Above- and belowground interactions in Jacobaea vulgaris: zooming in and zooming out from a plant-soil feedback perspective

Liu, X.

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Chapter 8

General discussion

Plant-soil feedback (PSF) has long been a topic of great interest in various fields (Bever 1994; Bever et al. 1997; van der Putten et al. 2013; Jing et al. 2022; Wang et al. 2023). Understanding the drivers of PSF and its temporal variation is important, as it not only enhances predictability but also provide implications for ecological applications (van der Putten et al. 2016; Wubs et al. 2016; Pineda et al. 2017; Jing et al. 2022; Chung 2023; De Long et al. 2023b). Furthermore, gaining insight into the role of PSF under natural conditions provides a lens to understand plant community dynamics and ecosystem functions (van der Putten et al. 2016; De Long et al. 2019). Therefore, my objective was to investigate the causes of temporal variation in PSF, explore their consequences on plant performance and aboveground interactions, and further examine the relevance of PSF in field conditions.

In this thesis, I first examined the influence of soil conditioning duration and the age or the size of response plants on the magnitude of PSF in *J. vulgaris* (Chapter 2). Additionally, I explored the changes in microbial communities and their effects on growth of responding plants in relation to the temporal variation in PSF of *J. vulgaris* (Chapter 2). Furthermore, I isolated bacteria from the roots of the roots of *J. vulgaris* and examined their effects on seed germination, plant growth and aboveground herbivores of *J. vulgaris* (Chapter 3). I then focused on two of these root-associated bacteria with negative effects on the growth of *J. vulgaris*. I carried out a series of experiments to understand the underlying mechanisms behind the negative effects two bacteria have on the growth of *J. vulgaris* and examined how specific these bacteria are by testing their effects on nine other plant species that cooccur with *J. vulgaris* (Chapter 4). Moreover, I investigated the role of soil nematodes, another group of the biotic drivers in PSF, by examining whether *J. vulgaris* provides associational resistance against nematodes to neighboring plants and the impact of soil nematodes on plant-plant interactions (Chapter 5). To gain a broader understanding of the importance of PSF in the field, I examined its role in the population structure of *J. vulgaris* plants in the field (Chapter 6). Additionally, I examined the relative importance of soil legacy effects and current neighborhood effects on plant growth and herbivory the focal plants endured using *L. vulgare* as another focal plant species (Chapter 7).

In the following sections, I highlight several main findings and indicate their implications. Next, I discuss some future directions for PSF research and provide my view on biological control options for common ragwort.

8.1 Understanding drivers of PSF

Although PSF is widely recognized as an important ecological process for plant species coexistence, plant-insect interactions, plant population dynamics, succession, and ecosystem functioning (Bever 1994; Kardol et al. 2006; Kostenko et al. 2012a; van der Putten et al. 2013; Bennett et al. 2017; Forero et al. 2022; Wang et al. 2023), the conclusions and implications that can be made from PSF studies are often still rather limited (van der Putten et al. 2016; Reinhart et al. 2021; Png et al. 2023). There are several reasons for this, and some of these are addressed in this thesis. An important reason for the relatively poor predictability of PSF, I propose, is that the causal agents of PSF are often still poorly understood. PSFs can arise from a multitude of effects. For example, root exudates of *J. vulgaris* can contribute to the negative PSF of this species (Wang et al. 2019). Plant chemical compounds (released i.e. via litter or root exudates) have also been found to negatively affect seed germination of *J. vulgaris*, an example of autotoxicity (van de Voorde et al. 2012a; Möhler et al. 2021). Some researchers have argued that extracellular self-DNA (i.e. small fractions of DNA from *J. vulgaris* from decaying roots or plant litter) can negatively affect plant growth and act as an biotic driver of PSF (Mazzoleni et al. 2015). It is currently unknown whether self-DNA also plays a role in the negative PSF that is typically reported for *J. vulgaris*. Plant parasitic nematodes are another group of soil organisms that have been confirmed as an important driver of negative PSFs (Wilschut et al. 2019). However, root-feeding nematodes were almost absent in *J. vulgaris* roots in a feedback experiment suggesting that they may not act as major drivers in the negative PSF of *J. vulgaris* (van de Voorde et al. 2012b). Similarly, I found that addition of soil nematodes had no impact on *J. vulgaris* growth, and the number of root-feeding nematodes present in roots or soil when *J. vulgaris* plants were grown in monoculture was low suggesting that this species suppresses root-feeding nematodes (Chapter 5). Other soil organisms, in particular soil microorganisms such as fungi have also been reported as drivers of negative PSF of *J. vulgaris* (Bezemer et al. 2006, 2013; Wang et al. 2019). In this thesis, I found bacteria that reside in roots of *J. vulgaris* can be drivers of the negative PSF of this plant species as well, as I found two of these bacteria can negatively affect seed germination and plant grow both through bacterial cells and through bacterial volatiles (Chapters 3 and 4).

Lastly, PSF can be influenced by abiotic and other biotic factors such as climatic conditions, herbivory, and competition with other plants. Competition can greatly influence the PSF of *J. vulgaris*, but various studies have shown that the effect of the neighbor plant on the focal plant, *J. vulgaris*, itself also depends on the plant species that conditioned the soil in which both plant species grow together (Jing et al. 2015a; Bezemer et al. 2018). The effects of herbivory on PSF vary greatly between studies. For example, Heinze and co-workers have highlighted that herbivory greatly affects PSF (Heinze and Joshi 2018; Heinze et al. 2020). By contrast, there is also evidence that aboveground herbivory has negligible effects on PSF (Bennett and Reynolds 2023). Hence these biotic effects on PSF appear rather species-specific. Interestingly, herbivory during the conditioning phase can also influence PSFs (Kostenko et al. 2012a; De Long et al. 2023a) and this is particularly evident for *J. vulgaris*, where the type of herbivory (aboveground or belowground herbivory) even alters the PSF effects (Kostenko et al. 2012a; Bezemer et al. 2013). In Chapter 5 of this thesis, although I did not conduct a feedback experiment, I observed that *J. vulgaris* experienced diverse outcomes in competition, which varied greatly among species and depended on the belowground herbivores, specifically soil nematodes.

It is also important that PSFs that are measured indoor in individual pots often do not correlate with PSFs measured in the field (Forero et al. 2019). Currently, experiments are moving from testing single species' PSFs in glasshouses and controlled conditions to testing the effects of PSF at community level and under natural conditions (Heinze et al. 2016; Schittko et al. 2016; Forero et al. 2019; Grenzer et al. 2021; Reinhart et al. 2021; De Long et al. 2023a). In Chapter 6, with field collected "home" and "away" soils, I found no evidence that PSF correlated with density- and distance-dependent seedling recruitment processes for *J. vulgaris*. While in Chapter 7 of this thesis, I found that soil legacy effects can influence the growth of another focal plant, *L. vulgare*. These studies suggest that there is still no a holistic picture of the drivers that influence the response of plants to soil conditioning by the same or other plants (De Long et al. 2019, 2023b).

8.2 Temporal changes in PSFs

In this thesis, in Chapter 2, I addressed temporal aspects of PSFs. One reason for duration-dependent changes in PSFs during the response or feedback phase is that the composition of the microbial communities changes over time. The plant that is growing in the conditioning or in the feedback phase in a control or "away" soil will convert the soil microbial community so that it resembles the one in "home" soil (Steinauer et al. 2023). If the microbial community is responsible for the PSF change, then when this phenomenon happens during the feedback phase this could result in feedbacks that become neutral over time, because the soil microbial communities in "home" and "away" soil become more and more similar over time and hence we may expect that their effects on plant growth also synchronize. Interestingly, even though these microbial legacies from the previous plant in the rhizosphere soil gradually diminish over time, recent work has shown that specific microbes from the previous plant can still be found inside the roots of the responding plant (Hannula et al. 2021). There, they could have specific effects on plant growth and hence still cause a legacy effect even though the microbial community in the soil seems not differ anymore. Therefore, it is important to examine how soil legacies change soil microbial communities in the soil but also inside the plant, how this is influenced by the later-growing plant, and how these changes influence plant growth. Such knowledge can enhance our understanding of PSF on plant growth and may improve its predictability. It is important to note in this context that fungal communities appear to be more stable than bacteria communities. In a recent study, the identity of the conditioning plant still explained fungal community composition after 5 months of growth of the response plant (Hannula et al. 2021). Interestingly, in Chapter 2, I found that only a few fungal OTUs significantly associated with the growth of *J. vulgaris* over the feedback phase. Finally, it is also important to note that several studies have shown that the soil communities of plants that grow in the "home" soil also change over time (e.g. Hannula et al. 2021; Steinauer et al. 2023). As possible explanations, I propose that this could be due to microbial community development after inoculation in sterilized soil, or due to changes after disturbances that occur in the soil during the change from phase 1 to phase 2 of the experiment.

In Chapter 2 of this thesis, in line with previous evidence, I found that PSF varied not only with the duration of the conditioning phase but also during the feedback phase. I found that plants became less sensitive to soil conditioning effects when they were larger/older leading to another reason for temporal changes in PSFs (Elger et al. 2009; Bezemer et al. 2018; Zhang et al. 2022a). In terms of the alterations in soil microbial communities, I found that the growth of *J. vulgaris* in soil conditioned by heterospecific plants ("away" soil) can lead to a decline in the relative abundance of beneficial and an increase in the relative abundance of detrimental bacteria. Such changes in the microbial communities can then lead to changes in PSF. An increase in harmful and a decrease in beneficial microbes suggests that the net effect on the plant becomes more negative in "away" soil and hence that the resulting negative PSF becomes less severe over time. Although clear and consistent patterns in soil microbial communities were lacking for *J. vulgaris* grown in “home” soil in Chapter 2, an alternative is that plants grow less bad over time in “home” soil, which can lead to a decreasing negative PSF as well. As the importance of rhizosphere bacteria on plant health is increasingly recognized (Berendsen et al. 2012), future studies should examine whether, over time, responding plants select rhizosphere bacteria to cope with the soil conditioning effects over time, particularly in “home” soil (Weller et al. 2002; Haas and Défago 2005; Mendes et al. 2011).

Previous studies have addressed that soil fungi can play an important role in PSF, while soil bacteria often do not seem to strongly influence PSF, probably due to their fast turnover (Allison and Martiny 2008). This is also the case for the negative PSF of *J. vulgaris* (Bezemer et al. 2006; Wang et al. 2019). However, as I found that detrimental bacteria accumulated in “away” soil, I further isolated bacteria that reside in roots of *J. vulgaris* to examine if they attribute to the negative effects on plant growth in Chapters 3 and 4. Two of these bacteria can negatively affect seed germination and plant growth of *J. vulgaris*, but they are not specific to *J. vulgaris* and can also affect some other plant species (Chapter 4). It is worth noting that I isolated bacteria from roots of *J. vulgaris* plants that were collected from natural grasslands using a general growth medium for bacteria. An alternative way is to select microbes from roots of plants grown during the feedback phase, where plants are exposed to different soil microbial communities. Further, by adding e.g. root or leaf extracts to the growth media, more specific bacteria could be selected that are able to grow in *J. vulgaris* roots that contain high concentrations of pyrrolizidine alkaloids (Joosten and van Veen 2011).

8.3 Diluted PSF effects under natural conditions

PSF can play an important role in determining plant community structure and plant species diversity (Petermann et al. 2008; Pugnaire et al. 2019; Goossens et al. 2023). However, most of the evidence for the role of PSF in structuring plant community and plant species diversity comes from indoor experiments, and few studies have examined the role of PSF under natural settings (Heinze et al. 2016, 2020; Heinze and Joshi 2018; Grenzer et al. 2021). Evidence regarding the influence of PSF on plant species dominance is not consistent. Some studies have reported that dominant species have net positive PSFs or weaker negative PSFs than rare species (Klironomos 2002). Conversely, other studies have found that dominant plant species accumulate more species-specific pathogens, and negative frequency dependent effects maintain diversity in plant communities (Crawford et al. 2019; Reinhart et al. 2021; Goossens et al. 2023). Furthermore, and few studies have examined the link between PSF and plant population structure (But see e.g. van de Voorde et al. 2012b), as well as the relative importance of soil legacy effects compared to current neighborhood effects. Therefore, in Chapters 6 and 7, I examined the role of PSF in relation to population structure of *J. vulgaris* (Chapter 6) and tested the relative importance of soil legacy effects compared to the current neighborhood effects in the field for *L. vulgare* (Chapter 7).

Jacobaea vulgaris exhibits a hump-shaped temporal population pattern both in an experimental field and in a chronosequence of abandoned old-fields (van de Voorde et al. 2012b). PSF has been found to play an important role in shaping this temporal population development. A positive relationship was observed between the number of *J. vulgaris* plants in the field and the biomass reduction when plants were grown in the soil collected from these different fields compared to when plants were grown in sterilized bulk soil (van de Voorde et al. 2012b). Such a conspecific density-dependent effect was confirmed in another study where plants grew worse in soil inoculated with soil collected from plots with high densities of *J. vulgaris* than in soil from plots with low densities (Kos et al. 2013). Interestingly, in this latter study, in pure live soil collected from the field, the relationship between plant growth and the number of *J. vulgaris* per m^2 was opposite and positive in plots with low density of *J. vulgaris*, probably because differences in soil abiotic properties such as nitrogen availability explained plant abundance in the field and plant performance in soil collected from the field (Kos et al. 2013). In my field studies where I focused on this focal species, the growth of *J. vulgaris* did not differ significantly in soil collected underneath flowering *J. vulgaris* plants compared to soil collected further away from flowering *J. vulgaris* plants (Chapter 6). It is important to note that the soil samples used in my study were collected from fields with mixed plant communities, resulting in diluted species-specific soil conditioning effects (Kuťáková et al. 2023). Also, the soil samples may have come from different neighborhood compositions, leading to an increased variation/noise in the results.

In the analysis of data from a field experiment in the Veluwe area where different plant communities were established (van der Putten et al. 2000; Bezemer and van der Putten 2007), I clearly detected the effects of soil legacies on the growth, but not on herbivore damage to the focal plant, *Leucanthemum vulgare*. Over the course of the field season, the influence of soil legacy effects on herbivory experienced by the focal plants gradually diminished while the influence of the current neighborhood increased. Neighboring plant communities can affect herbivory damage on focal plants via associational effects and by changing the quality of the focal plants (Kostenko et al. 2012b, 2017; Kos et al. 2015c). In line with other studies, the surrounding plants can have a strong influence on insect communities associated with the transplated focal plant. However, despite these strong neighborhood effects, my work shows that soil legacy effects from known plant communities can be detected in field and community settings. By combining the findings from Chapters 6 and 7, it becomes evident that the relationship between PSF and observed ecological patterns in the field is likely context-dependent. Additionally, neighborhood conditions, especially the composition of neighboring species, can lead to diluted PSF effects under natural conditions, making detections and predictions about these PSFs challenging.

8.4 Above- and belowground interactions and biological control

The potential use of microorganisms (i.e. fungi and bacteria) as biocontrol agents for unwanted invasive plants has gained increasing attention in the past decades (Johnson et al. 1996; Imaizumi et al. 1999; Weissmann and Gerhardson 2001; Bo et al. 2020; Fang et al. 2022). There are advantages of using microorganisms as biological control agents for invasive plants compared to insect herbivores. For example, the microorganisms are more easily prepared and easier applied than insect biocontrol agents. However, as belowground microorganisms are able to affect aboveground organisms (i.e. insect herbivores, pollinators and parasitoids) (Kostenko et al. 2012a; Kos et al. 2015a, b; Wang et al. 2019), insight into the direct and indirect effects of these microorganisms on aboveground herbivorous insects, particular on those that are used as insect biological control agents, is important, but this is rarely considered. In Chapter 3 of this thesis, I examined the potential of using root-associated bacteria that were isolated from the roots of *J. vulgaris* as biocontrol agents for this unwanted outbreak species. Two of the tested root-associated bacteria were identified that negatively affect plant performance and that have the potential to be used as biological control agents. However, inoculation with the bacteria strains overall also significantly influenced the preference of *T. jacobaeae* caterpillars on plants, probably by inducing changes in the plant as I did not detect direct effects of the bacteria on caterpillars. As *T. jacobaeae* can be used as biocontrol agent to control this plant species (Roberts and Pullin 2007; Leiss 2011), this Chapter exemplifies the complex interactions between aboveground and belowground subsystems, and stresses that biocontrol researchers and practitioners should be aware of the consequences of belowground microorganisms on aboveground herbivorous insects.

Similar to the use of insect biocontrol agents, the lack of host-specificity of microorganisms can also result in unintended damage on non-target native plants (Shahrtash and Brown 2021). In Chapter 4 of this thesis, I dissected the negative effects of two root-associated bacteria that have shown potential to act as biological control agents for *J. vulgaris*. However, I observed that both bacteria are not host-specific to *J. vulgaris*, which limits their potential use as biocontrol agents. I focused on individual strain of bacteria, but another approach could involve the development of microbial consortia, which are groups of microorganisms that collaborate in a community (Massot et al. 2022). The advantages of microbial consortia include their potential for enhanced effectiveness compared to individual microbial strain and their ability to withstand environmental fluctuations more robustly (Shahrtash and Brown 2021). Furthermore, if the objective of application is to decrease the tolerance of invasive weeds to abiotic and biotic stress and/or their competitiveness against native species, rather than directly inducing mortality in the target invasive weeds, the risk to non-target native plants may also be reduced (Shahrtash and Brown 2021).

I found that bacterial volatiles can have a detrimental effect on plant performance. This may also partly explain why the effects were not significant when seedlings were grown in soil, as volatiles may not be able to accumulate in soil as well as in para-filmed agar plates. Although the identification of the volatile compounds is currently missing, several interesting questions can be the topic of future studies. At a mechanistic level, I propose to explore gene expression patterns of plants that were exposed or not exposed to the bacteria volatiles. This may shed light on the activation of plant immune responses such as activation of the jasmonic acid (JA) and salicylic acid (SA) pathways and on the interaction of plant hormone production and bacteria. Recent studies have shown that bacterial volatiles can alter the nutrient status of plants (Martín-Sánchez et al. 2020) and induce intraspecific variation in plant traits (Raza et al. 2024), and this induced phenotypic variation ultimately results in enhanced productivity. The effects of belowground bacterial volatiles on *J. vulgaris*, such as changes in pyrrolizidine alkaloid concentrations or the systemic induced plant resistance, may have additional implications for understanding aboveground plant-herbivore interactions. There is considerable variation in the concentration of pyrrolizidine alkaloids in natural *J. vulgaris* populations (Vrieling et al. 1993). Besides the genetic variation in plants, belowground plant-microbe interactions may provide alternative explanations for the variation in pyrrolizidine alkaloids in *J. vulgaris*.

8.5 Belowground associational resistance and plant-plant interactions

In addition to rhizosphere bacteria, plant parasitic nematodes in the soil, which are well-known agricultural pests, represent a group of biotic organisms that can cause negative PSFs (Van der Stoel et al. 2002; Wilschut et al. 2019). Moreover, plants interact with multiple species, both above and belowground, in natural communities. Besides pairwise plant-plant and plant-herbivore interactions, the importance of indirect interactions such as associational effects or intransitive interactions, has been increasingly discussed in the past two decades (Barbosa et al. 2009; Gallien et al. 2017). Associational effects are intensively studied for aboveground plant-herbivore interactions (Barbosa et al. 2009). Plant neighbors can also strongly influence the interactions between herbivores and focal plants belowground (Huang et al. 2018, 2019). The model plant species used in my thesis, *J. vulgaris*, contains high concentrations of toxic secondary compounds, in particular pyrrolizidine alkaloids, which have negative effects on root-feeding nematodes (Thoden et al. 2009; van de Voorde et al. 2012b; Harkes et al. 2017) If this leads to a decrease in nematode abundance and/or in a reduction in feeding rates on other species adjacent to *J. vulgaris*, it could result in associational resistance for its neighboring plants. In Chapter 5, I confirmed that the presence of *J. vulgaris* provides associational resistance to certain neighboring plants, the ones that are good hosts for nematodes and hence plants that harbor high densities of plant feeding nematodes when grown in monoculture. Associational effects can be mediated by the influence of neighboring plants directly on abundance of herbivores by providing different quality of diet which is referred as consumptive effects. The effects can also be mediated by changes in the interactions between herbivores and the focal plant which are called as non-consumptive effects (Huang et al. 2018; Pessarrodona et al. 2019). Both consumptive and non-consumptive effects have been found to explain associational effects provided by neighboring plants, for example for the plant *Taraxacum officinale* against the insect herbivore *Melolontha melolontha* that feeds in belowground (Huang et al. 2018). In our study, the precise mechanisms underlying the associational resistance provided by *J. vulgaris*, including whether this is primarily driven by a reduction in the abundance of plant feeding nematodes or by alterations in their foraging behavior, remains unclear. I expect both effects attribute to the observed associational resistance. Future studies should clarify the relative importance of consumptive and non-consumptive effects in this system.

Apart from plant feeding nematodes other soil organisms including microbes also can play important roles in influencing plant-plant interactions and plant coexistence (Bever et al. 1997; Cardinaux et al. 2018; Lyu and Alexander 2022; Van Nuland et al. 2022). The impact of soil biota on plant-plant interactions and plant coexistence in the context of climate change has become a topic of current interest (Cardinaux et al. 2018; Lyu and Alexander 2022). For example, Cardinaux et al. (2018) investigated various scenarios of soil communities that plants may encounter during range expansion and how they can affect plant performance and plant-plant interactions in response to climate change. These findings highlight the contribution of plant-plant interactions and plant-soil interactions to the diverse spectrum of effects called neighborhood effects and provide valuable insights that help to understand novel interactions in the face of climate change.

8.6 Future perspectives

8.6.1 Improving the predictability and generality of plant-soil feedbacks

Recent studies, such as those predicting PSFs based on root traits, successional and phylogenetic positions, and commonness and rarity in communities, have aimed to uncover the generality and predictability of PSFs (Kempel et al. 2018; Wilschut et al. 2019; Rallo et al. 2022; Rutten and Allan 2023). To achieve this, it is important to study both temporal and spatial dynamics of PSF (Chung 2023). My thesis has primarily focused on the temporal variation of PSF and reported changes in both responding plants and soil microbiomes over time. The current dataset also provides an opportunity for the exploration of different perspectives. Although several recent studies report how rhizosphere microbial communities change over the course of feedback phase (Bezemer et al. 2018; Hannula et al. 2021; Steinauer et al. 2023), information about the core microbiomes that are typically associated with specific PSFs often remains missing. The term “core microbiome” has become widely accepted and used in the world of microbial ecology (Grady et al. 2019; Stopnisek and Shade 2021; Neu et al. 2021). Typically, the core microbiome is defined as the suite of members shared among microbial consortia from similar habitats (Shade and Handelsman 2012). An example related to the model system of *J. vulgaris* is the core microbiomes of the specialist caterpillar, larvae of the cinnabar moth *T. jacobaeae*. Part of the microbiome of these larvae remain consistent across different habitats (the core) while other parts of the microbiome vary between habitats (Gomes et al. 2020). In a similar way, if there is a buildup of the core microbiome following the conditioning phase that is typically associated with specific feedback outcomes, we may be able to trace the development of this known core microbiome to represent the conditioning rate. Furthermore, observing the fade-away or change of this known core microbiome during feedback phase may be able to indicate the decay rate of PSF. This could the ultimately enhance the predictability of PSF-mediated plant coexistence (Ke and Levine 2021). However, it is important to note that the core microbiome may not be correlated with the PSF outcomes all the time, as for some species only a few specific natural enemies such as soil-borne pathogens can contribute to negative PSFs while in other plant species an entire consortium of microbes may be responsible for the effects on plant growth. Further investigation on the role of core microbiomes vs. specific microbial taxa in PSF would enhance our conceptual understanding of plant-soil interactions.

8.6.2 Biological control of common ragwort

Jacobaea vulgaris is an invasive species in certain continents and considered an outbreak species in its native area of Europe. Two specialist herbivores, the cinnabar moth (*T. jacobaeae*) and the ragwort flea beetle (*L. jacobaeae*) have been introduced as biocontrol agents against common ragwort in the USA, Australia and New Zealand (Roberts and Pullin 2007; Leiss 2011). Application of both herbivores has led to an effective reduction in densities of common ragwort (McEvoy et al. 1991; Roberts and Pullin 2007). However, these herbivores mainly target large plants and the persistence of the seedbank of common ragwort could weaken the effectiveness of these herbivores in biocontrol of *J. vulgaris* (McEvoy et al. 1991). This plant species is known to produce thousands of seeds and the seeds can remain viable in the soil for many years there they are relatively invulnerable to herbivores (McEvoy et al. 1991; van de Voorde et al. 2012b). Therefore, a key implication for enhancing the biocontrol of this invasive weeds is to reduce the viability of seeds and seedlings in the soil. If using microorganisms can negatively affect early plant performance such as seed germination and seedling growth, this would be an ideal addition for the biocontrol of this species. In my work I identified bacteria that although they lack host specificity, they can significantly reduce seed germination and seedling growth. In the future, as I have discussed above microbial consortia and other specialist pathogens that are specific to *J. vulgaris* could be investigated for their potential in biocontrol. Furthermore, previous studies have indicated that seed germination of this species is limited by openness of the surrounding vegetation (van der Meijden and van der Waals-Kooi 1979; van de Voorde et al. 2012b). Therefore, reducing soil disturbance should be considered in land management to minimize the likelihood of *J. vulgaris* seed germination.

8.6.3 Putting plant-soil feedback theory to work: trends and opportunities

A major wave of enthusiasm for PSF research has emerged with the aim of applying the theory in practical settings. In this thesis, my focus was to explore whether bacterial drivers of the negative PSF of *J. vulgaris* can be used to enhance biological control of this invasive weed. Apart from this line of investigation, there are other avenues to explore in connecting PSF theory with ecological applications. Many studies have attempted to promote the restoration of vegetation by incorporating knowledge of PSF. For example, soil inoculation (where the soil inoculum contains a legacy of previous pant growth) has been proven a powerful tool for restoring degraded terrestrial ecosystems and promoting the development plant community and multifunctionality (Wubs et al. 2016; Han et al. 2022; Li et al. 2023). Besides soil inoculation directly from healthy natural ecosystems, soil microbiomes can also be engineered to select microbiomes with certain functions. For example, microbiomes that are associated with certain functions can be selected through several generations of constant selection such as drought, heat and herbivory (Monohon et al. 2021). The steered soil microbiomes can then help plants to cope with other abiotic stresses and defend themselves against aboveground insect pests (Pineda et al. 2010, 2017). In agricultural systems, positive soil legacies can also be optimized in the perspective of above- and belowground interactions to improve productivity of crop, reduce pests and pathogens and enhance the resistance and resilience of crops to climate change (Trivedi et al. 2017; Jing et al. 2022). These avenues represent promising future applications for research on host-microbiome interactions.

8.4 Final remarks

In this thesis, I focused on the above- and belowground interactions in *J. vulgaris*. I unraveled the temporal variation of negative PSF and examined the effects of root-associated bacteria on plant performance and its aboveground herbivores. Furthermore, I tested the role of PSF in relation to plant population structure, and test the relative importance of soil legacy effects under natural conditions. The results presented in this thesis demonstrate that changes in plant sensitivity and in the soil microbiome, particularly in a decline in the relative abundance of beneficial and an increase in the relative abundance of detrimental bacteria in "away" soil compared to "home" soil, during the feedback phase subsequently influence the PSF (Chapter 2). Although bacteria isolated from the roots of *J. vulgaris* can negatively affect plant performance, the inoculum can indirectly affect the preference of an aboveground herbivore, *T. jacobaeae*, and it can affect other plant species as well. Therefore, the bacteria used in the research in this thesis may not be used for biological control of common ragwort (Chapters 3 and 4). In addition, another group of biotic drivers of PSF, soil nematodes can mediate plant-plant interactions between *J. vulgaris* and other plant species, but this is often in favor of *J. vulgaris* (Chapter 5). In my field work, I uncovered that even though soil legacy effects are detectable under natural conditions (Chapter 7), the distance- and density-dependent seedling recruitment patterns of *J. vulgaris* in the field were not soil-mediated (Chapter 6). In summary, this thesis sheds light on the often overlooked temporal dimension of PSFs and demonstrates that changes in the sensitivity of responding plants and changes in soil microbes are underlying drivers. The insights gained from PSFs and above- and belowground interactions have the potential to reshape traditional approaches used for the biological control of invasive plants. Lastly, this thesis highlights the context-dependency of PSFs under natural conditions, emphasizing the need for experiments to transition from indoor to outdoor settings, and to considering various influencing factors simultaneously.