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

Liu, X.

Citation

Liu, X. (2024, May 15). *Above- and belowground interactions in Jacobaea vulgaris: zooming in and zooming out from a plant-soil feedback perspective*. Retrieved from https://hdl.handle.net/1887/3753963

Chapter 1

General introduction

Plants are influenced by both the abiotic and the biotic environment in terrestrial ecosystems. Regarding the biotic environment, interactions between plants and their neighboring plants, as well as interactions between plants and other above- and belowground organisms have been intensively studied over the past decades (Rees and Brown 1992; van der Putten et al. 2001; Rasmann and Agrawal 2008; Barbosa et al. 2009; Hambäck et al. 2014). In the past two decades, there has been a growing shift in focus from aboveground to belowground interactions, as ecologists have recognized the significant influence of belowground interactions on plants and their effects on aboveground interactions (Bardgett and Wardle 2010; Bardgett and van der Putten 2014). In the soil, microbes play an unseen but important role in influencing plant performance and the interactions with neighboring plants and aboveground insects (Kostenko et al. 2012a; Kos et al. 2015a, b; Blubaugh et al. 2018). Importantly, plants not only respond to their belowground environment but also gradually modify it. However, changes in species composition and abundance of soil organisms, particularly microbes, occur more rapidly than changes in the composition and abundance of plants or plant species that grow in the soil (Hannula et al. 2019). Therefore, gaining a mechanistic understanding of the temporal variation in plant-soil interactions will enhance our understanding of the diverse outcomes of the interactions between plants and soil organisms (Chung 2023).

Soil microbes play important roles in influencing plant performance and in above- and belowground interactions with other organisms (Kostenko et al. 2012a; Carrión et al. 2019; Hannula et al. 2019). The investigation of belowground microbes and their functions has become the current focus in research on host-microbiome interactions (Carrión et al. 2019; Nadarajah and Abdul Rahman 2021). Understanding how these microbial functions affect plant performance and the interaction of plants with other above- and belowground organisms may provide valuable insights into the ecological roles microbes play and how they might improve agricultural practices (Pineda et al. 2010, 2017; Jing et al. 2022). For example, many plant growth promoting-bacteria (PGPB) have the ability to enhance the host plants' ability to cope with abiotic stress and develop defenses against aboveground herbivorous insects (Mahapatra et al. 2022; Nannipieri et al. 2023). On the contrary, soil-borne pathogens and detrimental microbes can negatively affect plants and act as drivers of the negative density-dependent selection of plant recruitment in plant communities (Packer and Clay 2000; Liu et al. 2022). Understanding how detrimental microbes affect plant performance and the underlying mechanisms may be valuable for controlling invasive plant species and promoting sustainable land management (Harding and Raizada 2015; Lahlali et al. 2022).

In community ecology, plant-soil interactions are seen as an important driver of plant population dynamics, plant succession and ecosystem functioning (Kardol et al. 2006; van de Voorde et al. 2012b; van der Putten et al. 1993, 2013; Wang et al. 2023). However, most of the experimental work on plant-soil interactions has been carried out under highly controlled conditions, e.g. experiments in pots in greenhouses or growth chambers. More recently, researchers have acknowledged that knowledge on plant-soil interactions that is acquired from such artificial experiments may not be useful in predicting the outcomes of plant-soil interactions in real and complex systems (Heinze and Joshi 2018; Forero et al. 2019a; Heinze et al. 2020). In this thesis, I aim to investigate the causes of temporal variation in plant-soil interactions, explore their consequences on plant performance and aboveground interactions, and further examine the relevance of plant-soil interactions under field conditions.

1.1 Plant-soil feedbacks

Plants can modify the abiotic and biotic characteristics of the soil, and these changes in the soil can then influence the performance of succeeding plants that grow in the same soil (Bever 1994; van der Putten et al. 2013). This phenomenon is known as plant-soil feedback (PSF, Fig 1.1). If a plant grows worse in soil conditioned by a conspecific plant (referred to as “home” soil) than in soil conditioned by a heterospecific plant, sterilized soil or a soil mixture (referred to as “away” soil), this is referred to as negative PSF. The direction of PSFs can be positive, neutral or negative, with most plant species experiencing negative PSFs (Kulmatiski et al. 2008). Interest in PSFs has rapidly increased in the past two decades (Kulmatiski et al. 2008; Kardol et al. 2013; De Long et al. 2023b), because the concepts of PSF can be used to explain plant population dynamics, plant species coexistence, community diversity and dynamics, and ecosystem functioning (van de Voorde et al. 2012b; Kulmatiski 2016; Thakur et al. 2021; Chung et al. 2023). Current research focuses on understanding the mechanisms and disentangling the role of PSF in mixed, natural plant communities (De Long et al. 2023b).

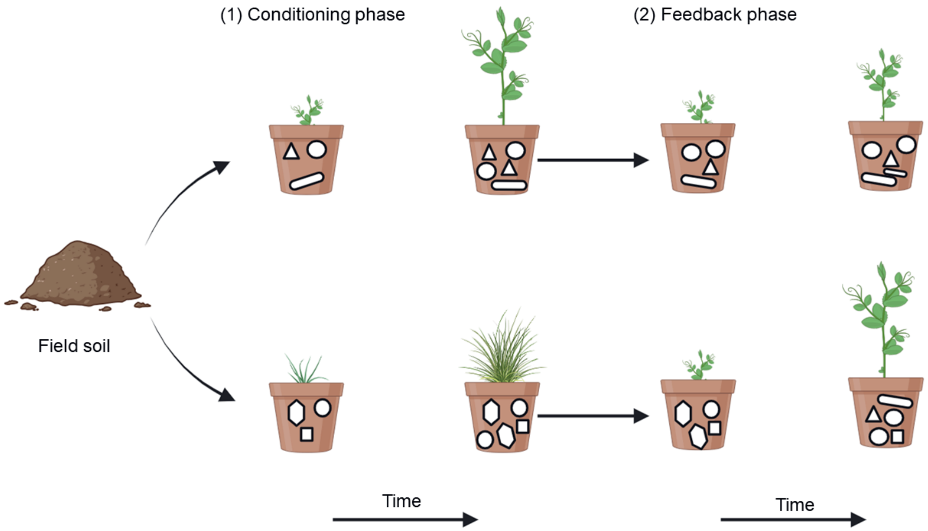

Fig. 1.1 A common plant-soil feedback experimental approach that involves a conditioning phase and a feedback phase that are both time-dependent. Different symbols indicate biotic characteristics such as microbes in the soil. Figure created using BioRender (https://biorender.com/).

1.2 Temporal variation in plant-soil feedbacks

Typical PSF experiments consist of two phases: the conditioning phase and the response phase (Fig. 1.1). The response phase starts after plants have conditioned the soil for a certain time period. Even though in nature these two phases do not necessarily operate separately (Chung 2023), in most experimental studies, there is a fixed time period for both phases. However, it is important to note that both the soil alternations by plants and the response to soil changes of plants are time-dependent (Kardol et al. 2013). During the conditioning phase, the impact of a plant on the microbial community or other characteristics of the soil depends on the duration of the plant's presence in the soil (Lepinay et al. 2018; Ke et al. 2021; Huberty et al. 2022). It is widely recognized that plants vary in their ability to take up nutrients, in the release of organic compounds in the soil via rhizo-deposits, and in their influence on soil properties such as moisture and pH (Bennett and Klironomos 2019; Nannipieri et al. 2023). Consequently, the longer a plant grows in a soil, the stronger the impact of this plant on this soil will be. Numerous studies have confirmed this hypothesis (e.g. Lepinay et al. 2018; Ke et al. 2021). For instance, Ke et al. (2021) demonstrated that non-leguminous species experienced increasingly negative PSF with longer soil conditioning periods, which was attributed to soil biota. This finding supports the notion that the duration of soil conditioning plays a crucial role in shaping the strength of PSF effects.

Although often overlooked, in the feedback phase, the responding plants not only respond to but also modify the microbial communities of the conditioned soil over time (Hannula et al. 2021; Steinauer et al. 2023). For example, responding plants have been observed to converge the bacterial communities between “home” soil and “away” soil over time (Steinauer et al., 2023). Previous studies have examined the temporal dynamics of PSF by harvesting plants at different time points during the feedback phase (Hawkes et al. 2013; Bezemer et al. 2018; Steinauer et al. 2023). These studies have found that plant sensitivity to soil conditioning effects varies with plant life stage, and that the magnitude of PSFs can change depending on the time of harvest. For example, the forb *Jacobaea vulgaris* exhibits a gradual shift from negative towards neutral PSF, while the grass *Panicum virgatum* shows a strengthening negative PSF over time (Hawkes et al. 2013; Bezemer et al. 2018). This approach provides insight into the temporal dynamics of PSF responses. However, it does not allow to differentiate whether the change in plant-soil feedback is due to changes in plant sensitivity over time, or due to the temporal effects of the modifications made by the plant currently growing in the soil to the characteristics of the conditioned soil. To better understand these PSF dynamics, it is therefore important to explore the changes in the soil microbial community during the feedback phase, such as changes in the relative abundance of crucial bacterial and fungal taxa over time, as this may provide an explanation for the variation in PSF effects.

1.3 Key drivers of plant-soil feedback

Determining the key drivers of PSF is crucial for improving our mechanistic understanding of this phenomenon. A recent review by De Long et al. (2023b) summarized the intrinsic and extrinsic drivers of PSFs. Intrinsic drivers of PSF are factors that are inherent to the conditioning plants themselves including plant maternal effects, plant traits and self-DNA. Maternal effects refer to the influence of a maternal plant on its offspring (De Long et al. 2021b). For example, the offspring of a plant that has experienced drought may be better equipped to withstand drought than a plant that originates from seeds of a mother plant that was not exposed to this environmental (Chen et al. 2022). Plant traits are the morphological, physiological and phenological features or characteristics that are associated with plant ecological strategies, performance and responses to the environment (Violle et al. 2007). On the one hand, plant traits determine the influence of plants on soil communities (Hu et al. 2018; Xi et al. 2021). On the other hand, traits are linked to the response of plants to soil communities (Rutten and Allan 2023). A recent study examining the linkage between plant traits and PSF, has revealed that fast-growing species with high specific leaf area (SLA), specific root length (SRL) and leaf nitrogen content (LNC) exhibit negative PSFs (Xi et al. 2021). It is argued that fast-growing species prioritize growth (high growth rate/nutrient acquisition) over defense against belowground enemies (i.e. soilborne pathogens) (Xi et al. 2021). Additionally, small DNA molecules (ranging from approximately 50 to 2000 base pairs) released by plants into soil (i.e. via litter) can have inhibitory effects on plants due to their direct auto-toxic effect, while they also can trigger the induction of plant defense responses against herbivores and pathogens via the jasmonate signaling pathway (Mazzoleni et al. 2015; Zhou et al. 2023).

Next to these intrinsic drivers, extrinsic drivers of PSFs are external factors in the environment that influence the interactions between plants and soil microorganisms. These drivers include both abiotic factors such as temperature, drought and flooding and pH, as well as biotic factors such as plant-plant interactions (i.e., competition), plant-herbivore interactions, and microbial and soil organism interactions (Kostenko et al. 2012a; Heinze et al. 2019, 2020; Semchenko et al. 2022; Kardol et al. 2023). Among these drivers, soil organisms have received considerable attention (Bennett and Klironomos 2019; Wang et al. 2019). For example, soil animals like plant feeding nematodes can play an important role in driving plant-soil feedbacks in dune systems (Brinkman et al. 2015; Wilschut et al. 2019). In particular, soil microbial communities have been recognized as a significant factor in influencing PSF (Bennett and Klironomos 2018). Many studies have highlighted the importance of soilborne pathogens such as fungi and oomycetes as major drivers of negative PSFs (Packer and Clay 2000; Maron et al. 2011; Wang et al. 2023). Alternatively, mutualistic symbionts such as mycorrhizal fungi and nitrogen-fixing bacteria have been associated with positive PSFs (Ke et al. 2015; Crawford et al. 2019). At plant community level, if soilborne pathogens are transmitted more rapidly among conspecific plants and when conspecifics are more aggerated, this will result in conspecific negative density-dependent effects and this limits this plant species to become dominant in the community, and hence promotes community diversity. In contrast, plants may also build mutualistic symbionts to benefit conspecifics, and this can also inhibit heterospecific species via competition and hence reduce community diversity (i.e. by altering soil pH) (Bever et al. 1997).

1.4 Putting PSF theory to work

A comprehensive understanding of the drivers of PSF has broader implications for future applications. For instance, when the objective is to enhance the performance of later growing target plants, steering beneficial microbes such as plant-growth promoting bacteria (PGPB) can be a valuable approach (Raaijmakers and Mazzola 2016; Pineda et al. 2017; Carrión et al. 2019; Jing et al. 2022). One example is the development of disease-suppressive soil through soil conditioning, which can provide microbe-mediated protection of crops against soil-borne pathogens (Raaijmakers and Mazzola 2016; Jing et al. 2022). In another study, inoculation with soil containing legacies from other plant species consistently induced resistance in chrysanthemum against thrips, *Frankliniella occidentalis* (Pineda et al. 2020). Therefore, investigating the functions of belowground microbes, particularly the selection of PGPB, has important implications, as it significantly contributes to enhanced plant performance and soil health. This is especially important in the contexts of agriculture and ecosystem management, as it has the potential to improve crop yields and reduce the use of chemical pesticides (Han et al. 2022; Jing et al. 2022).

Instead of steering microbes to create positive legacies, microbial drivers that cause negative PSF of invasive weeds, such as soilborne pathogens, might serve as potential biological control agents. The spread of invasive weeds can lead to the loss of biodiversity, degradation of ecosystem services, and public health issues (Vilà et al. 2011; Simberloff et al. 2013; Schaffner et al. 2020). Various methods have been employed to control invasive weeds, including mechanical approaches (i.e. removal of individuals and their propagules), chemical methods, and biological control (Pearson et al. 2016). In recent decades, there has been increasing concern about the environmental and human health impacts of chemical herbicides, leading to the banning of several chemical herbicides (i.e., Paraquat and Atrazine have been banned in the European Union) (Pearson et al. 2016; Abbas et al. 2018; Schaffner et al. 2020). Since the 1990s, numerous studies have focused on weed control using fungi and bacteria (Harding and Raizada 2015). The majority of commercial microbial weed control products are based on fungal species, and their application can result in necrosis and/or rot in the plant. Also, several bacteria have been investigated as potential bioherbicide candidates (Harding and Raizada 2015). Among them, significant attention has been given to strains of *Pseudomonas fluorescens* and *Xanthomonas campestris*. For example, *P. fluorescens* strain D7, a rhizobacteria isolated from winter wheat (*Triticum aestivum*), has been shown to inhibit the growth and germination of downy brome (*Bromus tectorum*) (Kennedy et al. 1991, 2001). However, unraveling the microbial drivers of negative PSF and examining their influences in the context of biocontrol of invasive weeds remains an open question.

1.5 Direct and indirect microbial effects

The outcome of PSFs is often related to changes in soil microbial communities (Bezemer et al. 2006, 2018; Mangan et al. 2010; Ke et al. 2015). Many studies have reported the diverse roles of rhizobacterial communities in PSFs (Revillini et al. 2016; Bennett and Klironomos 2019). Larger plants can foster more diverse bacterial communities due to their ability to provide more resources and create more niches i.e. via microenvironments. This diverse microbial community can exhibit increased enzyme activity, which promotes soil nitrogen mineralization and, consequently, increased plant growth, resulting in positive PSFs (Weidner et al. 2015). Moreover, many rhizobacteria have been recognized for their ability to alleviate drought, stimulate hormone production, and protect plants from pathogens through induced systemic resistance (ISR) (Van Loon et al. 1998; Pineda et al. 2010, 2017; Pangesti et al. 2013; Friman et al. 2021a, b). Rhizobacteria can perform these functions through various mechanisms, including the production of secondary metabolites, volatiles and direct interactions with plants (Carrión et al. 2019; Lucke et al. 2020; Sunita et al. 2020). Secondary metabolites secreted by rhizobacteria include antibiotics, siderophores and many phytohormones such as auxins, gibberellins, abscisic acid and indole-3-acetic acid (IAA) (Lucke et al. 2020). Antibiotics can protect plants from pathogens and siderophores can enhance the availability of irons. Phytohormones can stimulate plants growth. Volatiles released by rhizobacteria can also directly influence plant growth and defense (Garbeva and Weisskopf 2020). Notably, the effects of phytohormones and volatiles secreted by rhizobacteria also depend on their concentration. For example, the overproduction of auxins by root-associated bacteria, particularly indole-3-acetic acid (IAA), can also have an inhibitory effect on root growth (Sarwar and Kremer 1995). Hydrogen cyanide (HCN) emitted by bacteria can stimulate plant growth and induce systemic resistance at low concentrations, but it can be toxic to plants at higher concentrations (Rijavec and Lapanje 2016; Ossowicki et al. 2017; Anand et al. 2020).

In a nutshell, the influences of soil microbes on plants via induced ISR and changes in plant quality can indirectly influence interactions between plants and other aboveground organisms (Pangesti et al. 2013; Friman et al. 2021b). There is ample evidence supporting this (Berendsen et al. 2012; Kostenko et al. 2012a; Kos et al. 2015a, b). Except for the induced changes in plants, recent studies have reported that the movement of rhizobacteria can also directly affect interactions between plants and aboveground herbivores (Hannula et al. 2019; Kim et al. 2019). In a study, Flury et al. (2016) found that many *Pseudomonas* strains isolated from rhizosphere of plants can directly cause the death of caterpillars of a model insect. Despite these findings, the direct consequences of belowground microbe transmission on aboveground herbivores remain overlooked. Furthermore, many PSF studies have not delved deep into exploring important microbes and testing for their effects in the perspective of above- and belowground interactions.

1.6 Effects of belowground organisms on plant-plant interactions

Plant-soil interactions are influenced not only by soil microorganisms (i.e., soil fungi and bacteria) but also by soil fauna including nematodes, protozoa, or collembolans (De Deyn et al. 2003; Wilschut et al. 2019). For example, a study revealed a positive correlation between the strength of negative PSFs and the number of plant-feeding nematodes (Wilschut et al. 2019). Plants exhibit significant variation in the content of allelochemicals in their roots. For example, the plant, *J. vulgaris*, has high concentrations of root pyrrolizidine alkaloids (Kostenko et al. 2013). Several studies have shown that pyrrolizidine alkaloids can have negative effects on root-feeding nematodes (Thoden et al. 2009a, b; van de Voorde et al. 2012b; Harkes et al. 2017). Therefore, growing next to a neighboring plant species that has a high content of pyrrolizidine alkaloids in its roots, can indirectly reduce the risk of the focal plant being damaged by root herbivores, such as plant-feeding nematodes. Such indirect interactions between plants are known as associational effects (associational resistance or susceptibility) (White and Andow 2006; Barbosa et al. 2009; Underwood et al. 2014; Kos et al. 2015c).

Plant-plant interactions, particularly plant competition, have been a key focus in ecology for over a century (Brooker 2006). Clearly, the performance of a plant is not only influenced by its interactions with soil organisms, but also by interactions with neighboring plants via competition for nutrients, water and light. Some studies have shown that root herbivores can mitigate competition between plant species and reduce the competitive disadvantage of the inferior species (Rees and Brown 1992; Ramsell et al. 1993; Haag et al. 2004). Similarly, soil microbes have been found to alleviate plant competition (Siefert et al. 2018; Chung et al. 2023). Although both root herbivores and soil microbes can alleviate plant competition and promote plant-plant coexistence, the underlying mechanisms may differ. Several authors have argued that superior competitive plants may have a higher likelihood of encountering and being consumed by (root) herbivores and this then reduces their competitive advantage (Rees and Brown 1992). Alternatively, it is also possible that a (root) herbivore or pathogen exhibits a preference for one of two plants, independent of its abundance (e.g. when the herbivore is a specialist), and this can also result in modifications in plant-plant interactions in the presence of herbivores or pathogens.

1.7 Plant-soil feedbacks and legacy effects in the field

For over 150 years, community ecologists have been striving to use established theories or develop new ones to understand how species are assembled in communities and how species interactions respond to environmental changes (Vellend 2010). Following the conceptual work of Vellend in his book “The theory of ecological communities” (Vellend 2016), PSFs can be categorized as a selective effect. For instance, Kardol and coworkers showed that early-successional species exhibit negative PSFs, mid-successional species show neutral feedback, while late-successional species exhibit positive PSFs (Kardol et al. 2006). This provides evidence that PSFs can act as a negative frequency-dependent selection force and drive community succession. Many studies have examined PSFs in greenhouses using pot-conditioned or field-collected “home” and “away” soil, and then extrapolated the results to plant abundances in the field (Klironomos 2002; Petermann et al. 2008). However, indoor-measured PSFs do not consistently correlate with PSFs measured in the field (Heinze et al. 2016; Forero et al. 2019b; Reinhart et al. 2021). Therefore, several studies have recommended conducting PSF experiments in the field, as the development of plants and soil organisms is influenced by numerous abiotic and biotic factors under natural conditions such as herbivory (Heinze et al. 2016; Bennett et al. 2020; De Long et al. 2023a). Ecologists have argued that analysis of spatial patterns of plant species such as plant structures within populations in the field can be used to infer potential ecological processes operating in natural environments (Wiegand and Moloney 2004). This is because various ecological processes, such as species interactions, environmental filtering and dispersal, can leave footprints on spatial distributions of plants (Wiegand and

Moloney 2004; Velázquez et al. 2016; Ben-Said 2021). However, whether the spatial distribution patterns are related to PSFs is not yet clear.

As mentioned earlier, most indoor PSF experiments have focused on understanding the importance of PSFs using individually grown plants in pots. However, it remains unclear whether these findings hold true when testing for soil legacy effects in complex multispecies outdoors settings. For instance, evidence suggests that PSFs in the field may be masked by above- and belowground interactions, such as the presence of aboveground herbivores and soil heterogeneity (Kos et al. 2013, 2015c; Heinze et al. 2016; Heinze and Joshi 2018). Conversely, there is substantial evidence indicating that legacy effects left by previously grown plants in the soil can influence the growth of focal plants and their interactions with herbivores (Kostenko et al. 2012a; Ristok et al. 2019). Variation in plant species composition within a community or in the relative abundance of plant species can also create soil legacy effects (Heinen et al. 2020). The soil legacy created by a plant community, which is a mixture of plant species, is also referred to as plant-soil community feedback (van de Voorde et al. 2011). A recent review suggests the inclusion of soil legacy effects into PSFs (De Long et al. 2023b). In the final research chapter of this thesis, the results of a field experiment are reported where we examine the relative importance of soil legacy effects generated by different plant communities and the effects of the current neighborhood communities on the growth of a plant species, *Leucanthemum vulgare*, that is common in these communities, as well as on its susceptibility to aboveground herbivory.

1.8 Focal plant species

1.8.1 *Jacobaea vulgaris*

Jacobaea vulgaris Geartn. subs. *vulgaris* (syn. *Senecio Jacobaea* L.; Asteracaea), commonly known as common ragwort, is a monocarpic perennial forb that is native to Europe. It has become an invasive weed in North America, Australia and New Zealand. *J. vulgaris* typically thrives in dry and sandy soils and establishes well following soil disturbance. This species exhibits a strong negative PSF (van de Voorde et al. 2011). It forms a rosette in the first year and a stem with flowers in the second year (Harper and Wood 1957). However, flowering of this species can be delayed and takes three or more years e.g. due to herbivory (van der Meijden and van der Waals-Kooi 1979). In the native area flowering stems usually emerge from May to June, and its flowers bloom from July until October. After flowering and seed production, the plant dies. This species can produce a large number of seeds, ranging from 1000 to 30,000 achenes per plant in the Dutch coastal area Meijendel (van der Meijden and van der Waals-Kooi 1979). These seeds remain dormant in the soil as seedbank for many years and germinate in autumn or spring. At certain locations (e.g. the central region of the Netherlands, the Veluwe) the seedbank in the soil of natural grassland can contain more than 2000 viable seeds per square meter (van de Voorde et al. 2012b). Common ragwort is considered a problematic plant species in its native region due to its production of a significant amount of pyrrolizidine alkaloids, which are toxic to livestock. In countries where common ragwort has become an invasive weed, such as New Zealand, Australia, and North America, biological control methods have been employed, including the introduction of ragwort flea beetles (*Longitarsus jacobaeae*) and the cinnabar moth (*Tyria jacobaeae*) (McEvoy et al. 1991; Leiss 2011).

1.8.2 Plant-soil-insect interactions in *J. vulgaris*

Jacobaea vulgaris has been widely used as a model plant for studying novel plant-soil-insect interactions (e.g. Bonsall et al. 2003; Joshi and Vrieling 2005; Doorduin and Vrieling 2011). *J. vulgaris* exhibits strong negative PSF (van de Voorde et al. 2011), and its population structure shows a hump-shaped pattern with an increasing abandonment time in a chronosequence of old-fields (van de Voorde et al. 2012b). Negative PSF has been reported as one of the key drivers shaping this hump-shaped pattern, while local variation in PSF of *J. vulgaris* has also been observed in the field depending on nutrient availability and density of *J. vulgaris* plants (van de Voorde et al. 2012b; Kos et al. 2013). Numerous studies have focused on understanding the intrinsic drivers of PSF in *J. vulgaris*. There is evidence that this species exhibits autotoxic effects on its own seed germination and seedling growth (van de Voorde et al. 2012a). A recent study also found that litter of this species can negatively affect seed germination, but in this study it also exhibited a positive effect on seedling growth (Möhler et al. 2021). Furthermore, soil fungi and root exudates secreted by the plant have been reported to contribute to the negative PSF as well (Bezemer et al. 2006; Wang et al. 2019).

Plant-plant interactions and plant-insect interactions have been found to significantly affect PSF in *J. vulgaris* as well (Jing et al. 2015b). For example, in competition with *Holcus lanatus*, *J. vulgaris* experienced an exacerbated negative PSF (Bezemer et al. 2018). Exposure to above- and belowground herbivory during the conditioning phase can affect the performance of *J. vulgaris* plants that grow later, as well as its above- and belowground interactions as well (Kostenko et al. 2012a; Bezemer et al. 2013; Wang et al. 2019). Furthermore, co-occurring plant species exhibit species-specific PSF effects on plant performance of *J. vulgaris* and its aboveground interactions, and these interactions can also be altered by abiotic factors (Jing et al. 2015a; Kos et al. 2015b, c). In the field, when *J. vulgaris* were growing in a plant community, neighboring plant communities can determine the abundance of insects associated to this plant via associational effects from aboveground (Kos et al. 2015c; Kostenko et al. 2017).

1.8.3 *Leucanthemum vulgare*

Leucanthemum vulgare Lam. (Asteraceae), commonly known as common daisy, is a perennial species that is native to and widely distributed in Europe and North America (Clements et al. 2004). While this plant species has been reported as a weed in some countries, many other studies have not considered it a significant risk to crop production, likely due to its shallow root systems (Smith et al. 1999). Common daisy tends to be more abundant in moist climates, although it also exhibits drought tolerance and can establish in dry sandy soils.

1.9 Research questions and objectives of this thesis

Many studies have investigated the effects of PSF on plant performance and its aboveground herbivorous insects (Kostenko et al. 2012a; Kos et al. 2015a, b), revealing temporal variation in PSF effects (Bezemer et al. 2018; Hannula et al. 2021; Steinauer et al. 2023). Other studies have emphasized the significance of PSF as an ecological process in influencing plant population dynamics, plant succession and plant diversity (Bever et al. 1997; Kardol et al. 2006; van der Putten et al. 2013). In this thesis, I ask what the causes are of the temporal variation in PSFs, what their consequences are for plant performance and aboveground interactions between the plant and foliar feeding insects, and what the relevance is of PSFs under field conditions.

This thesis focuses on studying aboveground and belowground interactions in *J. vulgaris* from a PSF perspective. I examine patterns of temporal variation in the PSF of *J. vulgaris* in an attempt to bring these overlooked dimensions of PSFs into the light (chapter 2), examining functions of rhizosphere bacteria in these PSFs (chapter 3 and 4). Additionally, soil nematodes are used as soil organism group to investigate the effects on plant-plant interactions (chapter 5). Taking a broader perspective on PSFs, two field-based studies were conducted to explore the role of PSF on plant performance in natural conditions (chapter 6) and to determine the relative importance of soil legacy effects (chapter 7). In most of this thesis, *J. vulgaris* serves as the focal plant species, with the exception of the last research chapter where *L. vulgare* was used as model plant species. Based on the aforementioned knowledge gaps and research aims, this thesis has two perspectives: zooming in and zooming out on PSFs. The first part of the thesis focuses on zooming in on the variation of PSFs and the effects of bacterial drivers and soil nematodes on above- and below-ground interactions in *J. vulgaris*. This is covered in Chapters 2 to 5. The final two research Chapters 6 and 7, zoom out on PSF and explore its role in plant population dynamics and examine the relative importance of soil legacy effects in the field (Fig. 1.2).

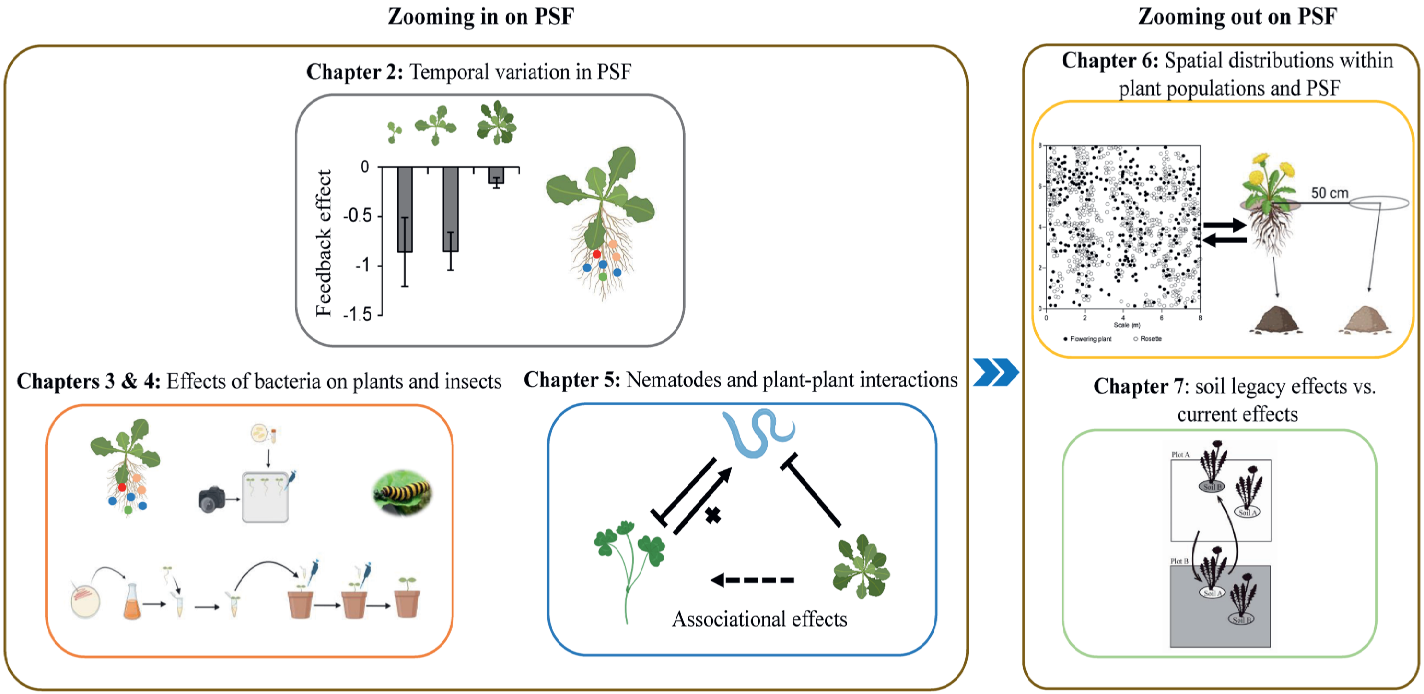

Fig. 1.2 The organization of chapters of this thesis to study above- and belowground interactions: zooming in and zooming out from a plant-soil feedback perspective. Figure created using BioRender (https://biorender.com/)

The thesis follows the following structure:

Chapter 2: In order to investigate whether PSFs vary with the duration of soil conditioning and with the age (size) of the response plant, I conducted a two phased PSF experiment. The soil was conditioned by two plant species, the focal species *J. vulgaris* and the grass *H. lanatus*, for 2, 5 and 8 weeks. During the feedback phase, 2-week-old, 5- week-old, and 8-week-old *J. vulgaris* plants that were grown in sterilized soil and fresh seedlings were transplanted into the soils that had been conditioned for different periods of time. The feedback phase lasted for four weeks, after which the plants were harvested, and the relative biomass increase of each plant was determined. Furthermore, using two existing PSF datasets of experiments with the same species, I examined the temporal dynamics of the relative growth rates of *J. vulgaris* and of rhizosphere microbes during the feedback phase.

Chapter 3: The objective of this chapter is to examine whether root-associated bacteria can be potential biocontrol candidates for *J. vulgaris*. For this purpose, I utilized twenty root-associated bacteria that I isolated from the roots of *J. vulgaris* to investigate the effects of inoculation with single bacterial isolates on seed germination and growth of *J. vulgaris*. Additionally, I tested whether these bacteria can directly and indirectly (via induced changes in plants) affect the performance and health of aboveground herbivores, particularly for a herbivorous insect, *T. jacobaeae*, that has been used in biocontrol of *J. vulgaris*.

Chapter 4: In this chapter, I focus on two Gammaproteobacteria, *P. brassicacearum* and *S. plymuthica*, which had previously shown inhibitory effects on *J. vulgaris* in Chapter 3. The objective of this chapter is to dissect the negative effects of bacterial inoculum on *J. vulgaris* and evaluate whether these two bacteria are specific to *J. vulgaris*. To achieve this, I first examined whether the negative effects depend on the concentration of bacterial inoculum by root inoculation and if the negative effects are due to metabolites secreted by the bacteria. I also examined whether these two bacteria can negatively affect seed germination and seedling growth of *J. vulgaris* at a distance through volatiles. Lastly, I investigated the host specificity of these bacteria by testing their effects via root inoculation on nine other plant species that cooccur naturally with *J. vulgaris*.

Chapter 5: In this chapter, I employed another group of soil fauna, soil nematodes, to investigate their effects on growth of *J. vulgaris* and thirteen cooccurring species. Furthermore, I explored whether the unpalatable focal plant species, *J. vulgaris*, that produces alkaloids provided associational resistance to neighboring plants against nematodes. Additionally, I examined how soil nematodes altered plant-plant interactions.

Chapter 6: To gain a broader perspective of PSF, in this chapter, I examined whether there is conspecific distance- and density-dependent plant recruitment in *J. vulgaris* in the field followed by the predictions of Janzen-Connell effects. To achieve this, in total 27 replicated 8 × 8 m^2 plots were established at two sites in The Netherlands and I mapped the positions of rosette-bearing and flowering *J. vulgaris* within each plot. Then spatial point-pattern analysis was employed to explore the distance- and density-dependent predictions of the Janzen-Connell hypothesis. Furthermore, I tested whether the observed distance- and density-dependent patterns were soil-mediated by conducting a series of indoor microcosm and pot experiments

Chapter 7: In this chapter, I analyzed an unpublished dataset of a study in which *L. vulgare* was the focal species. Plants were grown in tubes filled with soil collected from different plots from a field experiment with plant communities that differed in composition and the tubes were placed back in different field plots and hence in different plant communities and plant growth and herbivory were measured for three months. Using this design, the question this chapter addressed was what is the relative importance of the current neighborhood, and of the soil legacy of the previous community. To answer this question, I analyzed how above- and belowground characteristics of the current neighboring community and of the previous community contributed to the growth and aboveground herbivore damage on the focal *L. vulgare* plants.

Chapter 8: In this final chapter, I synthesize the results of all chapters, discuss the implications of these studies for our understanding of plant-soil feedbacks and discuss the potential for biological control of *J. vulgaris*, which is an unwanted outbreak species.