

**The role of soil microbiota in the interaction between the invasive
plant *Lupinus polyphyllus* and three native herbs**

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Master's thesis
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ABSTRACT

Species invasions are a significant element of global change. There are several mechanisms in which invasive species dominate the natives in their invaded range. One of those mechanisms is plant-soil feedback. In my master's thesis I assessed the role and importance of soil microbiota in the interactions between the herbaceous invader *Lupinus polyphyllus* (garden lupin) and three native herbs commonly found in human-affected habitats representing different attributes: *Trifolium repens* (white clover), *Centaurea cyanus* (cornflower) and *Taraxacum spp.* (dandelion). I evaluated the effects of competition and the presence of soil microbes by growing *L. polyphyllus* together with each of the three native plant species in a common garden pot experiment. A soil inoculum crafted from around lupin roots was used to introduce soil microbes into the sterilized substrate. The results of this study suggest that competition (the presence of *L. polyphyllus*) has an effect on the studied native herbs' growth and these effects vary among species. The effects were not all negative, as *T. repens* grew more shoots when growing with *L. polyphyllus* and *C. cyanus* was unaffected. *Taraxacum spp.* however, showed a significant decrease, especially in root growth. Soil microbes associated with *L. polyphyllus* also affected the growth of the studied natives, but species responded to the microbe addition similarly, growing less roots. Also, in turn, the species *L. polyphyllus* was growing with affected its growth. The biomass of *L. polyphyllus* was negatively affected by the presence of *C. cyanus*. *Lupinus polyphyllus* nodule growth was tentatively affected by microbes and competition together, as with *Taraxacum spp.* the microbes seemed to affect the growth of the nodules negatively. I conclude that the benefits of mutualistic soil microbes may vary with the species the invader is competing with. I also suggest that the effects of soil microbes, both positive and negative, may differ in different competition settings. Hopefully, these results can add to the knowledge of mechanisms of invasions, important in planning conservational efforts and screening for possible invaders.

KEYWORDS: *Lupinus polyphyllus*, invasive species, plant-soil feedback, competition, plant ecology

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1 INTRODUCTION

A number of human activities have altered the world's ecosystem composition and dynamics. Alongside land transformation and habitat fragmentation, species invasions are widely recognized as one of the major drivers of global biodiversity loss (e.g., Vitousek et al. 1997). Biodiversity, in turn, has been recognized of being one of the most significant factors facilitating the stability and productivity, affecting the nutrient dynamics and (again) the invasibility of ecosystems (Tilman 1999). As we face the biggest environmental crisis in our history, all of its contributing factors require our attention.

1.1 Species invasions – definition, mechanisms and effects

Terminology around many ecological phenomena can be varying, ambiguous and context dependent. Encyclopedia Britannica defines the term “invasive species” as follows: “Invasive species, also called introduced species, alien species, or exotic species, any nonnative species that significantly modifies or disrupts the ecosystems it colonizes. Such species may arrive in new areas through natural migration, but they are often introduced by the activities of other species. Human activities, such as those involved in global commerce and the pet trade, are considered to be the most common ways invasive plants, animals, microbes, and other organisms are transported to new habitats.” (Rafferty 2019). In other words, the above cited definition also includes species that have arrived in the area on their own, without human impact. Some scientists also include natives that have become dominant under the term “invasive”, and it has been suggested that just as there are non-invasive non-natives, there's also invasive natives (e.g., Alpert et al. 2000). However, a strong consensus prevails in the different use of “invade” and “colonize” in the scientific context, as “invade” means to “enter in hostile manner” (Oxford English Dictionary 1989). While definitions vary, in this thesis, I use the term “invasive” to describe a species non-native to the area, introduced there either intentionally or unintentionally by humans, either directly or via vectors. After their introduction, invasive species usually expand their range on their own, causing disturbance or being likely to cause ecological changes in the invaded range. This definition is supported by a comprehensive literary study that gathers the most commonly used terminology together and aims to shed light on the numerous synonyms and concepts that are in use in the field of biological invasions (Falk-Petersen et al. 2006).

Numerous studies, reaching back to the 1980's, have shown that introduced plant species tend to have a higher impact on coexisting native species than dominant native species have on the same competitors (Vitousek et al. 1987, Hejda 2013, Sun & Junod 2017). In other words, even the most dominant species very rarely form monocultures in their native range. However, the pattern of becoming dominant outside one's native range can be described as a classic paradox, because it is not consistent with the very fundamental ecological idea of local adaptations and the benefits resulting from coevolution of species (Rout & Callaway 2009). This is one reason why invasive species are such an interesting phenomenon to study.

The effects of invasive species often span several different levels of biological organization, from individuals to ecosystems. When invasive plant species become dominant in the community, competition dynamics between native and invasive plant species often characterize the community-level impacts (e.g., Kandlikar et al. 2019). Very early in the study of invasion ecology, it was suggested that as invasion changes resource availability, the community composition can be largely determined by the newly influenced competitive outcome (Tilman 1985). These community-level impacts of invasive plants are likely to vary depending on the characteristics of native species and their ability to tolerate or even to thrive in the presence of invasive species (Tilman 1999, Hejda 2013, Verbeek & Kotanen 2019). Because invasions alter the ecosystems, communities and population structures, they act as a whole new type of "disturbance", one that can be added to the list of natural ones, alongside fires and pathogen outbreaks (Vitousek 1990). One of the most cited ecologists in the field of invasions, Peter M. Vitousek has suggested that the study of invasions provides means to intergrade methods and approaches of population and ecosystem ecology. By studying the effects of invasions, biologists can analyze population processes both in basic and applied ways (Vitousek 1990).

Individual level impacts can, for example, be changes in the allocation of growth. Using two species of strawberries, native and invasive to Europe, Littschwager et al. (2010) studied nitrogen uptake and its role in the competition dynamics. This study suggests that strategies plants have in resource allocation between above- and belowground plant parts may change both in response to different types of competitive pressures and in response to changes in the soil nutrient composition (Littschwager et al. 2010).

There are many mechanisms by which invasive plants may outcompete and replace native ones. A successful invader has to be able to adapt to a range of new environmental conditions, and thus its native range needs to be large in size or otherwise varying in conditions (Pysek et al. 2009). Invaders are also often characterized by high productivity, fast germination and high growth rate, which can lead to greater water and nutrition uptake, as well as shading or root space competition (Ehrenfeld 2003).

Apart from being adaptive, fast growing and easily spreading, several invasive plant species are able to alter the soil microbial community (e.g., Wolfe & Klironomos 2005). A multi-year study done with two invasive herbs, *Centaurea solstitialis* and *Aegilops triuncialis*, shows that rhizosphere microbial communities significantly differ when comparing non-invaded and invaded areas. (Batten et al. 2006). Batten et al. (2006) also suggested that the soil microbial community continues to grow more different than the original community, which can be seen when comparing newly invaded areas to areas invaded several years ago. These invasion-induced changes in the soil microbial community may lead to a self-reinforcing loop of positive feedback, increasing the dominance of invasive species in an ecosystem (Batten et al. 2008). Comparisons done with locally dominant natives and invasive herbs suggest that the interactions and competition dynamics are fundamentally different in these two species groups (Sun & Junod 2017). Sun et al. (2017) showed with a combination of experiments, that while competition between natives is often driven by resource competition, the impact of some invasive species is driven by other mechanisms such as novel plant-soil interactions. Such interactions are only a part of the complex dynamics that determines invasion success and invasibility of ecosystems.

Invasions are strongly impacted by human activities, such as land use and global commerce (Vitousek et al. 1997). As human activities are most likely only increasing and becoming more global, and at the same time the climate is changing, new regions are very likely to be invaded in the future (e.g., Wolfe & Klironomos 2005). Often, invasions are irreversible (Vitousek et al. 1997), and thus the screening of potential invaders and prevention of harmful invasions is vital. This requires a better understanding on both the invaders as well as the species they come in contact with. Knowledge of different mechanisms of competition and methods of dominance will help in trying to predict the small- and large-scale effects invasions have on ecosystems. All of this is required when

planning effective conservation undertakings and facing species invasions as a conservational issue.

1.2 Plant associated soil micro-organisms and plant-soil feedback

All plants have close interactions with multiple different types of microbes, both above- and belowground. These interactions are vital to several different plant functions and can be seemingly neutral, mutualistic or pathogenic. Among other microbes, plants cultivate a community of soil organisms that can change soil properties, such as nutrient composition. (e.g., Bever et al. 1997)

The effect of plants on soil and *vice versa*, is called plant-soil feedback (PSF). This feedback acts through mechanisms involving all aspects of plant growth, morphology and physiology, and all the physical, chemical and biological properties of the soil the plant grows in (Ehrenfeld et al. 2005). Several studies have shown that plant-soil feedback has a vast scale of effects and it can provide mechanisms for plant diversity, succession and invasion (Parker 2001, Ehrenfeld et al. 2005, Niu et al. 2007, Kulmatiski et al. 2008, Weidenhamer & Callaway 2010, Rout & Callaway 2012, Verbeek & Kotanen 2019). As is true probably in all interactions with microbes, PSF can be negative or positive, depending on the microbe composition and plant species (e.g., Bever 2003).

James D. Bever et al. published a pioneering mathematical model incorporating PSF interactions to plant population dynamics in 1997. In this paper, the authors showed substantial evidence on soil microbial pathways maintaining species diversity (Bever et al. 1997). Bever himself extended this model in 2003 considering the possible simultaneous competition between plants (Bever 2003). An even newer model further extending Bevers work was published in 2019. This model shows that plant-microbe pathways, a component of PSF, can drive fitness differences in pairwise competition. As plants differ in their ability to both benefit from mutualistic microbes and tolerate pathogenic ones, microbes mediate fitness differences in plants and can drive plant coexistence or competitive exclusion (Kandlikar et al. 2019).

Apart from mathematical models, several experimental studies also indicate that soil microbial pathways, both mutualistic and pathogenic, may have a considerable role in the competition dynamics between native and invasive plants. One of the first studies to show

that plant invasion induced changes in the soil-microbial communities can negatively impact native plants was done with an invasive grass (*Aegilops triuncialis*) and two plants native to the US (*Lasthenia californica* and *Plantago erecta*) (Batten et al. 2008). Batten et al. (2008) showed that these changes can be observed very quickly and can be seen to affect the native plants' performance within a relatively short greenhouse experiment of one growing season.

Inderjit (Centre for Environmental Management of Degraded Ecosystems in India) and van der Putten (2010) suggested that there are three ways in which soil microbes and invasions jointly influence the invaded ecosystems. First, PSF interactions are often neutral to positive in the case of the invader, whereas native plants are often constrained by negative feedback. Second, invasive plants can manipulate the soil biota by increasing pathogen levels or disrupting mutualistic interactions, while tolerating these disturbances themselves. Third, invasive plants may produce allelochemicals that the local soil communities are unable to detoxify, or that become more toxic following microbial conversion (Inderjit & van der Putten 2010).

A recent quite comprehensive study on the effects of invasion-induced PSF on seed germination and the early growth of native herbs showed that natives competing with the studied invader Canada thistle (*Cirsium arvense*) had a very varied response to the invasion, depending also on the microbes that the invader cultivates (Verbeek & Kotanen 2019). Verbeek and Kotanen (2019) also demonstrated that while the invader can significantly disturb the germination and growth of natives via changes in soil microbial community, the invader itself can remain unaffected. Furthermore, the positive PSF effect of soil microbes can lead to a positive loop and also facilitate further invasions by other species (Jordan et al. 2008, Batten et al. 2008). Another recent study shows that soil microbes can have an effect on plant flowering time as well as on plant fitness in response to competition (both intraspecific and interspecific) and drought (Fitzpatrick et al. 2019).

As has been showed both in theory and practice, invasive herbs have both direct and indirect effects on the invaded community and on its soil nutrient composition. Direct effects are a result of litter and exudates, while indirect effects may result from herbicides used to control invasions (Weidenhamer & Callaway 2010). In the field of invasion ecology, the "novel weapon hypothesis" is one of the hypotheses used to explain how invasive species can become such strong competitors in their invaded range, even though

they have no local adaptations or coevolution with symbiotic species. This hypothesis suggests that various biochemical exudates of invading herbs can function as powerful allelopathic agents or as mediators of new plant-soil interactions (Callaway & Ridenour 2004). In other words, the “novel weapons” in the hypothesis are the exudates that may provide the invader an advantage when encountering new plant species in new communities, even though neighboring plants in the native community of the invader may be adapted to them.

One of the direct effects of plant-soil feedback are the effects on biochemical cycling, nutrient composition and richness (e.g., Wolfe & Klironomos 2005). In many ecosystems, nitrogen is the main factor limiting net primary productivity (e.g., Tilman 1985). One group of organisms that affects biochemical cycling through PSF are nitrogen-fixing bacteria, rhizobia and actinobacteria, which are found especially in the nodules of plants. Nodules, a plant part especially developed for this purpose, are root organs forming and activating in suitable microbial conditions. Nodules are often big enough to be visible to the eye, even though the microbes themselves are naturally more difficult to observe. Root nodule symbiosis (RNS) is an adaptation that allows plants to acquire nitrogen from the atmosphere, where it naturally exists in a form unusable for plants. Legumes are probably the most well known example of RNS plants, forming symbiosis with rhizobia. Less known, are the diverse group of plants, known as actinorhizal, associated with actinobacteria. (Svistoonoff et al. 2014)

The enrichment of soil nutrient content can in itself promote invasions (Niu et al. 2007). However, its effects can vary depending on the invaded ecosystem and the plant community (Rout & Callaway 2009). The effect of soil enrichment on native plants can also be positive, as some plants may, in turn, benefit from the change in the nutrient composition of the soil (Hejda 2013). A meta-analysis of 94 studies showed that while decreasing species diversity, soil-altering invaders significantly increased net primary productivity (Liao et al. 2008). This paradox is explained with differences in the PSF interactions. Liao et al. (2008) showed that N-fixing plants tend to have a great impact on both N and C cycles and have a profound influence on ecosystem processes. These changes may lead not only to a successful invasion, but to changes in the relative composition of native plants in their community, as some species may become more abundant while others diminish (Verbeek & Kotanen 2019, Kandlikar et al. 2019). Studies done on *Hieracium pilosella* also showed that in some circumstances, the invaded ecosystem can benefit from invasions in the short term, as a result of increase in

productivity (Scott et al. 2001). These effects, however, may only be seemingly positive as the long-term effects are naturally harder to observe.

In turn, the lack of mutualistic rhizobia has been suggested to work as a constrain on invasion success of legumes (Parker 2001). However, some symbionts can be supported at the population level and, thus, this natural constraint can be insufficient. Studies done in a young volcanic site in Hawaii with the nitrogen-fixing invading fruit tree *Myrica faya*, showed the positive effects of an increase in fixed nitrogen on total productivity, in an ecosystem where nitrogen is limiting the growth of native plants (Vitousek et al. 1987). In this case, the invader has a mutualistic relationship with actinobacteria, a symbiosis that is naturally lacking in the Hawaiian flora even though rhizobia are present. Regardless of this, *M. faya* has been successfully invading several sites in early succession.

Deyn et al. (2004) showed, that by comparing mixed plant communities on sterilized and non-sterilized soil, it can be observed that soil microbiota may significantly reduce the effects of nutrient supply, important in succession, restoration and patterns in plant community ecology (Deyn et al. 2004). By studying a model of mutualist-mediated competition, Abbott et al. (2015) found significant connections of microbial mutualism with invasion success and invasiveness. They suggest that a native plant that hosts mutualistic microbes can be particularly vulnerable to invasion because if either the herb or the microbes are temporarily decreased in density, a “window of opportunity” is opened for invasion (Abbott et al. 2015).

The connection of PSF processes and invasions is, as I already noted, very complex. A meta-analysis of 52 native-exotic pairwise feedback comparisons in 22 studies (Suding et al. 2013) suggests that results are not always consistent with predictions and invasions promoted by PSF processes do not always result to monocultures. While the connection between positive plant soil feedback and invasions is widely recognized, this analysis emphasizes on the importance of dispersal and competitive advantages, disturbances and other factors affecting invasion dynamics together with PSF-interactions. Studies done with *Lespedeza cuneata*, an invasive plant belonging to the nitrogen fixing legume-family, showed that competition may in itself influence the direction of PFS. In the presence of competition, the positive PSF effect on the invader disappeared (Crawford & Knight 2017).

One very profound question that has not yet been fully answered in the field of invasion ecology, is why most invasive species form monocultures only in their invaded range. Soil microbes can be the key to this question. As important as the micro-organisms present in the new range are, so are the ones the plant has “left behind”. Some specialized pathogenic microbes can help control a certain species from becoming dominant, and when missing from the invaded soil, can be important in their absence. This commonly cited mechanism affecting invasions in general is called the “enemy-release hypotheses”. (Reinhart & Callaway 2006, Kulmatiski et al. 2008, Huangfu et al. 2019)

Plants and their associated soil organism form holobionts and interact with the environment together. It has been suggested that the role of microbiota in plant population dynamics has been overlooked, even though microbiota and nutrition cycles have long been known to be linked (Bever et al. 1997) Bever (1997) states, that many of the effects of invasive species and PSF interactions can be delayed and thus noticeable only when the invader is already spread beyond control. There is an increasing interest towards the role of microbiota in plant ecology, and as human activities continue to change plant communities. I think that this “holobiotic” way of thinking is ever so needed in both plant ecology and microbiology.

1.3 The aims and hypothesis of this study

When studying invasions and the possible threats they pose, the key questions are: What makes a species capable of successful invasion? What makes a community invulnerable? What are the consequences of invasion?

In my thesis, I use a well-known invasive herb (*Lupinus polyphyllus*) because it has several properties that enhance its competitive success. One of these properties that interest me the most, is the above explained RNS and the plants ability to fix nitrogen from the air. *Lupinus polyphyllus*, (hereafter also referred as lupin) has been studied quite extensively since its establishment as a widespread invader. However, because it has such a complex effect on the invaded community and because the species keeps on spreading, the topic is still very relevant. In Finland, lupin has been declared as a harmful invader, as it threatens the herbs that have settled on to roadsides and other habitats resembling some of the traditional habitats lost with changes in land use. Many of the typical flora native to Finland are adapted to low nutrient soil and, thus, lupin and its ability to enrich

the soil can pose a threat to these species (The Finnish Advisory Board for Invasive Alien Species 2021).

To assess the effects *L. polyphyllus* can have on some of the typical species of Finnish flora, I grew it with three co-existing native herbs, *Trifolium repens*, *Centaurea cyanus* and *Taraxacum spp.*. These study species were selected in order to investigate how different types of plant species coexisting with *L. polyphyllus* can interact and compete with this invader, and what role its root microbes might have in this interaction. I also wanted to see, if I could observe the effects of soil microbes on *L. polyphyllus* itself, or if the plant that was neighboring *L. polyphyllus* would affect its growth. As a measure of growth, I used plant height and root, shoot, and total biomass (and in the case of *L. polyphyllus* also nodule biomass).

My study questions are:

1. How does *Lupinus polyphyllus* affect the biomass and height of the three native herbs, *Trifolium repens*, *Centaurea cyanus* and *Taraxacum spp.*?
2. How do soil microbes associated with *L. polyphyllus* affect the biomass and height of *T. repens*, *C. cyanus* and *Taraxacum spp.*?
3. Is the growth of *L. polyphyllus* affected by the presence of its associated soil microbes or the presence of *T. repens*, *C. cyanus* and *Taraxacum spp.*?

As the three native species studied differ from another, my main hypothesis is that these species differ in the way they grow both in the presence of *L. polyphyllus* and its associated soil microbes. The null-hypothesis thus being, neither *L. polyphyllus* nor the inoculum containing soil-microbes have any effect on the three native species.

I predicted that *L. polyphyllus* would have a negative effect on the height or the shoot biomass of the co-existing species. I also expected that the effect of competition might be visible on the root biomass, possibly affecting the root growth of both the natives and lupin negatively. More specifically, based on species' properties, I expected that the less-common *C. cyanus* would be most negatively affected, while the "weed" *Taraxacum spp.* would be least affected by the presence of *L. polyphyllus*.

I predicted that the soil microbes that *L. polyphyllus* hosts would have a considerable role in the way the invader dominates other species, benefitting *L. polyphyllus* over its

competitors. However, I expected the effects of lupin associated soil microbes to be less negative in the case of *T. repens* that is also a nitrogen-fixing species. More especially, I was interested in seeing if the effect of soil microbe inoculation would be visible in the roots of the studied herbs. Based on similar experiments and the possible effects of PSF induced biochemical changes, I considered an increase in total plant productivity also very possible.

Regarding the third study question, I predicted that *L. polyphyllus* would grow better in soils inoculated with their associated soil microbiota than in soil with sterilized inoculum added. I had no reason to expect the co-existing native species having any particular effect on the growth of *L. polyphyllus* other than, as already mentioned, the simple effect of competition of root-space or sunlight in the pot. If there is such competition, I predicted the big-rooted *Taraxacum spp.* could have the biggest negative effect on *L. polyphyllus*. However, as *L. polyphyllus* is not grown alone in this experiment, competition itself is not a treatment tested on it.

2 MATERIALS AND METHODS

2.1 Study species

2.1.1 *Lupinus polyphyllus*

Lupinus polyphyllus (Lindl.), is a perennial herb belonging to the family *Fabaceae*. This 50 – 150 cm tall herb has large colorful flowers, rosette shaped leaves and intensely branched root system. *Lupinus polyphyllus*, commonly named garden lupin is native to the north-western parts of North America with an oceanic climate: Canada (British Columbia), United States (Alaska, west Oregon, west Washington, north California). *Lupinus polyphyllus* was introduced to Europe for ornamental purposes in the early 1800's as it was brought first to England in 1826. (Fremstad 2010)

Today, *L. polyphyllus* is a very well-known invader in many parts of Europe, New Zealand and Tasmania (Hejda et al. 2017). Since its introduction, *L. polyphyllus* has been spreading out of gardens and into roadsides and other open areas and is a very common sight on the road verges in Finland. The invasion rate has been increasing in the past decades (Saarinen et al. 2006). *Lupinus polyphyllus* is listed as a harmful invader of national concern, which means it is prohibited to release this species into the environment or bring it into the country from outside or within EU, cultivate it intentionally, to transport or to sell (The Finnish Advisory Board for Invasive Alien Species 2021).

Lupinus polyphyllus has been shown to form monocultures more often in its invaded ranges, but it sometimes becomes dominant also in its native range (Hejda et al. 2017). The typical habitats this species is found in differs along its invaded range. In Finland lupin is very commonly found on roadsides and wasteland, and although not completely missing, is less common in forest understories, recently found to have invaded sandy and dry pine forests (The Finnish Advisory Board for Invasive Alien Species 2021).

This species of lupin has several qualities that facilitates its tendency to outcompete native plant species and lower species richness. *Lupinus polyphyllus* reproduces primarily with seeds (Fremstad 2010). Roadside maintenance reports show that one individual can produce hundreds of seeds and spread them up to a distance of few meters (Saarinen et al. 2006). The seeds are preserved germination-ready in the soil for several years (Luontoportti 2021).

Lupinus polyphyllus is a legume plant that hosts rhizobia in its root nodules. Through this symbiosis, lupins are able to fix atmospheric nitrogen into a form useable for plants. While not yet comprehensively studied, most lupins are associated with bacteria belonging to the genus *Bradyrhizobium* (Ryan-Salter et al. 2014). As the microbes lupins host alter the nutrient content of the soil, the introduction of lupin often results in soil becoming too rich for some of the native species adapted to low-nutrient soil (The Finnish Advisory Board for Invasive Alien Species 2021).

In addition to the plants ability to alter the nutrient cycle and spread efficiently via seeds, lupin has a high growth rate and can overshadow smaller plants with its rosette shaped leaves. Lupin can also tolerate drought and is well adapted to different climate conditions. (Luontoportti 2021)

As *L. polyphyllus* is widely recognized as a strong invader in the Finnish landscape, several studies have been done to study its effects. A long-term study ordered by the public roads administration to assess the threats *L. polyphyllus* poses to native flora and fauna was published in 2006. This study was done on *L. polyphyllus* invaded road verges and it shows that the invasion has decreased the cover of other plant species (Valtonen et al. 2006). The researchers observed that on *L. polyphyllus* invaded road verges the average total species richness was almost eight species less than in the non-invaded verges. This report shows, that in the plots that had *L. polyphyllus* in them, the average cover of the plant was 70%, which quite well describes the monoculture that is, the typical Finnish roadside invaded by *L. polyphyllus*. High abundancies of *L. polyphyllus* suggest that this invader has the potential to keep spreading outside its current range also in the future (Hejda 2013).

Vascular plants have been observed to be declining after the introduction of *L. polyphyllus* across all of its main habitat types: meadow, forest, road verge and wasteland (Ramula & Pihlaja 2012). Not all studies, however, reveal associations between lupin invasions and changes in plant community structures. In some cases, lupins may enhance the productivity or reproductive output of native plants. However, there may be negative effects found when looking at the whole life cycle of the plant (Ramula & Pihlaja 2012). *Lupinus polyphyllus* does not produce nectar (e.g., Haynes & Mesler 1984), and thus, pollinators are rewarded only with pollen when visiting the flower. Lupin is mostly pollinated by generalist pollinators such as bumblebees (Haynes & Mesler 1984). While

bumblebees are often abundant in lupin invaded areas, lupin has the potential to negatively affect the total abundances of arthropods (Ramula & Sorvari 2017). Lupin invaded road verges have been observed to be less abundant in butterflies as lupin does not provide food for butterflies (Valtonen et al. 2006). Both of these recent studies (Ramula and Sorvari 2017 and Valtonen et al. 2006) suggests that lupin invasion thus not only has a negative bottom-up effect on the species richness of the community but can also alter the community structure on a larger scale.

2.1.2 Native herbs co-existing with *L. polyphyllus*

For the common garden experiment, I selected three native or archaeophyte (prehistoric) herbs to grow both alone and together with *L. polyphyllus*. In the nature, these herbs, hereafter also referred as “natives”, are co-existing with lupin but few studies have been done to examine if they have been directly affected by the invasion. Even though there is no evidence pointing towards there being any imminent conservational concern for these particular species because of lupin, I wanted to create a competition setting between these common herbs to examine what the competition dynamics could look like.

Trifolium repens (L.), commonly known as white clover is a perennial herb belonging to the pea family – *Fabaceae*. White clover is also associated with nitrogen-fixing root bacteria. White clover grows typically 10 cm – 30 cm tall, partially growing following the ground surface by stem creeping. It has white flowers on top of its branched stems. Flowers produce a lot of nectar and white clover is thus one of the best honey plants in Finland. The plant is often used for ground covering purposes in lawn mixtures, but it also grows naturally on roadsides and meadows. White clover and its close relative species are also grown for livestock feeding. White clover is considered to be an archaeophyte in Finland, growing very commonly especially in habitats highly impacted by humans (Luontoportti 2021). White clovers native range reaches from northwestern Macaronesia to Africa (Egypt to Zimbabwe) and from Europe to Mongolia and Himalaya. The herb has also been introduced to the Far East, Australia, South Africa, western South America and few parts of Central- and North America (POWO 2021).

Centaurea cyanus (L.), commonly known as cornflower, is an annual plant belonging to the family *Asteraceae*. The stems can grow up to 80 cm in height, with a single, usually blue or violet shaded, flower on top (Luontoportti 2021). Cornflower’s native range is Central- and Eastern Mediterranean. From there, it has spread globally along the

temperate zone (POWO 2021). Very recently, a first record of new “invasion” was made in northeastern India. The species was found in the area, for the first time, in the summer of 2020 (Shankar 2020). Shankar (2020) states it is yet to be determined, whether cornflower has the potential to spread beyond control and become invasive. In Finland, cornflower is classified as an archaeophyte, believed to have arrived from the eastern parts of Europe a few thousand years after the ice age. It then started to thrive as a result of agriculture in the 18th and 19th century. Cornflower (named after rye in Finnish) used to be very common “weed” in rye fields, turning the fields blue when flowering. With the intensified modern agriculture and the decline in the farming of rye, cornflower was very close to disappearing in the previous century, but has since somewhat recovered and returned to fields, meadows and gardens (Luontoportti 2021).

The third herb selected for this experiment is *Taraxacum spp.* (F.H. Wigg), commonly known as dandelion. It also belongs to the family *Asteraceae*. The genus of *Taraxacum* can be found nearly all over the temperate zones of the globe (POWO 2021). In Finland, a collective name of *Taraxacum officinale* has been given to this group consisting of approximately 500 micro-species, difficult to accurately distinguish. In this group, there are micro-species considered to be natives and archaeophytes, and while less so, also some believed to have arrived more recently (Kurtto et al. 2019). This very common perennial plant is generally considered a weed in gardens and farmlands. Its distribution follows closely to those of human habitats and can be found anywhere from wastelands to city centers and gardens. Apart from several different types of human associated habitats, dandelions can be found in shores and wetlands (Luontoportti 2021). While considered a weed and often treated with herbicides, dandelion is an important plant for many pollinators (Larson et al. 2014). With the growing concern about pollinator decline, appreciation for the pollinator-rewarding dandelion is starting to increase and I expect its status as an unwanted weed might be changing.

The three species were selected to represent typical plants growing in close proximity with human affected habitats, in other words, areas likely to be affected by plant invasions. The white clover, a rewarding plant for pollinators and an important plant to agriculture, is sold commercially in almost any gardening store. In this case, the white clover represents a similar species to the invader, being from the same family as lupin and enriching the soil with nitrogen fixing. Cornflower, a mostly annual, typical meadow and crop field plant, represents a plant thriving in traditional cultural environments,

declining in some areas with the intensified agriculture and changes in land use, while at the same time also spreading into new ones. Dandelion represents a plant that while also important plant for pollinators, is considered a weed, growing strong almost everywhere and spreading easily with human activities. These species differ both in morphology and ecology.

2.2 Experimental design

This common garden experiment was conducted in the Botanical garden of Ruissalo, University of Turku, Finland. Ruissalo is an island in the western part of Turku, connected to the mainland by a bridge. The 23-hectare area of the garden is partially fenced but not absolutely protected from herbivores or other disturbances. The study site is situated in the small area of the garden officially excluded from visitors, but also not fenced off (60.434356, 22.171856). As the study site is a non-covered area, most of the watering during the summer was expected to come naturally with rainfall. During periods low rainfall and high temperatures, a watering hose was used to ensure sufficient watering.

To examine the effects of *L. polyphyllus* and its associated soil microbiota on the growth of *T. repens*, *C. cyanus*, and *Taraxacum spp.* the native herbs were grown either in the presence or absence of lupin (yes/no) in two different types of soil inoculum (lupin associated root microbes/sterile). To account for the possible effect of the soil addition itself, half of the pots were inoculated with soil containing microbes, while the other half had the same amount of soil added, only it was sterilized beforehand. A repetition of 20 was decided to be sufficient and feasible, as the limiting factors were the area of the study site and supplies provided. The resulting four treatments on native herbs were as follows:

T1: growing with lupin, sterilized inoculum

T2: growing without lupin, sterilized inoculum

T3: growing without lupin, non-sterilized inoculum

T4: growing with lupin, non-sterilized inoculum

2.3 Processing of seeds and soil

The lupin seeds used in this experiment were from three different populations, sites (lat., long.) listed here. The locations of the populations were: Lieto, open wasteland population (60.507998 N, 22.392266 E), Turku airport population, wasteland with trees

(60.512062, 22.289962), Paimala open wasteland population (60.520985, 22.348884). The seeds had been collected before the set-up of my experiment in July 2018, put in paper bags and kept in room temperature.

Seed preparation for lupin got started on 20.6.. To prevent pre-existing microbes from contaminating the test pots, the lupin seeds used in the experiment were surface-sterilized in 0.5% natriumhypoclorite solution for 15 minutes and rinsed with deionized water three times. In order to facilitate faster germination, I scarified the lupin seeds using a scalpel. Seeds were then set on a moist paper towel inside petri dishes and kept in room temperature and undirect sunlight for four days until germinated and big enough (>2 cm) for potting.

I acquired the seeds of the native herbs in the first week of June and planted them a week before the fast-germinating lupin seeds. Cornflower and white clover seeds were bought from a gardening store. I collected the dandelion seeds from the Ruissalo Botanical garden (60.434184, 22.172965), from 30 different individuals. Dandelion seeds were gathered in one paper bag and mixed together. I planted all of the seeds in a sterilized (method described in the next chapter) low nutrient soil. They were kept in a greenhouse for germination and planted together with lupin when all the plants needed were large enough to be transferred outside (the range of starting heights by species: white clover 2.5-7 cm, cornflower 5-18 cm, dandelion 3-7 cm). The final planting and transferring of all seedlings in the pots outside took place on 24.6.2019.

The soil used as a substrate for the common garden experiment was a 1:1 mixture of sand and a peaty substrate, both commercially sold and bought from a gardening store. The sand used is “Kekkilä leikkihiekkä”, a sieved natural sand with a grain size of 0-4 mm. The peaty substrate is “Kekkilä Karkea ruukutusseos W R8014”, pH 5.5. The substrate was mixed in a large container by hand. This mixture of light weighted peat and heavier sand was done in order to make the soil easy to handle and remove from the roots. The soil also had to be relatively low in nutrients, while still providing a good enough substrate with a proper capability to hold water for all of the plants in the experiment. For sterilization, the mixed soil was autoclaved in the botanical garden’s autoclave in the temperature of 120 Celsius and the pressure of 1 bar for 20 minutes and then left to cool down for at least an hour. The sterilized soil was handled with sterilized equipment and

packed in double layered plastic bags before it was used in planting to prevent contamination.

Before the planting, the soil for the inoculums was gathered from the three lupin populations listed above. In each location, a bucket of 10 liters was filled with soil taken from the depth of approximately 10 cm, around the roots of at least 5 different lupin individuals per site. The shovel used for soil gathering was sterilized in diluted chlorite and rinsed between locations. After soil collection, all of the soil was mixed together to form a mixture of the soil microbiotas. Half of the soil was taken apart and the previously described treatment of sterilization with an autoclave was performed to it. As a result, there was approximately 15 liters of sterilized soil and 15 liters of unsterilized soil. The soils were stored in closed double layered plastic bags before their usage.

1.2 L plastic pots were filled with approximately 1 L of the sterilized and mixed substrate. A plastic plant label with treatment details and a running identification number was placed in the pots and all pots were watered thoroughly with tap water before planting. Seedlings in the no-competition treatment were planted individually, close to the middle of the pot. When growing a native plant together with lupin, approximately 3 cm was left in between the two seedlings. According to the treatments assigned, a 1 dl inoculum of either the sterilized or the unsterilized soil was added to the pot after the planting. This was estimated to be a sufficient amount for a direct soil transfer inoculation, based on a study done on the methodology of microbiome transfer (Howard et al. 2017). The inoculum was scattered on top of the soil around the seedling without covering it. A different measuring cup was used for the sterilized and unsterilized soil. The pots were placed outdoors in four blocks (described below). This was done in the end of June and starting measurements were taken immediately after planting. The different lupin populations were used as evenly as possible in all blocks and all treatments. Some lupin populations seemed to have lower germination success and these populations were slightly less represented. Also, mortality of the seedlings was checked during the first week of the experiment and seven of the lupins were replaced, hence the final distribution of lupin populations was not absolutely equal. However, as total survival was nearly 95% and my interest was not to study the differences between lupin populations, this was not used in statistical analysis. All of the natives survived the first week of the experiment and no replacements had to be made.

With a repetition of 20, three native herbs and four treatments, the total $n=240$. The 240 pots were divided into four blocks of 60 pots in order to take in consideration the amount of light and changes in water puddling in the site. The treatments and species of each pot were assigned randomly using Microsoft Excel's randomization tool, while making sure all treatments and species were divided evenly into the four blocks. The pots were set in rows of four, with the space of one row between every 60 pots to make the blocks easier to detect set apart. The pots were situated behind a greenhouse, on top of a tarp on the ground, in a direction from east to west. The difference in conditions between the blocks was observed during the experiment. Block 1. was getting most sunlight, with only few vegetation shading it during the day (**Figure 1.**). Block 2. was shaded by nearby bushes and got least, although enough, sunlight during the course of the day. Blocks 3. and 4. experienced some puddling of water during heavy rain or watering due to the unevenness of the base of the site. I estimated blocks 3. and 4. to be exposed to average amounts of light and shade compared to the other two blocks. The differences in between blocks were, however, quite marginal.



Figure 1. Block 1 of the common garden setting on 24.6.2019

2.4 Measurements and data collection

During the 10 weeks following the planting and taking the starting measurements (28.6.), I measured the height of the native plants three times, approximately every 20 days (17.7., 6.8., 26.8.). The measurement was taken using a tape measure and rounding the

measurement to the nearest 0.5 cm. The plant was measured from the soil surface to the tip of the longest part of the plant, a possible flower included. In addition to the measurements taken, I also recorded observations about flowering, herbivory intensity and mildew throughout the study in case they would be needed. However, as all cornflowers flowered while the other species did not (apart from one white clover individual in the end of summer), and herbivory and mildew were observed to be very evenly distributed among individuals, these observations were not used as explanatory factors in statistical analysis. No pesticides or fertilizers were used. Halfway through the experiment, I observed that due to their rapid growth, cornflowers were not staying upright. A wooden support stick was placed in all of the cornflower pots to prevent them from falling over.

A total of three natives (two cornflowers and one white clover) died before the end of the experiment, all growing without lupin. There was a period of high temperatures in the end of July and seven of the lupins were observed to be dead in the third measurement of the experiment (6.8.) and one more had died before the end of the experiment. Lupin mortality was so evenly distributed within treatments, and competition was present for the majority of the duration of the experiment, lupin mortality was not taken into the analysis as a variable. Mortality of natives however was naturally considered as no biomass data was gathered of them, thus the final $n=237$.

The experiment lasted as long as the weather conditions allowed it, as the plants had to be harvested before they withered. The termination of the experiment started on 26.8. when the final measurements were taken. In the following four days, pots were taken to the greenhouse and soil was carefully removed and washed from the roots (**Figure 2.**). Below- and aboveground parts of plants were cut apart with gardening scissors and all plant parts were left to dry on the table for at least an hour before bagging, to prevent the growing of mold. Semi-dry plants were put in individually marked paper bags. As was done with the native plants, lupins were also harvested, washed and bagged individually, roots and shoots in separate bags. The paper bags were stored loosely as they were waiting to be dried. Samples were taken to the University of Turku laboratory and dried at 65 Celsius for 48 hours. Immediately after drying, I weighed the samples to the nearest third decimal (0.001 g) using a standard laboratory precision scale. To examine the differences in the parts essential to the nitrogen-fixing of lupins, lupin roots were first weighed with nodules on them, after which nodules were taken apart and weighed separately.



Figure 2. a) *Trifolium repens* growing with *L. polyphyllus* b) *Centaurea cyanus* growing with *L. polyphyllus* c) *Taraxacum* spp. growing with *L. polyphyllus*

2.5 Data analysis

For the analysis, I first compiled all of the gathered data using Microsoft Excel. I used SAS 7.1 for all the statistical analysis. Because of both fixed and random effects, I selected a mixed analysis model (in SAS, Proc Mixed). In all of the analysis, I used the block (1 to 4) as a random factor, to take in consideration any differences in the growing conditions that might have affected the site unevenly. In order to take in consideration, the possible effect of the starting measurement of the plant, the first height measurement taken was used in the model as a fixed covariate in all of the analysis done on the data on native herbs. Tukey-Kramer adjustment for multiple comparisons was used to avoid getting falsely significant p-values. Example codes for the analysis are found in the appendix.

To examine my study questions 1. (How does *Lupinus polyphyllus* affect the biomass and height of the three native herbs, *Trifolium repens*, *Centaurea cyanus* and *Taraxacum* spp.?) and 2. (How do soil microbes associated with *L. polyphyllus* affect the biomass and height of *T. repens*, *Centaurea cyanus* and *Taraxacum* spp.?), the explanatory factors tested in the model were species, inoculum (microbes), competition and possible interactions of all the above. To detect possible allocational effects, root and shoot biomass were used as a response variable both separately and as a total biomass. The

repeated height measurements of the natives were analyzed using the previously described mixed model with an added option for repetition.

To examine my study question 3. (Is the growth of *L. polyphyllus* affected by the presence of its associated soil microbes or the presence of *T. repens*, *C. cyanus* and *Taraxacum spp.*?), I ran the previously described analyses with the data collected from lupins, using either root, shoot, nodule or total biomass as a response variable. Only species and inoculum (and the interaction of these two) were used as explanatory factors as lupin was not grown alone and thus competition was not tested on it.

Normality of the data was visually inspected from the diagnostic plots of residuals, and data was transformed to fit the model assumptions. Logarithmic transformation was found to be the most appropriate to the repeated height measurements of natives and to the total biomass, root biomass and nodule biomass of lupin. The biomass of lupin shoots as well as all the biomasses of the natives were square root transformed. Back transformed values of estimates of Least Standard-means and standard error data were used for figures presented in the results section. All figures were done using Microsoft Excel. Averages and percentual differences presented in the results section were calculated from the original data.

3 RESULTS

3.1 The effect of *L. polyphyllus* on the biomasses of natives

Statistical analysis shows that competition (the presence of *L. polyphyllus*) affected the shoot, root and total biomasses of *T. repens*, *C. cyanus* and *Taraxacum spp.* (**Table 1**). Naturally, biomasses of all plant parts were also affected by species and starting height (**Table 1**). A significant interaction was observed between species and competition for shoot, root and total biomass, indicating natives responded to competition differently (**Table 1**). *Taraxacum spp.* was smaller in total biomass ($p=0.0062$), and in root biomass ($p=0.0172$) when growing with *L. polyphyllus*, while total and root biomasses of *T. repens* and *C. cyanus* were not affected by the presence of *L. polyphyllus*. On average, *Taraxacum spp.* was 25% smaller in both total- and root biomass when growing with *L. polyphyllus* (**Figures 3. and 4.**). *Trifolium repens* showed a tendency of growing larger shoots (23% larger) when growing with *L. polyphyllus* (**Figure 4.**).

Table 1 Results of linear mixed model analysis: Natives' biomass (root, shoot, total). The effects of *L. polyphyllus* and its associated soil microbes on *T. repens*, *C. cyanus* and *Taraxacum spp.*. Red color indicates statistical significance.

Natives' biomass									
	Root biomass			Shoot biomass			Total biomass		
Effect	Num Df/ Den Df	F-value	P	Num Df/ Den Df	F-value	P	Num Df/ Den Df	F-value	P
Species	2/220	198.4	<.0001	2/222	50.2	<.0001	2/225	11.8	<.0001
Competition	1/220	6.3	0.0128	1/221	0.2	0.6545	1/224	0.6	0.4406
Microbes	1/220	4.2	0.0424	1/221	2.4	0.1216	1/224	3.1	0.0799
Starting height	1/221	4.3	0.0405	1/223	9.1	0.0029	1/227	13.4	0.0003
Species * Competition	2/220	4.1	0.0173	2/221	3.4	0.0355	2/223	5.5	0.0045
Species * Microbes	2/220	1.1	0.3505	2/221	1.2	0.3185	2/224	1.7	0.1844
Competition * Microbes	1/220	0.1	0.7890	1/221	0.7	0.3928	1/224	0.1	0.8045
Species * Microbes * Competition	2/220	0.2	0.8325	2/221	0.1	0.9018	2/224	0.2	0.8362

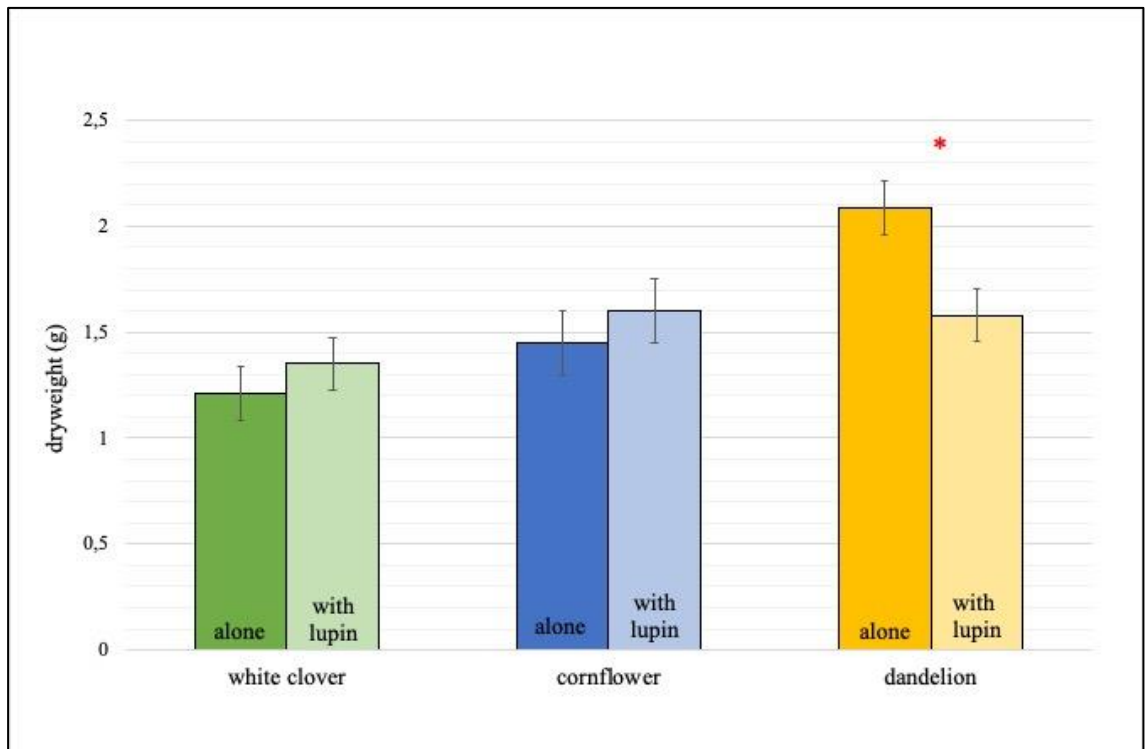


Figure 3. The effect of *L. polyphyllus* on the total biomass of *T. repens* (white clover), *C. cyanus* (cornflower) and *Taraxacum spp.* (dandelion). Columns depict the species-specific LS-means estimates of biomass, with standard errors. Asterisk between columns indicates statistical significance ($p < 0.05$).

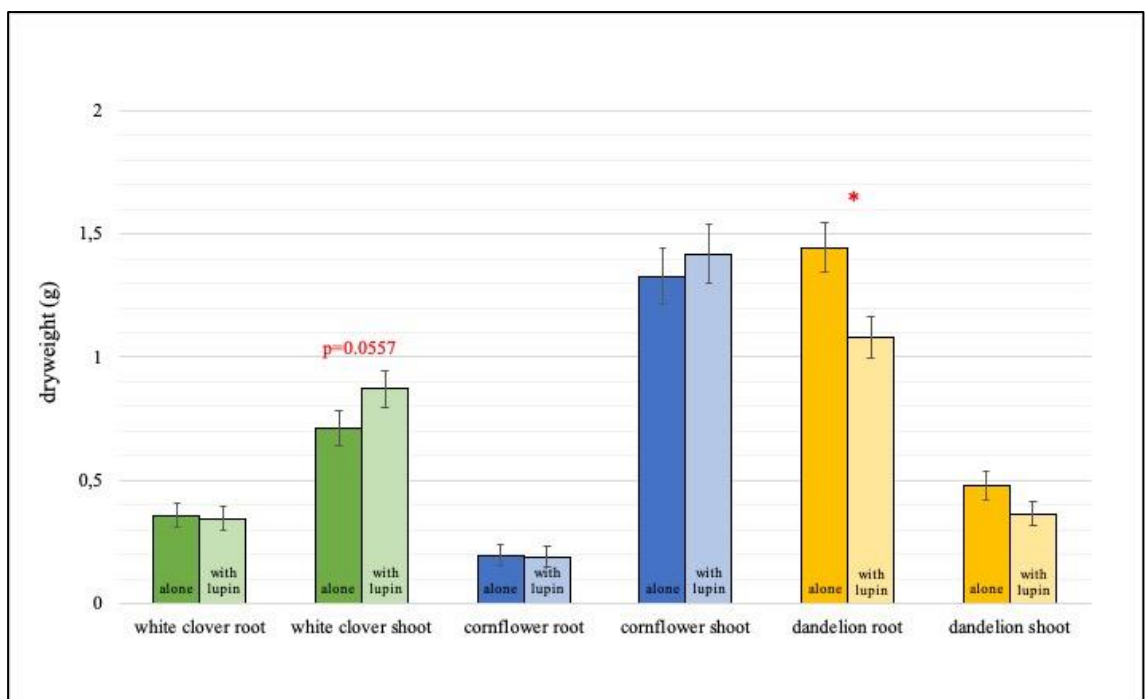


Figure 4. The effect of *L. polyphyllus* on the root and shoot biomass of *T. repens* (white clover), *C. cyanus* (cornflower) and *Taraxacum spp.* (dandelion). Columns depict the species-specific LS-means estimates of biomass, with standard errors. Asterisk between columns indicates statistical significance ($p < 0.05$). In addition to true statistical significance, p-values indicating tendency are marked.

3.2 The effect of microbe addition on the biomasses of natives

Statistical analysis shows that microbe addition (the presence of *L. polyphyllus* associated soil microbes) affected the biomasses of *T. repens*, *C. cyanus* and *Taraxacum spp.*. No interactions between species and microbes were observed. (Table 1)

Microbe addition had a significant effect on natives' root biomass (Table 1). On average, the natives grew 17% smaller roots when growing with lupin associated microbes (Figure 5.). Microbe addition had a tentative effect on natives' total biomass (Table 1). On average, the natives grew 15% smaller in total biomass when growing with lupin associated microbes. (Figure 5.)

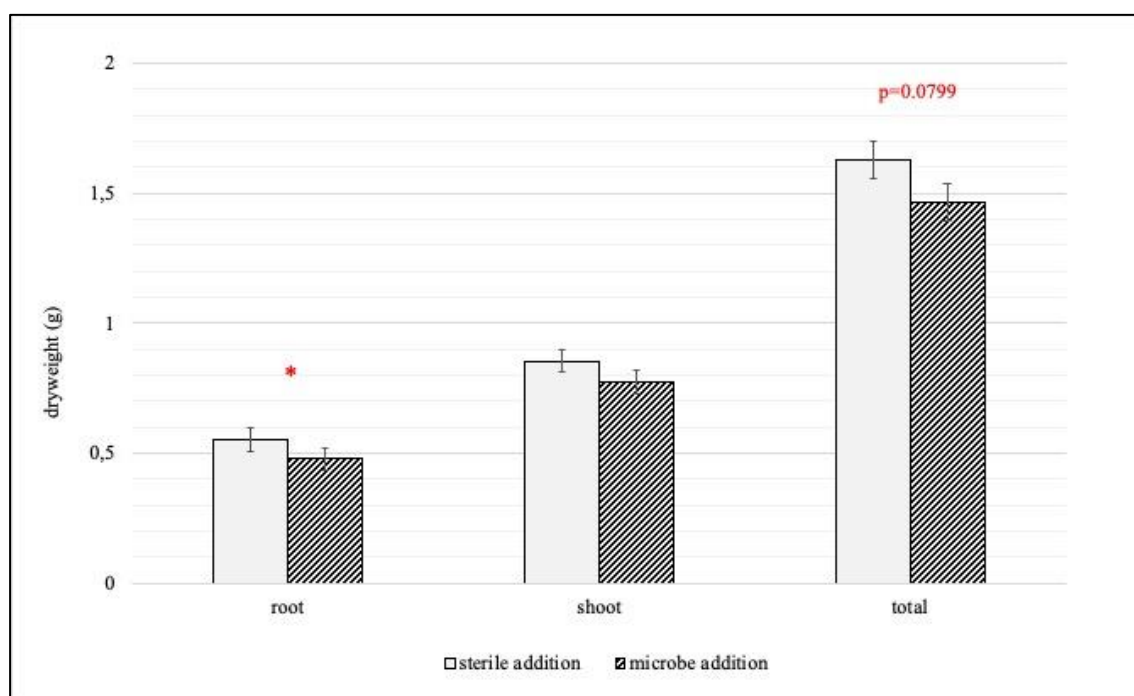


Figure 5. The effect of *L. polyphyllus* associated microbes on the root, shoot and total biomass of *T. repens*, *C. cyanus* and *Taraxacum spp.*, all species together, as no interaction of species and microbes was found. Columns depict the LS-means estimates of plant biomasses, with standard errors. Asterisk between columns indicates statistical significance ($p < 0.05$). In addition to true statistical significance, p-values indicating tendency are marked.

3.3 The effects of *L. polyphyllus* and microbe addition on the height of the natives

The height of the natives was studied with repeated measurements. Statistical analysis shows that the heights of *T. repens*, *C. cyanus* and *Taraxacum spp.* were affected by a three-way interaction of competition, species and measurement time, meaning the natives differed in their response to competition in different measurement times. Microbe addition did not affect the height of the natives. (**Table 2**)

Trifolium repens showed the biggest differences in height between plants growing with *L. polyphyllus* and plants growing alone. Throughout the experiment, plants growing with *L. polyphyllus* were higher than plants growing alone. The difference was at its highest in the middle of the experiment. On the 3rd measurement time, the *T. repens* growing with *L. polyphyllus* were on average 15.2 cm tall (\pm SD 4.2), while the *T. repens* growing alone were on average 12.5 cm tall (\pm SD 3.8). In other words, at this point in the growth of *T. repens* the plants growing with *L. polyphyllus* were on average 21% taller than the plants growing alone. (**Figure 6.**)

In *C. cyanus*, the difference in height between competition treatments was low all throughout the experiment. The difference was at its highest in the end of the experiment. The direction of the difference changed after the 2nd measurement time. On the 4th measurement time, the *C. cyanus* growing with *L. polyphyllus* were on average 55.0 cm tall (\pm SD 6.9), while the *C. cyanus* growing alone were on average 53.4 cm tall (\pm SD 8.8). In other words, at this point in the growth of *C. cyanus* the plants growing with *L. polyphyllus* were on average 3% taller than the plants growing alone. (**Figure 6.**)

In *Taraxacum spp.*, the difference in height between competition treatments was low all throughout the experiment. The difference was at its highest in the middle of the experiment. The direction of the difference changed after the 2nd measurement time. On the 3rd measurement time, the *Taraxacum spp.* growing with *L. polyphyllus* were on average 15.0 cm tall (\pm SD 3.7), while the *Taraxacum spp.* growing alone were on average 15.9 cm tall (\pm SD 3.8). In other words, at this point in the growth of *Taraxacum spp.* the plants growing with *L. polyphyllus* were on average 6% shorter than the plants growing alone. (**Figure 6.**)

Table 2 Results of linear mixed model analysis with repeated measurements: Natives height (four measurement times). The effects of *L. polyphyllus* and its associated soil microbes on *T. repens*, *C. cyanus* and *Taraxacum spp.*. Red color indicates statistical significance.

Natives' height			
Effect	Num Df/ Den Df	F Value	P
Species	2/219	997.5	<.0001
Competition	1/219	6.4	0.0123
Species * Competition	2/219	1.8	0.1691
Microbes	1/219	1.5	0.2181
Species * Microbes	2/219	0.6	0.5609
Competition * Microbes	1/219	0.9	0.3417
Species * Competition * Microbes	2/219	0.1	0.8985
Measurement	3/220	2015.6	<.0001
Species * Measurement	6/294	87	<.0001
Competition * Measurement	3/221	0.6	0.6086
Species * Competition * Measurement	6/294	2.7	0.0152
Microbes * Measurement	3/221	0.1	0.9508
Species * Microbes * Measurement	6/294	1.5	0.1733
Competition * Microbes * Measurement	3/221	1.1	0.3531
Species * Competition * Microbes * Measurement	6/294	0.4	0.8877

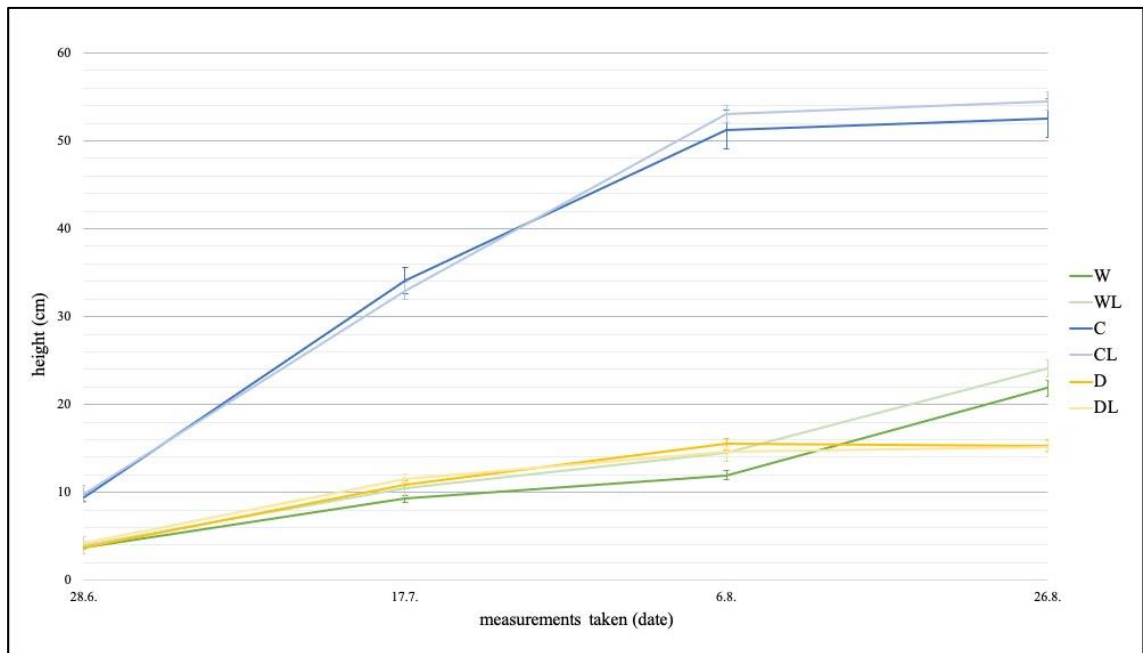


Figure 6. The effect of *L. polyphyllus* on the height of the natives during the repeated measuring. (W = white clover (*T. repens*), C = cornflower (*C. cyanus*), D = dandelion (*Taraxacum spp.*) L = lupin (*L. polyphyllus*)). Lines depict the species-specific LS-means estimates of heights at the time, with standard errors.

3.4 Growth of *L. polyphyllus*

Statistical analysis shows that *T. repens*, *C. cyanus* and *Taraxacum spp.* affected the biomass of *L. polyphyllus* (all four biomass measures considered: **Table 3**). An interaction trend was observed with species and microbes in nodule growth, but microbes alone did not affect the growth of lupin (**Table 3**).

Lupinus polyphyllus grew significantly smaller in all of its parts (total biomass, roots, shoots and nodules) when growing with *C. cyanus* than when growing with the other two native herbs (**Figure 7**). On average, *L. polyphyllus* growing with *Taraxacum spp.* were 95 % larger than *L. polyphyllus* growing with *C. cyanus*.

While the microbe addition alone did not seem to make any significant difference in *L. polyphyllus* growth (**Figure 8**), there was a marginally significant interaction between species and microbes for nodule biomass (**Table 3**). When *L. polyphyllus* was growing with *Taraxacum spp.*, microbes tended to reduce the biomass of *L. polyphyllus* nodules (**Figure 9**). On average, nodules were 29% smaller when *L. polyphyllus* was growing with *Taraxacum spp.* and microbes were present in the inoculum, than when the inoculum was sterile.

Table 3 Results of linear mixed model analysis: Lupin biomass (root, shoot, nodule and total). The effect of *L. polyphyllus* associated microbes and the effects of *T. repens*, *C. cyanus* and *Taraxacum spp.* to *L. polyphyllus*. Red color indicates statistical significance.

Lupins' biomass												
	Root biomass			Shoot biomass			Nodule Biomass			Total biomass		
Effect	Num Df/ Den Df	F Value	P	Num Df/ Den Df	F Value	P	Num Df/ Den Df	F Value	P	Num Df/ Den Df	F Value	P
Species	2/104	12.5	<.0001	2/102	8.7	0.0003	2/101	12.3	<.0001	2/100	11.5	<.0001
Microbes	1/104	0.0	0.9089	1/102	1.3	0.2535	1/101	0.0	0.8954	1/100	0.7	0.4055
Species * Microbes	2/104	1.9	0.1515	2/102	0.9	0.3966	2/102	2.9	0.0591	2/100	1.1	0.3376

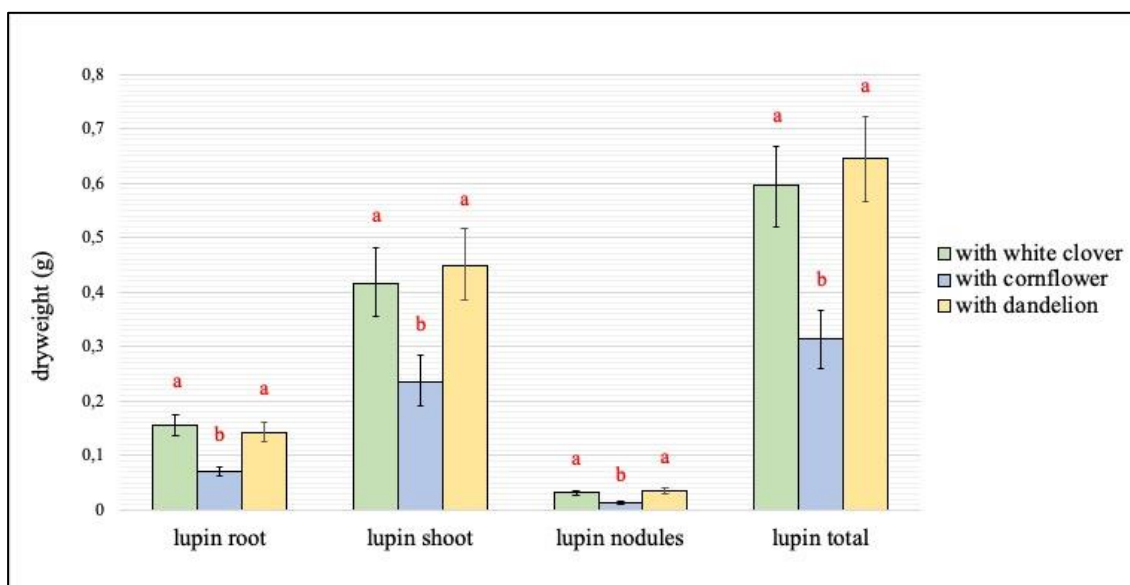


Figure 7. The effect of the co-existing natives (*T. repens* (white clover), *C. cyanus* (cornflower), *Taraxacum spp.* (dandelion)) to the growth of *L. polyphyllus*. Columns depict the estimates of species-specific LS-means of biomasses of lupin, with standard errors. Differing letter (a or b) on top of columns indicates a difference with statistical significance ($p < 0.05$) within each tissue.

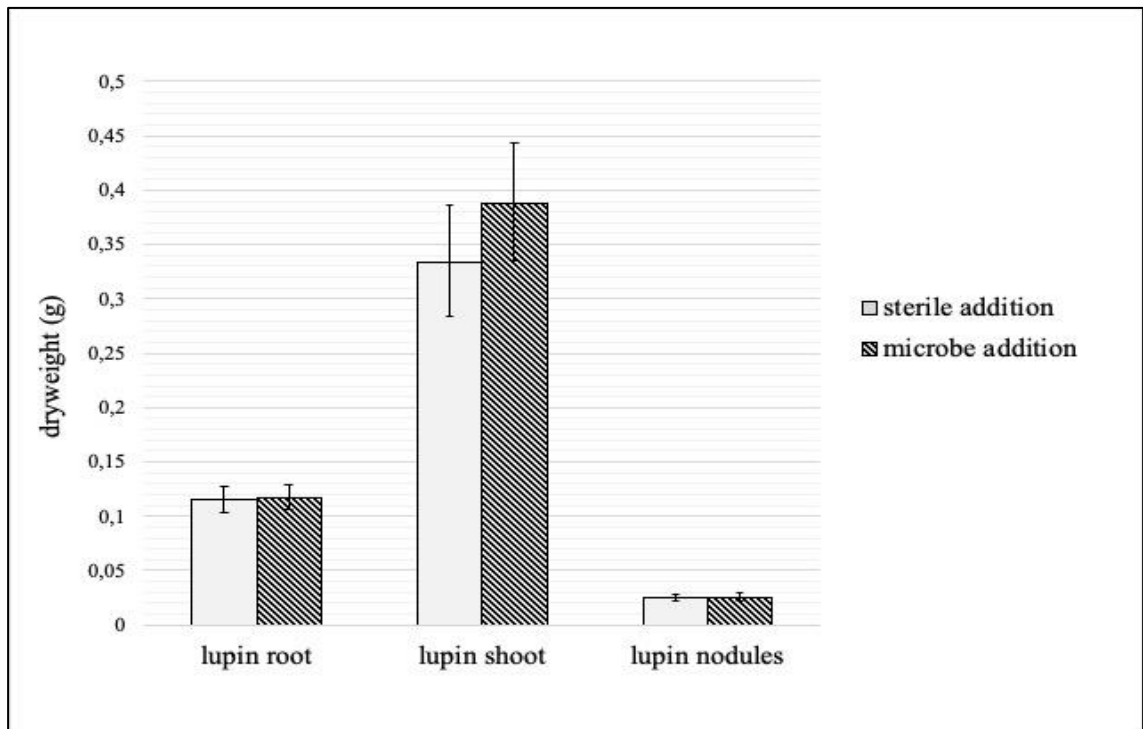


Figure 8. The effect of *L. polyphyllus* associated microbes to the growth of *L. polyphyllus*. Columns depict the estimates of LS-means, with standard errors. No significant or tentative effects were observed.

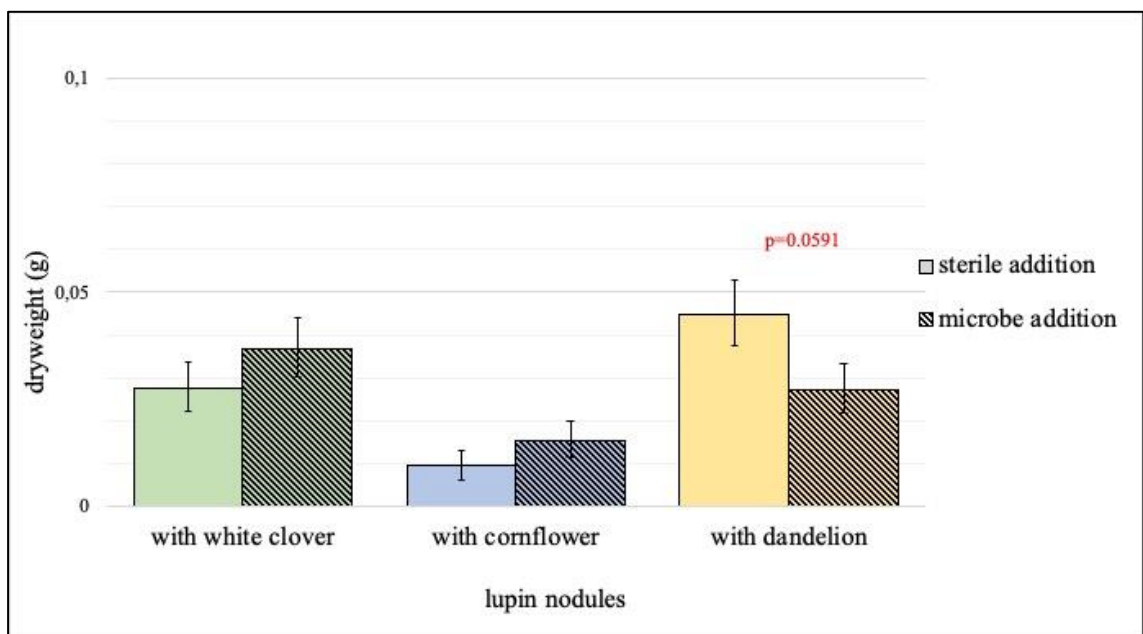


Figure 9. The interactional effects of *L. polyphyllus* associated microbe addition and co-existing natives (*T. repens* (white clover), *C. cyanus* (cornflower), *Taraxacum spp.* (dandelion)) to *L. polyphyllus* nodule growth. Columns depict the estimates of species-specific LS-means, with standard errors. P-values indicating tendency are marked in between columns.

4 DISCUSSION

I found that the main hypothesis that the three native plant species would differ in their response to *L. polyphyllus* was partially supported by my results. However, the effect of *L. polyphyllus* associated microbes was not species-specific as all of the natives seemed to respond to the microbe inoculum similarly.

4.1 The effect of *L. polyphyllus* on the growth of natives and *vice versa*

Lupinus polyphyllus had varying effects on the growth of the native herbs considered, and not all of them were negative. *Trifolium repens* grew higher (or in the case of *T. repens*, longer ground-creeping shoots) when growing with *L. polyphyllus* than when growing without it. While just below the line of true statistical significance if following the strict (and artificial) line of $p < 0.05$, this positive trend can also be seen when comparing the shoot biomasses of *T. repens* growing alone and with *L. polyphyllus*. I suggest that legumes can positively affect each other's growth, as also *L. polyphyllus* grew well with *T. repens*. While not considered explicitly in the analysis of biomasses, I observed that *T. repens* had developed root nodules. It is thus possible that *T. repens* had acquired symbiotic microbes on its own regardless of the treatment.

Opposite to my predictions, the possible competition of root-space was only truly visible in the case of *Taraxacum spp.*, as it was negatively affected by *L. polyphyllus*. I suggest that the sturdy roots of *Taraxacum spp.* weren't able to properly grow in the presence of *L. polyphyllus* in the same pot. As there was no increase in shoot growth, the decrease of root biomass could not be the result of changes in resource allocation. This negative effect corresponds with the various observations on native species decreasing in cover in the presence of *L. polyphyllus* (e.g., Valtonen et al. 2006).

The possible competition of root-space, however, was not visible in the root growth of *L. polyphyllus*, as all of its plant parts were affected negatively only when growing with *C. cyanus*, an herb with relatively small roots compared to the other two natives. I suggest that the effect of competition of root-space on *L. polyphyllus* might have risen later on if the experiment was continued, as the size of the pot could have started to limit the roots. This however is purely speculative, and only stated here in order to note the need for a longer-term experiment. Especially when studying perennials, a long-term study with

more observation times, and the added observation on reproduction would likely be able to reveal more trends and lead to more conclusive results.

The non-existing effect of *L. polyphyllus* on *C. cyanus* was somewhat unexpected, and so was the observation that *C. cyanus* in turn had a strong negative effect on *L. polyphyllus*. All in all, *C. cyanus* grew very fast and flowering success was great. I can only infer that the conditions of this common garden experiment were optimal for the annual *C. cyanus* and the perennial *L. polyphyllus* was not able to compete with it in the duration of this experiment. While the mechanism of how *C. cyanus* was able to reduce the growth of *L. polyphyllus* remains unclear, I find these results promising. If more similar results are found, plants or a plant community capable of controlling the growth and spread of *L. polyphyllus* could be found.

4.2 The effect of microbe addition on the growth of natives

While the effects of *L. polyphyllus* associated soil microbes was not species-specific, I suggest that my prediction about them negatively affecting the growth of the natives held true as the combined root- and total biomasses of natives were in fact negatively affected. This negative effect, however, was not visible in the height measurements of the natives. I find it interesting, and also encouraging in terms of the reliability of this experiment, that the effect of microbe inoculation was visible mostly in the roots, as that is of course where the soil microbes are.

As all of the native plant species were affected similarly, this experiment did not reveal any specific plant properties that would indicate the species being at a special risk when considering the effects of invaders capable of soil nutrient modification through rhizobial symbiosis. So far, the best indicators of being in danger of being outcompeted by *L. polyphyllus* seem to be related to habitat requirements, with plant species growing on meadows and roadsides and being adapted to low nutrient soils are particularly sensitive (The Finnish Advisory Board for Invasive Alien Species 2021).

As it has been suggested, soil microbes can mediate the fitness differences within plant populations and communities (Kandlikar et al. 2019). This means, the effects of inoculation (or in turn soil sterilization) may not only benefit some species over others, but also favor coexistence. I suggest that a more comprehensive greenhouse experiment

could show the effects of soil microbe addition on native herbs more efficiently. In a more controlled setting, it would be possible to better control the microbiota involved.

4.3 The effect of microbe addition on the growth of *L. polyphyllus*

Opposite to my predictions, the soil microbes did not affect the growth of *L. polyphyllus* in this experiment. Even though this was a null-result in itself, I wanted to depict it together with the rather interesting result of neighboring species affecting the nodule growth together with microbes. The microbe addition not only was not affecting the nodule growth of *L. polyphyllus* when it was growing with the other natives, but in the case of growing with *Taraxacum spp.*, microbe addition seemed to in fact reduce the biomass of nodules. These results are similar to those of Crawford & Knight in 2017, a study also done on an invading legume (*L. cuneata*). They observed that competition can in fact overwhelm the positive PSF effect on the invader (Crawford & Knight 2017). I find these effects very interesting, as the varying success and resource allocation of *L. polyphyllus* with different herbs could possibly take future research in the direction of predicting the invasibility of communities.

As the relationship between rhizobia and *L. polyphyllus* is in fact mutualistic (e.g., Ryan-Salter et al. 2014), my results of the seemingly non-existing benefits of soil microbes on the biomass of *L. polyphyllus* do not correspond with literature in this case. Again, it is possible that my experimental design just did not reveal the benefits this symbiosis provides for *L. polyphyllus* in this time frame. Future research should therefore pay more attention to the inoculum used. Another study done with an invasive legume showed that legumes have a threshold density of nitrogen fixing bacteria, necessary for nodule development (Parker 2001). While the inoculation method I used was based on the literature (Howard et al. 2017), it is still possible that the density of bacteria in my inoculums was not high enough for legumes, as it was not checked.

4.4 General conclusions

I conclude that the soil microbes definitely have a role in the dynamics of *L. polyphyllus* and in the growth of plants native to its invaded range. However, the importance and details of this role require further research. My results were partially in line with results from similar experiments. Studies done on the competition dynamics together with soil microbes show that the importance of plant soil feedback may change with changes in

the competitive interactions between invaders and natives (Shannon et al. 2012, Crawford & Knight 2017). In addition, as plant parts were affected differently, I suggest that *L. polyphyllus* invasions can have allocational effects on natives, and that also *L. polyphyllus* can allocate resources in response to different neighboring species in its invaded range.

A pioneer of invasion ecology Charles S. Elton suggested already in 1958 that diversity positively affects productivity (Elton 1958). This idea has since been supported by several studies and mathematical models (e.g., Tilman et al. 1996). Although legumes such as *L. polyphyllus* may increase plant productivity, an even greater increase on productivity may result from species diversity and niche complementary (van Ruijven & Berendse 2003). Van Ruijven et al. (2003) observed an increase in the productivity of grassland communities in absence of legumes. This increase was observed not to result from an increase of highly productive species, but from the increased performance of several low-productive species. This too, can be added to the list of benefits resulting from high diversity.

As this is a thesis, my goals were also to learn how to design and conduct a working common garden experiment. Working with plants has its own benefits and restrictions, of which I wanted to learn more of. While some improvements could be done if planning a similar experiment again, I find the methods of my one-growing-season experiment appropriate and the results interesting.

4.5 Plant invasions in Finland, management and restoration

In Finland, there are several invasive plant species that are posing a threat to the native plants. Some of the most commonly known invaders are *Lupinus polyphyllus*, *Rosa rugosa*, *Impatiens glandulifera* and *Heracleum mantegazzianum & persicum* (Saarinen et al. 2006). These species are among the “worst”, as nine invasive plant species in Finland have been included in the EU List of invasive alien species of Union concern (The Finnish Advisory Board for Invasive Alien Species 2021). Many of these invaders have found a place on road verges, a habitat important to the meadow flora and fauna which has been decreasing since the agricultural modernization (Valtonen, Jantunen et al. 2006).

A total of 98 herbs have been listed as invasive by the Finnish Advisory Board for Invasive Alien Species. There are several herbs capable of soil modification through nitrogen fixation in the list of invasive plants that are threatening native flora and fauna.

These species are often also capable of causing economical damage to landowners and farmers. Among these species are *Lupinus nootkatensis*, an herb closely related to *L. polyphyllus*. This species of lupin originates from Alaska and is adapted to colder climate than *L. polyphyllus* and thus may be capable of invading areas outside the range of *L. polyphyllus*. This species has not yet spread beyond control, and thus eradication or management is still possible. Other examples of invasive nitrogen fixers are *Pueraria montana var. lobata*, *Galega orientalis* and *Lespedeza cuneata*, the latter of which has not yet spread to Finland but is believed to pose a serious threat also in our climate and is thus under special interest. (The Finnish Advisory Board for Invasive Alien Species 2021)

Many of the invasive species found in Finland have spread to the nature from gardens and thus tend to have large and colorful flowers that may seem like a pleasant sight to some. In fact, one very beneficial feature for any organism is being in the favor of humans. However, due to active informing and media attention, most are aware of the problems resulting from monocultures in the natural habitats, even if they seem visibly pleasing. Especially in the case of *L. polyphyllus*, attempts of control and eradication have been made, often highlighting the importance of voluntary work done by citizens. While planting and growing harmful invasive species is prohibited by law, landowners are not obligated to eradicate the plants if their presence is not a result of intentional actions (The Finnish Advisory Board for Invasive Alien Species 2021).

At this point, total eradication of *L. polyphyllus* is often considered impossible. However, efforts can be made to protect areas with special conservation values. As several studies have suggested (e.g., Valtonen et al. 2006), lupin invaded areas require regular mowing before the lupin seeds are ripe. This, and the removal of the cuttings is at the moment the most efficient management option. I also think that we need more studies done on *L. polyphyllus* and the invaders "strengths and weaknesses", in order to plan further management measures.

Soil microbes can play a role also in management and restoration of invaded communities. Studying the effects of leafy spurge (*Euphorbia esula*), smooth brome (*Bromus inermis*) and crested wheatgrass (*Agropyron cristatum*), Jordan et al. (2008) not only came to the same conclusion that soil microbiota can facilitate future invasions, but also that PSF has potential to be a tool used in restoration efforts. After the removal of

invasive species, with the knowledge on soil communities, soil can be modified to again fit the requirements of the natives' (Jordan et al. 2008).

4.6 The larger scale

When assessing the threats plant invasions pose, one needs to learn from habitats of high conservation value and the threatened species they may maintain. As the media often focuses mainly on mammal, bird and amphibian extinctions, it is important to note that plants are vital to all wildlife and human well-being and thus should not be overlooked in studying extinctions and planning conservation efforts. In 2015, it was estimated that 27% - 33% of all plant species (known and unknown) are likely threatened (Pimm & Joppa 2015). These estimates are based on current threats and do not take in consideration possible further development of the environmental crisis. Pimm and Joppa (2015) argue that there are in total 450 000 flowering plants and the rate of extinction of the ones threatened is 1000 to 10 000 times the background rates. As we try to prevent extinctions, conservation efforts often require prioritizing. Knowing which attributes make species susceptible to become threatened, and which promote them in becoming invasive, helps in predicting future conservation needs and directing resources. This knowledge can only result from comprehensive studies done in greenhouses, common gardens and in the nature. I think it is safe to say new mechanisms of invasion and chains of effects of species invasions are also likely only waiting to be found.

I believe global biodiversity loss through invasions could be the next equivalent to the climate change. The scale of ecological damage seems to be comparable when studying how habitats are invaded and species are threatened with decline and extinction. We humans not only diminish the number of natural environments but also homogenize the ones that are left. I want to end this thesis in a citation that I find ever so relevant even though, it was stated over two decades ago and since its time, many of the compositions and dynamics of the world's ecosystems have already been changed irreversibly.

” In a very real sense, the world is in our hands – and how we handle it will determine its composition and dynamics, and our fate.” (Vitousek et al. 1997)

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7 APPENDIX

SAS Enterprise Guide 7.1 code for plant biomasses:

```
proc mixed covtest data=gradu.muunnettudata2
plots=residualpanel(conditional blup)
plots=boxplot(fixed);
class species competition microbes block;
model totaldw=species competition microbes startingheight
species*competition species*microbes competition*microbes
competition*microbes*species
/solution cl ddfm=kenwardroger outpred=residuals;
random intercept / subject = block ;
lsmeans species species*microbes competition microbes competition*microbes
species*competition/cl adjust=tukey;
```

SAS Enterprise Guide 7.1 code for repeated height measurements:

```
proc mixed covtest data=GRADU.gradudatatatoistomuunnettu
plots=residualpanel;
CLASS species competition microbes block measurement individual;
MODEL logheight = species|competition|microbes|measurement
/solution cl ddfm=kenwardroger outpred=residuals;
random block;
REPEATED measurement / SUBJECT=individual
type=un rcorr;
lsmeans species|competition|microbes|measurement/cl adjust=tukey;
```