB. 1997. Plant competition underground. Ann Rev Ecol Syst. 28:545-570. https://doi.org/10.1146/annurev.ecolsys.28.1.545

Comas LH, Callahan HS, Midford PE. 2014. Patterns in root traits of woody species hosting arbuscular and ectomycorrhizas: implications for the evolution of belowground strategies. Ecol Evol. 4(15):2979-2990. https://doi.org/10.1002/ece3.1147

Eckhardt MM, Baldwin IL, Fred EB. 1931. Studies of the root-nodule organism of *Lupinus*. J Bacteriol. 21(4):273-285. https://doi.org/10.1128/jb.21.4.273-285.1931

Forsman A. 2013. Effects of genotypic and phenotypic variation on establishment are important for conservation, invasion, and infection biology. P Natl Acad Sci - Biol. 111(1):302-307. https://doi.org/10.1073/pnas.1317745111

Funk JL. 2013. The physiology of invasive plants in low-resource environments. Conserv Physiol. 1(1). https://doi.org/10.1093/conphys/cot026

Harvey IC, Seyb AM, Warren AFJ, Van Den Ende H. 1996. The biological control of Russell lupin in riverbeds with endemic plant pathogens. Proc 49th NZ Plant Protection Conf. 49:119-125. https://doi.org/10.30843/nzpp.1996.49.11441

Hurlbert SH. 1984. Pseudoreplication and the design of ecological field experiments. Ecol Monogr. 54(2):187-211. https://doi.org/10.2307/1942661

Kalske A, Blande JD, Ramula S. 2022. Soil microbiota explain differences in herbivore resistance between native and invasive populations of a perennial herb. J Ecol. 110(11):2649-2660. https://doi.org/10.1111/1365-2745.13975

Kasper S, Christoffersen B, Soti P, Racelis A. 2019. Abiotic and biotic limitations to nodulation by leguminous cover crops in south Texas. Agriculture. 9(10):209. https://doi.org/10.3390/agriculture9100209

Keane RM, Crawley MJ. 2002. Exotic plant invasions and the enemy release hypothesis. Trends Ecol Evol. 17(4):164-170. https://doi.org/10.1016/S0169-5347(02)02499-0

Kong D, Wang J, Wu H, Valverde-Barrantes OJ, Wang R, Zeng H, Kardol P, Zhang H, Feng Y. 2019. Nonlinearity of root trait relationships and the root economics spectrum. Nat Commun. 10(article 2203). https://doi.org/10.1038/s41467-019-10245-6

Kramer-Walter KR, Bellingham PJ, Millar TR, Smissen RD, Richardson SJ, Laughlin DC. 2016. Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic spectrum. J Ecol. 104(5):1299-1310. https://doi.org/10.1111/1365-2745.12562

Kumar A, Shahbaz M, Koirala M, Blagodatskaya E, Seidel SJ, Kuzyakov Y, Pausch J. 2019. Root trait plasticity and plant nutrient acquisition in phosphorus limited soil. J Plant Nutr Soil Sci. 182(6):945-952. https://doi.org/10.1002/jpln.201900322

Leuschner C, Gebel S, Rose L. 2013. Root trait responses of six temperate grassland species to intensive mowing and NPK fertilisation: a field study in a temperate grassland. Plant Soil. 373:687-698. https://doi.org/10.1007/s11104-013-1836-4

Maron JL, Klironomos J, Waller L, Callaway RM. 2013. Invasive plants escape from suppressive soil biota at regional scales. J Ecol. 102(1):19-27. https://doi.org/10.1111/1365-2745.12172

McCormack ML, Adams TS, Smithwick EAH, Eissenstat DM. 2012. Predicting fine root lifespan from plant functional traits in temperate trees. New Phytol. 195(4):823-831. https://doi.org/10.1111/j.1469-8137.2012.04198.x

McCormack ML, Iversen CM. 2019. Physical and functional constraints on viable belowground acquisition strategies. Front Plant Sci. 10:1215. https://doi.org/10.3389/fpls.2019.01215

Mitchell CE, Power AG. 2003. Release of invasive plants from fungal and viral pathogens. Nature. 421:625-627. https://doi.org/10.1038/nature01317

Nuñez MA, Horton TR, Simberloff D. 2009. Lack of belowground mutualisms hinders Pinaceae invasions. Ecology. 90(9):2352-2359. https://doi.org/10.1890/08-2139.1

Nürnberger B. 2013. Ecological genetics. In: Levin SA, editor. Encyclopedia of biodiversity. 2nd ed. Amsterdam (NL): Academic Press. p. 714-731. https://doi.org/10.1016/B978-0-12-384719-5.00038-1

Ostonen I, Püttsepp Ü, Biel C, Alberton O, Bakker MR, Lõhmus K, Majdi H, Metcalfe D, Olsthoorn AFM, Pronk A, et al. 2007. Specific root length as an indicator of environmental change. Plant Biosyst. 141(3):426-442. https://doi.org/10.1080/11263500701626069

Parker MA, Malek W, Parker IM. 2006. Growth of an invasive legume is symbiont limited in newly occupied habitats. Divers Distrib. 12(5):563-571. https://doi.org/10.1111/j.1366-9516.2006.00255.x

R Core Team. 2021. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing. https://www.r-project.org/

Reich PB. 2014. The world-wide 'fast–slow' plant economics spectrum: a traits manifesto. J Ecol. 102(2):275-301. https://doi.org/10.1111/1365-2745.12211

Reinhart KO, Callaway RM. 2006. Soil biota and invasive plants. New Phytol. 170(3):445-457. https://doi.org/10.1111/j.1469-8137.2006.01715.x

Reinhart KO, Packer A, Van der Putten WH, Clay K. 2003. Plant–soil biota interactions and spatial distribution of black cherry in its native and invasive ranges. Ecol Lett. 6(12):1046-1050. https://doi.org/10.1046/j.1461-0248.2003.00539.x

Richardson DM, Allsopp N, D'Antonio CM, Milton SJ, Rejmánek M. 2000a. Plant invasions – the role of mutualisms. Biol Rev. 75(1):65-93. https://doi.org/10.1111/j.1469-185X.1999.tb00041.x

Richardson DM, Pyšek P, Rejmánek M, Barbour MG, Panetta FD, West CJ. 2000b. Naturalization and invasion of alien plants: concepts and definitions. Divers Distrib. 6(2):93-107. https://doi.org/10.1046/j.1472-4642.2000.00083.x

Ryser P. 1996. The importance of tissue density for growth and life span of leaves and roots: a comparison of five ecologically contrasting grasses. Funct Ecol. 10(6):717-723. https://doi.org/10.2307/2390506

Schultheis EH, MacGuigan DJ. 2018. Competitive ability, not tolerance, may explain success of invasive plants over natives. Biol Invasions. 20:2793-2806. https://doi.org/10.1007/s10530-018-1733-0

Stępkowski T, Banasiewicz J, Granada CE, Andrews M, Passaglia LMP. 2018. Phylogeny and phylogeography of rhizobial symbionts nodulating legumes of the tribe Genisteae. Genes. 9(3):163. 25p. https://doi.org/10.3390/genes9030163

Sun ZK, He WM. 2010. Evidence for enhanced mutualism hypothesis: *Solidago canadensis* plants from regular soils perform better. PloS One. 5(11):e15418. https://doi.org/10.1371/journal.pone.0015418

Sun K, McCormack LM, Li L, Ma Z, Guo D. 2016. Fast-cycling unit of root turnover in perennial herbaceous plants in a cold temperate ecosystem. Sci Rep. 6(article 19698). https://doi.org/10.1038/srep19698

Stock SC, Koester M, Boy J, Godoy R, Nájera F, Matus F, Merino C, Abdallah K, Leuschner C, Spielvogel S, et al. 2021. Plant carbon investment in fine roots and arbuscular mycorrhizal fungi: a cross-biome study on nutrient acquisition strategies. Sci Total Environ. 781. https://doi.org/10.1016/j.scitotenv.2021.146748

Valentine AJ, Kleinert A, Benedito VA. 2017. Adaptive strategies for nitrogen metabolism in phosphate deficient legume nodules. Plant Sci. 256:46-52. https://doi.org/10.1016/j.plantsci.2016.12.010

Wolfe LM. 2002. Why alien invaders succeed: support for the escape-from-enemy hypothesis. Am Nat. 160(6):705-711. https://doi.org/10.1086/343872

Younginger BS, Sirová D, Cruzan MB, Ballhorn DJ. 2017. Is biomass a reliable estimate of plant fitness? Appl Plant Sci. 5(2). https://doi.org/10.3732/apps.1600094

Wysokiński A, Kuziemska B. 2019. The sources of nitrogen for yellow lupine and spring triticale in their intercropping. Plant Soil Environ. 65(3):145-151. https://doi.org/10.17221/644/2018-PSE