Community perspectives of individual plant-soil interactions

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Contents

Summary

Low productive agricultural fields in Europe have been taken out of production to be converted into semi-natural grassland ecosystems. This transition process is also called old -field succession. During old-field succession, plant species enter and leave successional sequences according to a boom-bust pattern. The performance of these plant species is greatly influenced by feedback interactions with the abiotic and biotic soil environment. This means that plants change the soil environment, which in turn can change plant performance. The main aim of my thesis was to examine the importance of plant-soil interactions for the population dynamics of an early-successional plant species during old-field succession. In addition, I studied how plant community composition can influence plant-symbiont interactions of individual plants. As a model I selected the plant species *Jacobaea vulgaris* ssp. *vulgaris*, which is a strong determinant of early successional plant communities in the Netherlands.

I examined the population dynamics of *J. vulgaris* in ten fields that form a chronosequence of land abandonment. The old-fields were taken out of agricultural production between 2 and 25 years ago. *J. vulgaris* cover at the old-fields peaked about 5 years after agricultural abandonment, after which cover declined again. I hypothesized that these changes in the abundance of *J. vulgaris* were related to plant-soil feedback. In order to study the effects of the soil community on *J. vulgaris* performance, I conducted a greenhouse experiment with soil collected from the old-fields. There was a positive relationship between *J. vulgaris* density in the field and the level of control by the soil community. When the soil was conditioned first during one growth period by growing *J. vulgaris,* soils from all stages of the chronosequence developed a strong negative feedback effect to *J. vulgaris.*

In a common garden I planted intact vegetation turfs, collected from the chronosequence fields, and sew *J. vulgaris* in the turfs. Seedling establishment was significantly lower in turfs from older than from young fields. In a seed bank study the number of emerging seedlings declined with time since abandonment of the field, but the number of seedlings out-ruled the actual number of *J. vulgaris* plants in the field in all cases. Thus, the seed bank was not limiting, but establishment conditions were less favorable when time since abandonment proceeded.

Plant species that co-occur with *J. vulgaris* in the old-fields can also change the abiotic and biotic soil conditions, which may alter *J. vulgaris* performance. In a greenhouse experiment I tested soil effects of *J. vulgaris* on its own performance and on that of 30 co-occurring plant species. In addition, I examined the reciprocal soil effect of each species on *J. vulgaris*. Thus, I compared interspecific plant-soil interactions between *J. vulgaris* and co-occurring plants with intraspecific plant-soil feedback effects of *J. vulgaris* on itself. This study confirmed that *J. vulgaris* exhibits strong negative plant-soil feedback and it revealed there were profound differences among the co-occurring species in interspecific plant-soil

effects on *J. vulgaris* (approximately half the species reduced *J. vulgaris* performance, whereas the other half had no effect), as well as that soil conditioned by *J. vulgaris* had a positive or neutral effect on the growth of the co-occurring species. I suggested three mechanisms how the legacy of plant-soil interactions may, alone or in combination, enhance the rate of succession through priority effects: early successional plant species exert negative plant-soil feedback; co-occurring plant species cause negative interspecific plant-soil effects to the early successional species; and the early successional species has an overall positive interspecific plant-soil effect on co-occurring plant species.

Then I teased the soil community effects apart in relation to the size of the organisms involved. In a greenhouse experiment, I compared *J. vulgaris* performance in sterilized soil inoculated with live field soil that was sieved through a 1 mm mesh, with an aqueous soil suspension also sieved through a 1mm mesh, or with an aqueous microbial suspension sieved through a 20 µm mesh. Biomass of *J. vulgaris* was most reduced in pots inoculated with the sieved soil, whereas the growth reducing effect was lost when pots were inoculated with the microbial suspension. I also showed that results obtained with inoculating sieved soil or with a soil suspension are not necessary comparable, so that soil inoculum preparation can strongly influence the feedback effect. This needs to be considered when designing plant-soil experiments.

I also examined whether reduced growth of *J. vulgaris* in soil conditioned by conspecifics can be due to the release of autotoxic phytochemicals. I tested this hypothesis by examining the inhibitory effects of different strengths of aqueous *J. vulgaris* tissue extracts, as well as the effect of root fragments on the performance of *J. vulgaris* seedlings growing in soil or water. Performance of seedlings that were growing in water and received aqueous extracts was in some cases significantly reduced. However, seedlings growing in sterilized soil were not affected by *J. vulgaris* extracts. Incorporating root fragments reduced maximum root length significantly, also when seedlings were growing in sterilized soil. Therefore, I conclude that *J. vulgaris* may show autotoxicity under laboratory conditions, but since autotoxic effects were less strong in soil autotoxicity does not seem to play an important role in the decline of *J. vulgaris* abundance in old-fields.

To study the effect of plant communities on soil organisms that colonize individual plants, I determined the composition of arbuscular mycorrhizal fungi (AMF) colonizing individual *J. vulgaris* plants. These plants were growing in experimental plant communities. Half the plant communities were established by sowing mid successional plant species in arable land and the other half by natural colonization. The plant communities were 10 years old by the time we collected *J. vulgaris* plant roots and analyzed the AMF community in the roots, using a molecular fingerprinting method (T-RFLP). After 10 years, the unsown plant communities were more diverse and spatially heterogeneous than sown ones, but both treatments shared many plant species. AMF diversity did not differ between the plant communities, but there was higher AMF assemblage dissimilarity between individual *J. vulgaris* plants in the unsown plant communities. When we grew *J. vulgaris* plants in field

soil that was homogenized, in order to rule out spatial variation, there were no differences anymore in AMF dissimilarity between sown and unsown plots. Thus, plant community assembly history can influence the AMF community of individual plants growing in those plant communities.

In conclusion, my thesis study shows that plant-soil biota interactions are an important factor explaining the hump-shaped population development of *J. vulgaris*, but these interactions should be considered in relation to seedling recruitment and interspecific competition.

Chapter 1

General introduction

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Biodiversity is declining worldwide (Vitousek *et al.* 1997; Thomas *et al.* 2004). In order to conserve natural diversity and combat its decline, low productive agricultural fields in the Netherlands have been taken out of production and converted into semi-natural grasslands. These crop fields became available for nature restoration due to agricultural intensification. The conversion of agricultural land into species-rich grasslands is a common practice to restore and conserve diversity in many industrialized countries (Ejrnæs *et al.* 2003; Walker *et al.* 2004). After these fields are taken out of agricultural production, they undergo a transition from an arable system into a species rich grassland. This transition of plant communities associated to agricultural systems to those typical for semi-natural grasslands is a well accepted model for ecological studies on factors that drive changes in plant communities (*e.g.* Armesto and Pickett 1986; Myster and Pickett 1994). Understanding the development of grassland communities and the successional transitions on old-fields is becoming increasingly important from a conservation, restoration and social perspective (Cramer *et al.* 2008), and it can help to learn more about other ecological processes, such as invasion ecology (Meiners *et al.* 2009).

Succession can be defined as the change in composition of natural vegetation over time (Picket *et al.* 1987; Walker and Chapin 1987; Walker and del Moral 2003). Secondary succession starts after the disturbance of an already existing ecosystem, such as after forest fires, floods or agricultural abandonment. When secondary succession starts after abandoning an agricultural field this process is called old-field succession. During secondary succession, many plant species enter and leave successional sequences according to a boom-bust pattern (Olff and Bakker 1991; Bezemer *et al.* 2006a; Meiners *et al.* 2009). These temporal changes in the performance of individual species can be due to factors such as plant dispersal and recruitment dynamics, resource availability, control by the surrounding vegetation, as well as by natural enemies (Huston and Smith 1987; Olff and Bakker 1991). The multitude of possible factors that can affect the population dynamics of a species during succession make this a complex process to understand. In addition, the factors that determine the increase in local abundance of a species during the onset of succession can be entirely different from the characteristics causing its decline when succession progresses. Also, the strength and relative importance of each factor can change during the different life stages of a plant or between successional stages (Chapin *et al.* 1994; Anderson 2007; Luzuriaga and Escudero 2008).

After cessation of agricultural practices the process of succession starts on soils that are usually low in soil organic matter content and high in nutrient availability due to fertilization. In these early successional fields later-successional seeds are often absent in the seed bank (Bakker and Berendse 1999) and the presence of these species depends on the dispersal of propagules from the surrounding area (Bakker *et al.* 2000). Instead, in young fields the vegetation is dominated by fast growing plant species (Marrs 1993). These species are more limited by light than by nutrients (Tilman 1988), as the best competitors for nutrients typically dominate the plant community (Tilman 1982). As time

since abandonment increases soil pH decreases (Zeller *et al.* 2001), levels of nitrate and phosphorous decrease as they leach or are taken up by microorganisms and plants. The C:N ratio and amount of organic material increases as no harvestable product is removed while the fields are not fertilized or cultivated anymore (Inouye *et al.* 1987*;* Knops and Tilman 2000; Zeller *et al.* 2001; Baer *et al.* 2002; van der Wal *et al.* 2006). For long the focus in studies on successional changes has been on detecting how changes in abiotic conditions influence plant community composition and *vice versa* (*e.g.* Huston 1994; Olff *et al.* 1994; Wardle 2002).

Currently, recognition is growing that biota can also play an important role in the vegetation dynamics during succession. Especially on shorter temporal scales biotic processes can be important and can cause plant species to be replaced over successional time. Not only aboveground vertebrates and invertebrates can change plant community dynamics during succession (Brown and Gange 1992; Schädler *et al.* 2003; Veblen 2008), also belowground herbivores and (micro-)organisms can affect this process (*e.g.* Johnson *et al.* 1991; De Deyn *et al.* 2004; Kardol *et al.* 2006; van der Heijden *et al.* 2008; Eschen *et al.* 2009). Belowground organisms can have both direct and indirect effects on secondary succession via their beneficial or harmful effects on plants (Wardle *et al.* 2004). For example, pathogenic soil bacteria and fungi, root-feeding nematodes, soil invertebrates, and mycorrhizal fungi can have strong effects on the performance of individual plants, their long-term population dynamics and on plant community composition (*e.g.* Augspurger 1983; van der Putten *et al.* 1993; Bever 1994, van der Heijden *et al.* 1998b; De Deyn *et al.* 2003; Kardol *et al.* 2007). When the vulnerability of a species to soil pathogens differs from other plant species this can lead to the directional replacement of a species during succession (van der Putten *et al.* 1993). Soil biota may also affect the establishment and survival of new seedlings (van der Heijden *et al.* 2004; Pregitzer *et al.* 2010). In addition, soil organisms that belong to the decomposer community alter nutrient cycling during succession, which can influence plant performance indirectly (Wardle *et al.* 2004).

In this thesis I will mainly focus on the effects that soil biota have on the performance and replacement of a plant species during old-field succession. I will also investigate the role of neighbouring species, via the soil community, on the performance and population dynamics of the tested species in order to investigate the importance of individual plant-soil interactions in a community context.

Plant-soil interactions

The performance of a plant is greatly influenced by interactions with the abiotic and biotic soil environment (Wardle *et al.* 2004). Soil organisms can affect the performance of

individual plants, but they can also change plant species diversity, the productivity and composition of plant communities, as well as the functioning of ecosystems (*e.g.* van der Putten *et al.* 1993; Klironomos *et al.* 2000; van der Heijden *et al.* 1998b, 2008). The effects of soil organisms on plant performance depend on soil taxon, as well as on the plant species (Bradford *et al.* 2002; De Deyn *et al.* 2003). Plants also have an effect on soil biota and the abiotic soil environment (Bever 1994; Wardle 2002; Ehrenfeld *et al.* 2005), for example, via living or decaying roots and root exudates (Bardgett *et al.* 1999; Kulmatiski *et al.* 2008; Berg and Smalla 2009). In this way, plants can change the soil (microbial) community (Bever 1994), increase nutrient availability due to increased decomposition rates (Berendse 1998), or promote the build-up of soil-borne pathogen populations (Augspurger 1983; De-Rooij-van der Goes *et al.* 1995; Packer and Clay 2000, 2003). These effects of plants on the soil community and abiotic soil environment can differ greatly among plant species, as plant species culture their own soil community (Aerts and Chapin 2000; Klironomos 2003; Kardol *et al.* 2007; Ayres *et al.* 2009). The effect of a plant species on the soil community can show up at different locations in the soil food web (Bezemer *et al.* 2010). Plant-soil interactions have been shown a key process in connecting belowground and aboveground compartments in terrestrial ecosystems (Bardgett and Wardle 2010).

Plant-soil feedback

Plant species not only affect the soil biotic and abiotic environment in a species-specific way, they also respond to interactions with the soil community and the abiotic soil environment in a species-specific way (Bever 1994; Ehrenfeld *et al.* 2005). The two-step process that a plant changes the soil biotic and abiotic conditions, which subsequently alter the growth rate of plants of the same species is called plant-soil feedback (Bever *et al.* 1997; Bever 2003). Plant-soil feedback encompasses the direct effects of the changed soil community on the growth rate of individuals of the same species (Fig. 1.1). Indirect feedback effects can occur when the changed soil conditions alter plant performance of the studied plant by changing the competitive strength of neighbouring plants (Bever 2003). Feedback is called positive when the effects of belowground mutualistic symbionts and resource availability overrule the negative effects caused by belowground herbivores, pathogens, and resource immobilization (Bever *et al.* 1997). When the latter effects dominate, plant-soil feedback is called negative and plant growth will be reduced. For example, when the performance of individuals of plant species A (Fig. 1.1) is reduced when they grow in soil conditioned by plant A this is called negative plant-soil feedback. The negative feedback effect on individuals of species A will indirectly stimulate the performance of individuals of plant species B, due to the reduced competitive ability of plant A (Fig. 1.1, dashed arrow). Positive plant-soil feedback has been proposed to result in plant dominance and enhanced intraspecific competition, enhancing plant persistence

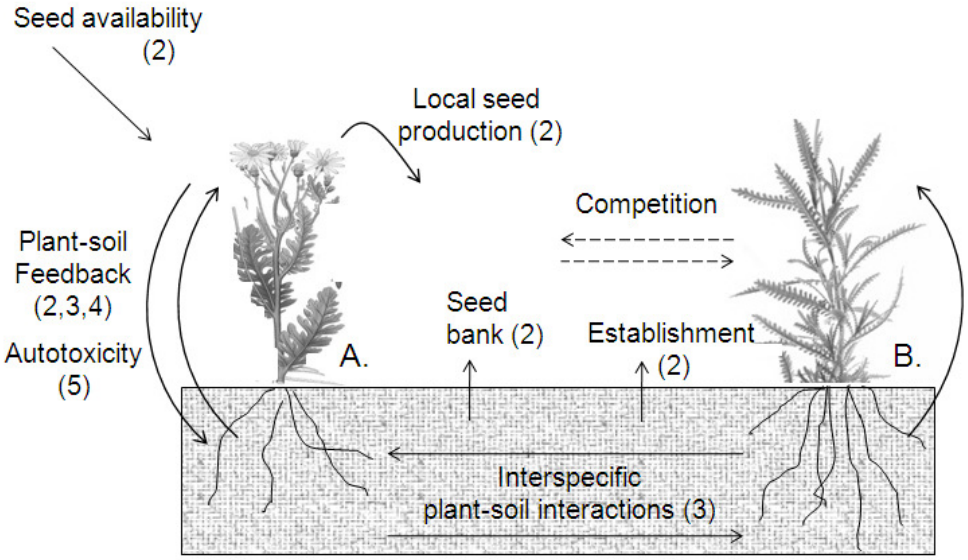

Fig. 1.1 Scheme of the mechanisms that are important for the performance and replacement of species A. These mechanisms involve plant-soil interactions with plants of the same species (species A; plant-soil feedback) and with plants of other species (species B; interspecific plant-soil interactions), seed availability, seedling recruitment, and indirect changes in competitive ability due to plant-soil interactions. The chapter in which the mechanism is studied is given between brackets. Dashed arrows indicate indirect effects.

(Bever 2003). Negative feedback is supposed to promote species co-existence due to reduced interspecific competition (Bever 2003).

By stimulating or constraining the performance of specific plants, soil biota can change plant community composition (*e.g.* van der Putten *et al.* 1993; van der Heijden *et al.* 1998a) and even the functioning of ecosystems (Bardgett and Wardle 2010). This requires plant species-specific soil feedback effects. Indeed, arbuscular mycorrhizal fungal (AMF) species differ in their effects on plant species (van der Heijden *et al.* 1998a; Van der Putten 2003; Klironomos 2003). In this way they can favour the performance of some plant species as compared to others, which can lead to differences in plant community composition or ecosystem functioning (van der Heijden *et al.* 1998ab; Bardgett and Wardle 2010). Plant species specificity is also known for soil pathogens. For example, van der Putten *et al.* (1993) showed that early successional plant species can accumulate soil pathogens that reduce their performance, but not that of later-successional species. In this way, the negative plant-soil feedback promotes the replacement of the early-successional species so that soil-borne pathogens can act as a driver of succession (van der Putten 2003).

The build-up of plant species-specific soil micro-organisms can also enhance secondary succession, for example, in old-fields (Kardol *et al.* 2006) or forests (Augspurger 1983). Kardol *et al.* (2006) showed that a negative plant-soil feedback can increase the rate of successional changes in old-fields in the Netherlands. Plant species indicative of early stages of old-field succession created a negative plant-soil feedback, which enhanced the replacement of these early successional plant species (Kardol *et al.* 2006; 2007). Mid-successional species had a neutral feedback, whereas late successional species changed the soil community in such a way that their own performance was stimulated, thus maintaining their importance in the community and retarding succession (Kardol *et al.* 2006; Kulmatiski *et al.* 2008). Although many feedback studies focus on the importance of soil micro-organisms on successional dynamics, also soil invertebrates, such as nematodes or insects, can change plant community composition, or enhance the rate of succession in grasslands (Bradford *et al.* 2002; De Deyn *et al.* 2003).

Plant-soil interactions with neighbouring plants

In nature, plants typically grow in mixed communities, and thus also interact with individuals of other species through competition for light, space, or nutrients (Tilman 2009) or through facilitation (Brooker *et al.* 2008). However, these plant-plant interactions can be influenced by biotic interactions between plants and aboveground or belowground higher trophic level organisms, including soil feedback (Bever 2003; Kardol *et al.* 2007; Fig. 1.1). Most of the interactions involving plant-soil feedback effects have focused on plants of the same species. However, although less well studied, there is also some evidence that points at the importance of plant-soil interactions between plant individuals that belong to different species (*e.g.* Kardol *et al.* 2007; McCarthy-Neumann and Kobe 2010; Fig. 1.1). Similar to the plant-soil feedback mechanism described before, via their effects on soil biota or abiotic soil conditions, plants can affect the performance of individuals of other species as well. In this way plants can facilitate (van der Heijden and Horton 2009; van der Putten 2009) or inhibit (Bonamoni *et al.* 2005; Casper and Castelli 2007) the performance of plant individuals that belong to other species. Depending on the temporal and spatial dimension of these plant-soil interactions, such effects are termed 'associative resistance or susceptibility' (Barbosa *et al.* 2009) when two plants physically grow together; or 'priority effects' when one plant follows after the other (Young *et al.* 2001; Grman and Suding 2010; Haussmann and Hawkes 2010). Although these processes are well studied for aboveground processes, interactions via the soil have not received much attention yet.

The changed soil biotic and abiotic conditions that are created by a plant cause legacy effects in the soil that not only affect the performance of co-occurring plants of the same and other species, but also the offspring of these species (Bonanomi *et al.* 2008). In this way, soil conditions that were altered by a plant can affect the establishment, growth, performance or reproduction of the later arriving plants. Priority effects have been studied

frequently within the context of community assembly (Young *et al.* 2001; Fukami *et al.* 2005). Many studies have shown that the arrival order of species is important for subsequent community composition and dynamics (Chapin *et al.* 1994; Fukami *et al.* 2005; Ejrnæs *et al.* 2006; Körner *et al.* 2008; Stevens and Fehmi 2009). Early arrival of a plant species could lead to space occupancy, both aboveground and belowground, which contributes to the success of that species (Körner *et al.* 2008). Timely arrival may confer a competitive advantage to a species that is normally an inferior competitor (Young *et al.* 2001). In this way priority effects could reduce the invasion success of new colonizers, when native plants are already present (Stevens and Fehmi 2009). Besides competitive interactions, priority effects can also arise from facilitative interactions between plant species, such as ameliorated micro-climate (Scowcroft and Jeffrey 1999), depleted nutrients (Davis *et al.* 2000) and soil chemical conditions (Rhoades *et al.* 1998). Via such environmental changes, early successional species can modify the environment that allows later successional species to enter the community (Young *et al.* 2001).

Although priority effects are well studied, the importance of priority effects due to soil legacies has received little attention. Especially priority effects due to biotic soil legacies have rarely been considered. Recently, Grman and Suding (2010) showed that soil legacies created by native or exotic grassland species can cause strong priority effects on the performance of subsequent native and exotic plant species. They showed that priority effects due to soil legacies indeed occur and that they can have large consequences for the growth of subsequent plant species. The strength of the priority effect depends on the strength of the plant-soil interactions and their time of endurance after the initial plant species has disappeared. This time of endurance is called the legacy effect (Kardol *et al.* 2007). However, little is known on the role of priority effects via soil legacies for plant population dynamics during secondary succession. In addition, the debate is still open whether or not interspecific plant-soil interactions play a significant role in determining individual plant performance and composition of mixed plant communities (Kulmatiski *et al.* 2008).

In this thesis I will call the effects of one plant species on another plant species through soil legacies 'interspecific plant-soil interactions' in order to distinguish them from the commonly studied 'intraspecific plant-soil feedback', which refers to the effect of one individual on individuals of the same species.

Plant-chemical interactions

Plants can affect other plants by interacting with the biotic soil environment, but they can also interact with each other via the soil in a different way. Due to foliar leaching, root exudation, plant decomposition or volatilization plants can release chemicals into the soil (Rice 1974; Inderjit and Nilsen 2003; Lipinska and Harkot 2007; Lambers *et al.* 2008).

These chemicals can inhibit the germination, nutrient acquisition or performance of neighbouring plants (Inderjit 1996). This is called allelopathy (Rice 1984). Many researchers use the broadest definition of allelopathy: "…allelopathy includes any direct or indirect harmful effect by one plant (including microorganisms) on another through the production of chemical compounds that escape into the environment" (Rice 1974). A special form of allelopathy is autotoxicity or autoallelopathy, which means that the released allelochemicals inhibit the performance of plants of the same species (Kumari and Kohli 1987; Lambers *et al.* 2008).

Many different plant secondary metabolites, such as phenolics, alkaloids, benzoic acids, can have allelopathic effects (Rice 1974). Allelopathic effects can be attributed directly to plant chemicals (Bais *et al.* 2003). However, soil microorganisms can also play an important role in allelopathy as they can (de-)activate toxic plant compounds or release phytotoxic compounds when decomposing plant tissues (Rice 1984; Inderjit and Nilsen 2003; Weir and Vivanco 2008). The properties of plant chemicals can also be influenced directly by the physical and chemical properties of the soil (Kobayashi 2004), for example, due to adsorption to soil particles (Wardle *et al.* 1998). The main focus of allelopathy studies has been on agricultural problems. For example, how to deal with weed competition, soil sickness and replanting problems (Rice 1974; Inderjit 2005; Liu *et al.* 2008). However, autotoxicity can also be important in natural systems (Hierro and Callaway 2003; Orr *et al.* 2005).

Although the importance of allelopathy in natural systems is not well studied (Rice 1984; Inderjit 2001), there are some interesting examples that show the possible effects of allelopathy in natural systems. For example, a considerable amount of work has been done on the role of allelopathy in the invasion of exotic plant species (Callaway and Aschehoug 2000; Vivanco *et al.* 2004; Orr *et al.* 2005). According to the novel weapons hypothesis exotic species can excrete allelochemicals that are new to the native plant community, thus providing the invader with a competitive advantage over the native plant species which may promote invasion and gaining dominance (Callaway and Ridenour 2004).

Allelopathy and autotoxicity can also play an important role in succession (Rice 1984). In old fields in Kansas, leachates of *Helianthus annuus* inhibited the performance of co-occurring early successional species as well as the performance of *H. annuus* itself during early secondary succession, but leachates did not reduce the performance of a later successional species (Wilson and Rice 1968). In this way allelochemicals of *H. annuus* enhanced successional replacement, suggesting that plant-chemical interactions can be important for the replacement of a plant species by later successional ones.

Symbionts colonizing individual plants in a grassland

Until now, I focused on plant-soil interactions and their effect on plant performance and community dynamics. However, the interaction between plants and soil organisms can also be studied from a soil organism viewpoint: the effect of the plant community on soil organisms. As a model to study the effect of plant communities on soil organisms I used arbuscular mycorrhizal fungi (AMF). AMF are obligate biotrophs living in close collaboration with plants. Therefore, we can expect that the plant community has a direct effect on the composition of the AMF community in the soil and in the roots of individual plants growing in that community.

AMF belong to the phylum *Glomeromycota* (Schüßler *et al.* 2001) and form symbiotic associations with approximately 60-80% of the terrestrial plant families (Trappe 1987; Schüßler *et al.* 2001). AMF colonize plant roots and extend hyphae into the soil. Via these hyphae the AMF can take up immobile nutrients, such as phosphorous in a wider range than the plant roots can (Smith and Read 2008). They exchange the nutrients and other plant benefits, such as drought and pathogen protection, for photosynthetically fixed carbon (Davies *et al.* 1993; Newsham *et al.* 1995; Borowicz 2001; Smith and Read 2008). The exchange takes place in arbuscules, which are structures formed by the fungi inside plant roots. AMF reproduce via spores, which can survive in the soil for many years. Although most plants benefit from AMF colonization, the costs of maintaining AMF can be higher than the benefits for the plant (Johnson *et al.* 1997). Therefore, the association between AMF and plants can range from mutualistic to parasitic depending on, for example, the AMF strain, the identity of the plant species (Klironomos 2003), the environmental conditions, or the developmental stage of the plant (Šmilauer 2001; Husband *et al.* 2002). Due to this differential effect on individual plant species (Johnson *et al.* 1997; Klironomos 2003; Rinaudo *et al.* 2010) AMF can change the outcome of plant competition (Koide and Dickie 2002; Scheublin *et al.* 2007), productivity (van der Heijden *et al.* 1998b; Klironomos *et al.* 2000), the success of invasive plants (Stampe and Daehler 2003), and plant community composition (van der Heijden *et al.* 1998a; Hartnett and Wilson 1999; Koide and Dickie 2002) and diversity (Klironomos and Hart 2002).

Besides the effect that AMF can impose on plant performance and ecosystem functioning, AMF themselves are also affected by environmental conditions. For example, the AMF spore community within the soil can be influenced by soil characteristics, such as disturbance history, soil type and chemistry (Helgason *et al.* 1998; Egerton-Warburton *et al.* 2007; Fitzsimons *et al.* 2008). Nevertheless, the composition of the spores does not have to reflect the actual AMF community colonizing plant roots. In a study investigating the composition of root colonizing AMF , Öpik *et al.* (2006) reported large differences between ecosystems worldwide, suggesting that environment is an important factor determining AMF colonization of plant roots. On a smaller spatial scale, Johnson *et al.* (2004) showed that in a microcosm study plant species composition influenced AMF

diversity in the plant roots significantly. Recently, Hausmann and Hawkes (2009) showed in a glasshouse study that besides plant diversity, also the identity of neighbouring plants can influence the composition of AMF colonizing a focal plant. They showed that the composition of the AMF community differed significantly in roots of plants that were growing in a monoculture and that were growing in a heterospecific perennial-annual mixture. Similarly, in field experiments in Utah and California Hawkes *et al*. (2006) found that the AMF assemblage colonizing native plant roots changed when the area was invaded by exotic grasses. Also, the presence of the invasive forb *Centaurea maculosa* changed the AMF community colonizing *Dactylis glomerata* plants (Mummey *et al.* 2005). These studies show that the community context is important for plant-AMF interactions and that there is a need for more field studies in order to understand how the composition of AMF communities is shaped.

Aim and research questions of the thesis

The main aim of this thesis is to examine the importance of plant-soil interactions via soil biota or the release of plant chemicals for the population dynamics of an early-successional plant species. In addition, I studied how the surrounding plant community can influence plant-symbiont assemblages in the roots of individual plants. In my thesis, I use *Jacobaea vulgaris* as a model, because this species is a strong determinant of early successional plant communities in old-fields.

The major research questions of this thesis are:

i. How does a *J. vulgaris* population develop during secondary succession on old-fields?
ii. What is the importance of plant-soil feedback for performance and population dynamics of *J. vulgaris* during old-field succession?
iii. Do co-occurring plant species create soil legacies that affect the performance of *J. vulgaris*? What is the effect of interspecific plant-soil interactions of co-occurring plant species on the performance of *J. vulgaris*?
iv. Does the plant-soil feedback effect differ between size classes of soil biota, and is there variation in effect size between plant populations?
v. Does autotoxicity affect the performance of *J. vulgaris*? Under which conditions is autotoxicity most likely to occur?
vi. What is the effect of the surrounding plant community on mutualistic symbionts (*i.e.* arbuscular mycorrhizal fungi) colonizing individual *J. vulgaris* plants?

Jacobaea vulgaris

In this thesis I use the plant species *Jacobaea vulgaris* ssp. *vulgaris* (Asteraceae) synonym *Senecio jacobaea* (Pelser *et al.* 2006) (Fig. 1.2). *J. vulgaris* is commonly known as (Tansy or Common) ragwort. In Dutch this forb is called 'Jakobskruiskruid'. *J. vulgaris* can become dominant in early successional plant communities and is native to most countries in Europe, including the Netherlands. In North America, Australia, and New Zealand *J. vulgaris* has been introduced and became invasive (Poole and Cairns 1940; Wardle 1987; Bain 1991). *J. vulgaris* occurs in all provinces of the Netherlands (van der Meijden 2005). In the western part of the Netherlands the subspecies *J. vulgaris* spp. *dunensis* (Dumort.) is more commonly found (van der Meijden 2005).

J. vulgaris occurs typically on relatively dry and sandy soils in northwestern Europe (*e.g.* van der Meijden and van der Waals-Kooi 1979; Crawley and Nachapong 1985; Bezemer *et al.* 2006a). In the Netherlands, *J. vulgaris* occurs on open or grazed, sandy soils, such as weed pastures, agricultural and natural areas (van der Meijden 2005; Bezemer *et al.* 2006a). Especially in disturbed areas or during periods of disturbance, such as overgrazing or pasture suppression, *J. vulgaris* can establish well (Harper and Wood 1957; Wardle *et al.* 1987) and become very abundant (Smittenberg 2005; Bezemer *et al.* 2006a).

J. vulgaris is a monocarpic perennial weed that spends its first year as a rosette (Fig. 1.2D). Seeds germinate in autumn or in spring, after which they form a rosette in the first growing season. From May-June of the next growing season, the flowering stem develops and the plant will flower from July-October. After flowering and seed set the plant dies. Flowering may take place in the second year, but is often delayed following herbivory as flowering will only occur when a certain threshold size has been reached (van der Meijden and van der Waals-Kooi 1979; Prins *et al.* 1990). The foliage of the plant is sometimes eaten almost entirely by (specialist) herbivores, but *J. vulgaris* has a strong capacity for re-growth after damage (van der Meijden *et al.* 2000). Dispersal occurs mainly via seeds that are dispersed via wind or gravity, but *J. vulgaris* can reproduce vegetatively via root or crown buds as well (Harper and Wood 1957). The seeds can differ in weight depending on plant performance and a small reduction in seed size can already strongly reduce plant fitness and the competitive ability of these seedlings (Crawley and Nachapong 1985). Given the right conditions, each plant can produce more than 100,000 seeds. These seeds can remain dormant for many years in the soil seed bank and retain high germination rates (*e.g.* Harper 1958; Thompson and Makepeace 1983; Wardle *et al.* 1987; McEvoy *et al.* 1991). Although seeds can remain viable in the soil for a long time, there is often a large discrepancy between the number of *J. vulgaris* seeds in the seed bank and the actual number of seedlings that are present in the field (Forbes 1977; Crawley and Nachapong 1985). One of the explanations given for this discrepancy is the absence of disturbances, so that non-germinated seedlings are not brought back to the soil surface (van der Meijden and van der Waals-Kooi 1979).

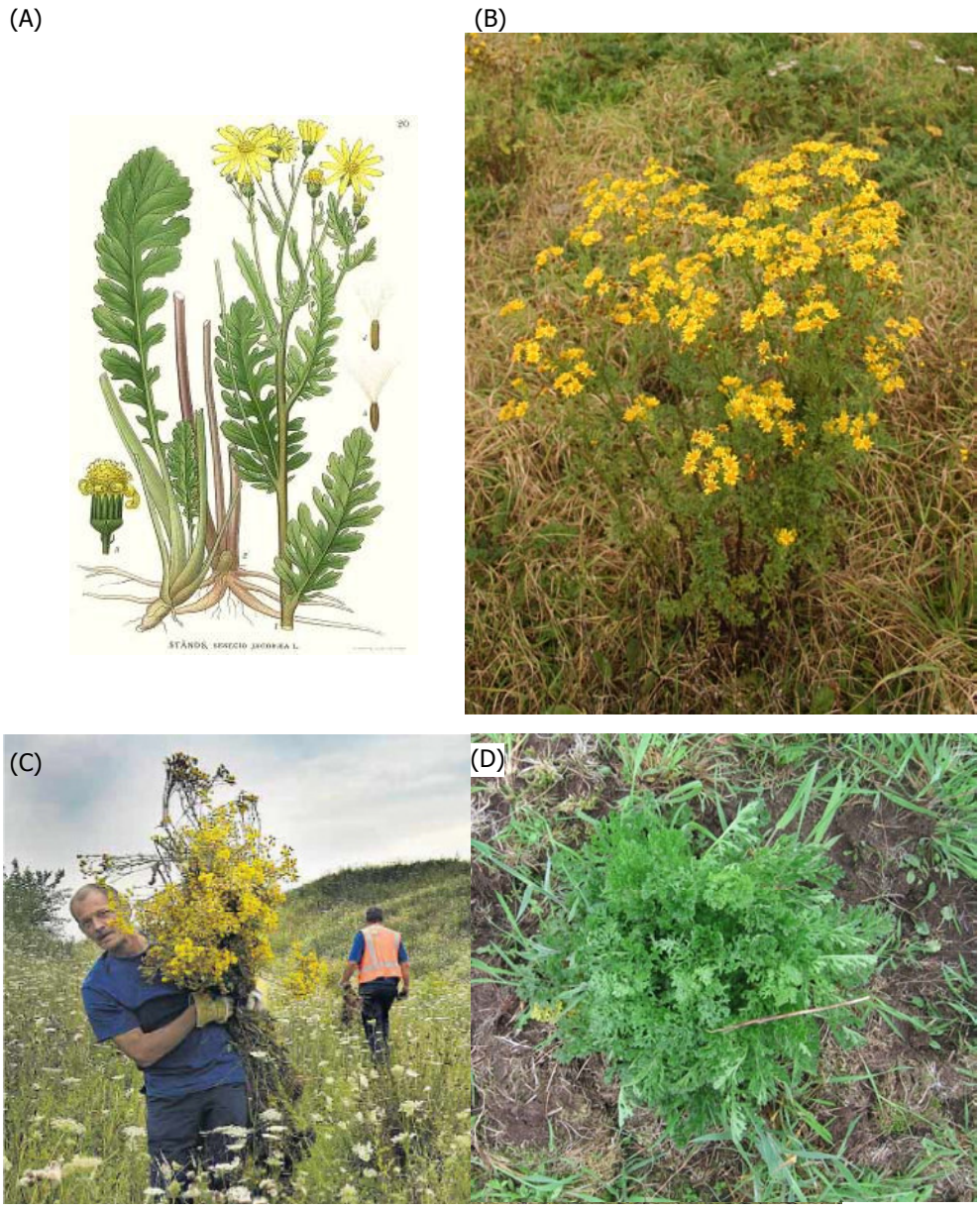

Fig. 1.2 *Jacobaea vulgaris* ssp. *vulgaris* (Asteraceae) synomym *Senecio jacobaea* L. (A) Drawing obtained from CAM Lindman (1901-1905), Bilder ur Nordens Flora; picture of flowering *J. vulgaris* plant (B); manual removal of *J. vulgaris* plants near Emmen, the Netherlands (Picture GPD/Jan Anninga in Leeuwarder courant ed. Zuid 13-11-2010) (C); *J. vulgaris* rosette (D).

The seed bank can be important for *J. vulgaris* to maintain population size, as was shown in a Dutch dune system by van der Meijden (1971). *J. vulgaris* is a plant species that prefers open, disturbed patches to colonize (Cameron 1935; Harper and Wood 1957) and germination success is higher in bare and disturbed micro-sites than in dense grassland vegetation (Cameron 1935; van der Meijden and van der Waals-Kooi 1979; Prins and Nell 1990). Dense grassland vegetation also lowers *J. vulgaris* establishment success, showing that *J. vulgaris* is a weak competitor before reaching the rosette stage (Harper 1958; Thompson 1980). However, once reaching the rosette stage, *J. vulgaris* can outcompete other plant species effectively (Harper 1958).

J. vulgaris produces a variety of pyrrolizidine alkaloids (PAs) in the roots (Hartmann and Dierich 1998). These PAs, which are also expressed in aboveground plant parts, can act as defense chemicals against mammals, insect herbivores and soil pathogens, such as fungi, bacteria and plant parasitic nematodes (Mattocks 1968; Vrieling *et al.* 1991b; Witte *et al.* 1992; van Dam *et al.* 1995; Hol and van Veen 2002; Macel *et al.* 2002; Kowalchuk *et al.* 2006; Thoden *et al.* 2009ab). Herbivory can also influence PA concentrations in roots and shoots (Vrieling *et al.* 1991a; Hol *et al.* 2004). Especially the interaction between *J. vulgaris* and aboveground insects has been studied intensively, in order to understand the importance of PAs on insects or *vice versa*, or the use of insects as biological control agents. In this thesis I will focus on interactions between *J. vulgaris* and belowground organisms.

The concentration of PAs in the roots and shoots is reduced when more nutrients for plant growth are available. This reduction is due to increased biomass production rather than due to decreased PA production (Hol *et al.* 2003). Ahmed and Wardle (1994) found that extracts made of *J. vulgaris* tissues can reduce the performance of grassland species. In the Netherlands and in many other countries *J. vulgaris* is considered a problem weed in pastures (McEvoy 1984; Smittenberg 2005) and abandoned arable fields that are used for nature restoration (Bezemer *et al.* 2006a). This is mainly due to its dominance (Wardle 1987; Bezemer *et al.* 2006a) and because it contains pyrrolizidine alkaloids that are toxic for livestock (Cameron 1935; Wardle 1987).

Besides the scientific interest in *J. vulgaris*, there is also much interest in *J. vulgaris* from the public and many hot-tempered discussions can be found on the internet. Landowners, farmers and horse keepers are annoyed by *J. vulgaris* invading their properties from neighbouring nature reserves and remove *J. vulgaris* plants manually (Fig. 1.2C). The reason for this uproar is that the PAs in *J. vulgaris* can cause acute and chronic liver damage when ingested by cattle and horses (Mattocks 1986). In the field, *J. vulgaris* plants are generally avoided by cattle unless the plants are very abundant (Wardle *et al.* 1987). However, when the plants are harvested and end up in hay, they are not noticed anymore by the animals and they will be eaten. The consumption of *J. vulgaris* plants can have serious negative health effects on cattle and horses. Besides the consequences for horse keepers and cattle farmers, the *J. vulgaris* problem has consequences for other

groups as well. For example, harvested grass from road sides can not be sold anymore as hay unless it is proven to be "*J. vulgaris*-free". The PAs in *J. vulgaris* can also be toxic for humans when ingested, however, the risks for humans by skin contact (www.ragwort.jakobskruiskruid.com) or via honey consumption (www.vwa.nl) are unlikely, but still under investigation.

Landowners, municipalities and provinces are currently investigating the increase in *J. vulgaris* abundance and the problems that this may cause in the Netherlands (Covenant Province Gelderland; Groningen; Friesland). Besides investigating the problem, some provinces and municipalities made covenants in which they exclude *J. vulgaris* from seed mixtures for road verges or made appointments about only selling hay without *J. vulgaris*. In addition, they have developed guidelines to mow areas with high *J. vulgaris* cover before seed-set (Province Gelderland 2007). However, mowing only reduces seed distribution, but it does not remove the plants, as it only removes aboveground plant parts of the plants. Therefore, in order to reduce *J. vulgaris* cover mowing has to be repeated frequently, which is a costly measure. In addition, frequent mowing will disrupt other plants and insects as well, which makes it an unfavorable management tool in natural areas. Removing the *J. vulgaris* plants manually, including their root system, has a better success rate, however this is very labour-intensive and therefore not suitable for large areas (Boekhoff 2005). Currently, some landowners are conducting experiments in which they test different maintenance practices against *J. vulgaris*. Nevertheless, *J. vulgaris* is a native plant species in the Netherlands and therefore the urgency of reducing its presence is not considered to be very high by all land owners and nature conservationists.

Study area

For the work described in this thesis I selected ten fields that form a chronosequence of agricultural land abandonment (Fig. 1.4). In a chronosequence space replaces time (Picket 1989). In order to do so, it requires that all other factors than time are constant, such as land use history, climate and substrate. The ten fields were taken out of agricultural production between 1982 and 2005, and the eight oldest fields are a subset of ones also used by Kardol (2007), van der Wal (2007) and Holtkamp (2010). All fields are larger than 1 ha and are situated at the Veluwe, which is a large nature reserve in the centre of the Netherlands. The Veluwe was created during the Saalien ice age and consists of sandy to sandy loam glacial deposits. The ten fields were selected because of their similarity in soil substrate, current management practices (extensive grazing), and similar arable crop growing history (maize, cereals; Kardol *et al.* 2005; van der Wal *et al.* 2006). After the agricultural practices were ceased, the fields were colonized naturally and since then they have been managed by low-intensive grazing by natural and introduced vertebrate herbivores (deer, horses, cattle). The maximum distance between two fields is

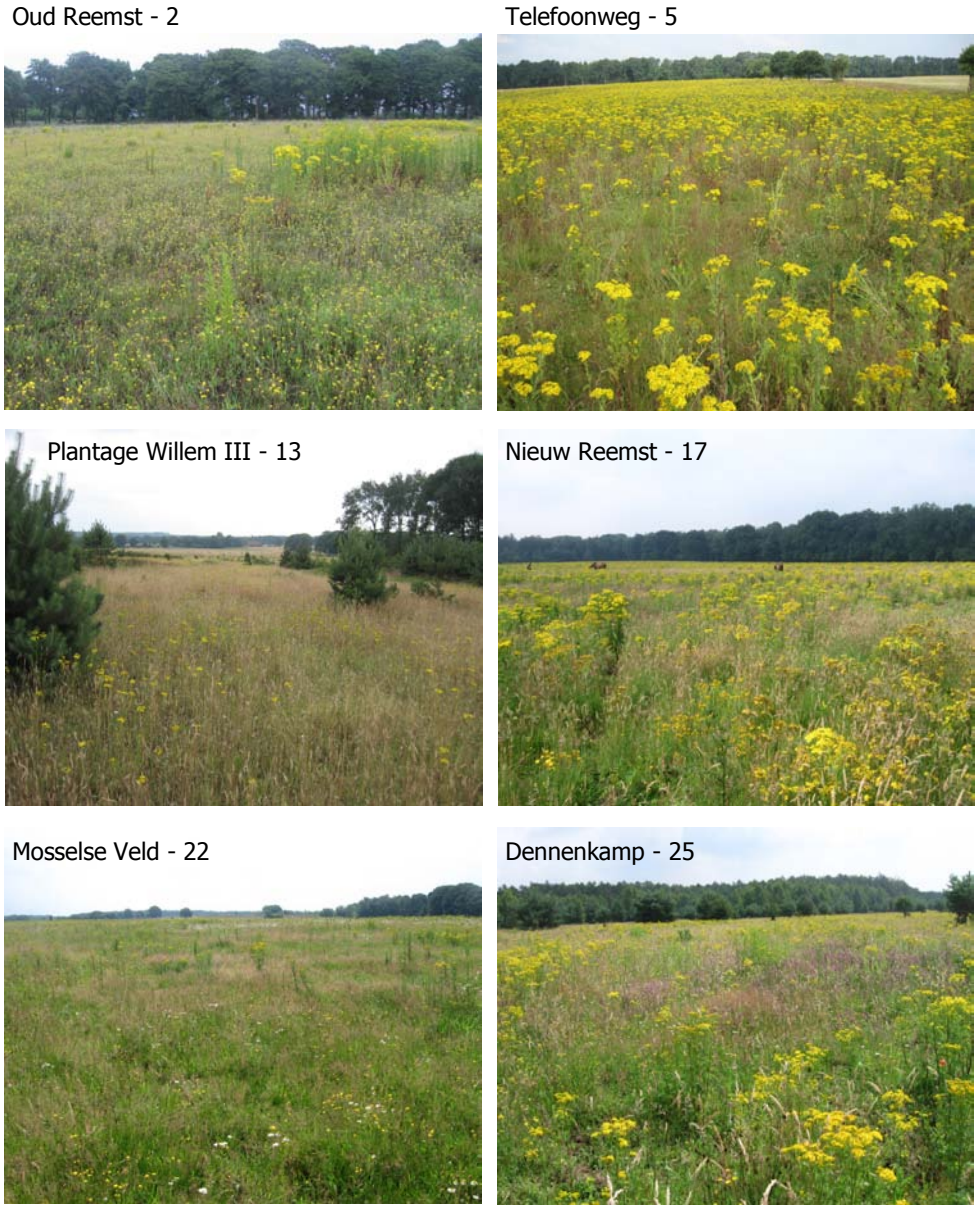

Fig. 1.4 *Jacobaea vulgaris* cover in the chronosequence of fields that were taken out of production 2, 5, 13, 17, 22 or 25 years ago.

approximately 35 km. An overview of locations of the chronosequence fields and the year in which they were taken out of production is shown in Table 1.1 and Fig. 1.3 & 1.4.

In order to test the strength of the assumption that space may replace time, I compared *J. vulgaris* abundance measurements at the chronosequence with measurements at a long-term experimental field site. The experimental field site was set up in 1996 as part of the European 'changing land usage, enhancement of biodiversity and ecosystem development' program (CLUE) under the Fourth Framework Environment and Climate Program of the European Commission (van der Putten *et al.* 2000). The experimental field site is also part of the chronosequence (Mossel; Table 1.1) and is located near Ede, the Netherlands (52°04'N, 05°45'E). In 1996, the 0.5 ha field experiment was started by cultivating the top soil of a corn field harvested in 1995. Then, plots of 10 x 10 m were sown with 15, 4, or 0 (Unsown) mid-successional grassland species. There are five replicate plots for each treatment, arranged in five blocks (Fig. 1.5). In addition to the treatments applied in 1996, agriculture was maintained in one treatment until 1999. After initial sowing, plots were left to be colonized by plant species from the seed bank and the surrounding area without any weeding. Once a year, at the end of the growing season, above-ground biomass was mown and removed from all plots (see van der Putten *et al.* 2000 and Bezemer *et al.* 2006a for further details). The plant community characteristics of the plots sown with 0, 4 and 15 species differed significantly and consistently over the years (Fukami *et al.* 2005; Bezemer and van der Putten 2007; Lepš *et al.* 2007).

Table 1.1 Characteristics of chronosequence fields and their properties: field code, name, year of abandonment and location.

Field code (age in 2007)	Field name	Year of abandonment	Latitide (°N)	Longitude (°E)
2A	Oud Reemst	2005	52.02	5.48
2B	Reijerskamp	2005	52.01	5.47
5A	Telefoonweg	2002	52.00	5.45
5B	Assel	2002	52.12	5.49
12	Mossel	1995	52.04	5.45
13	Plantage Willem III	1994	51.59	5.31
17	Nieuw Reemst	1990	52.04	5.47
19	Wolfhezerveld	1988	51.60	5.47
22	Mosselse veld	1985	52.04	5.44
25	Dennenkamp	1982	52.02	5.48

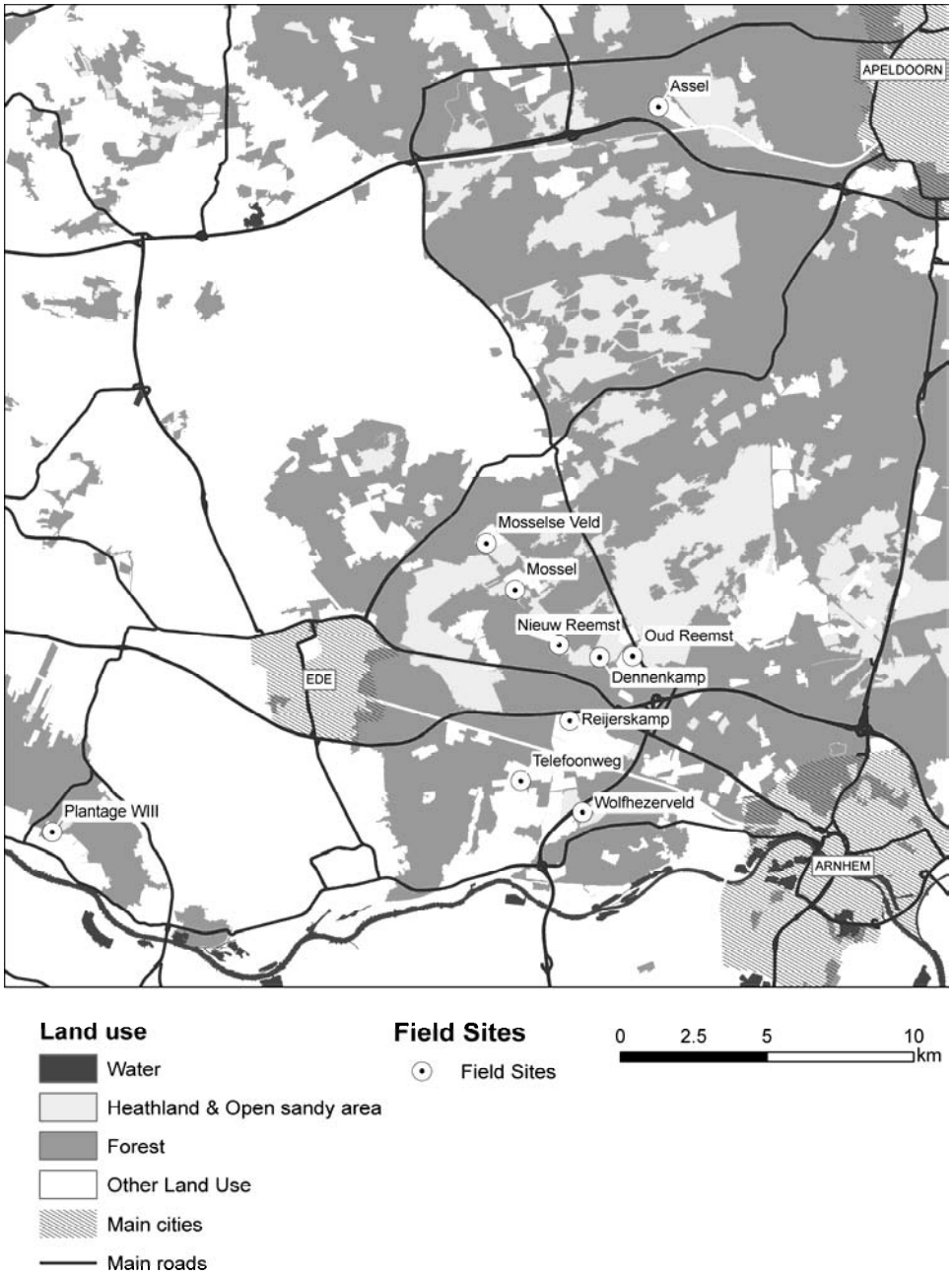

Fig. 1.5 Overview of the locations of the ten chronosequence fields in the Netherlands.

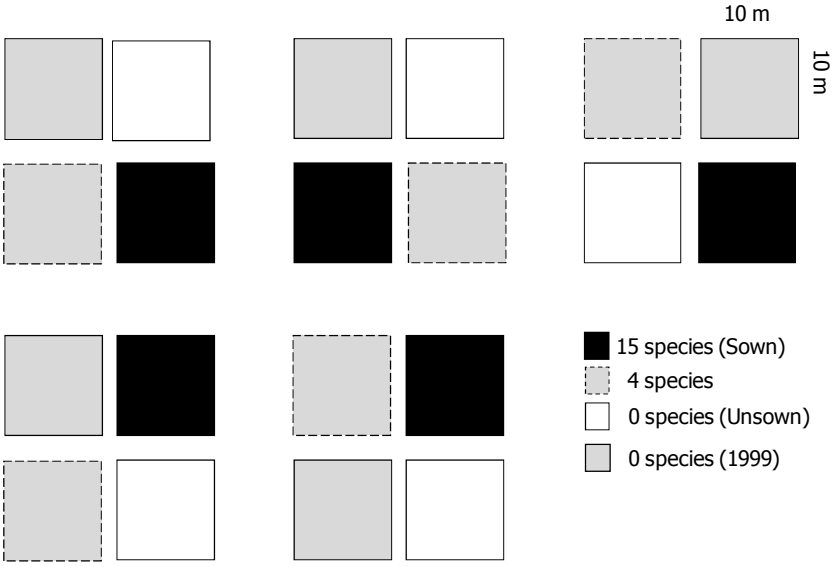

Fig. 1.6 Schematic overview of the long term experimental field site.

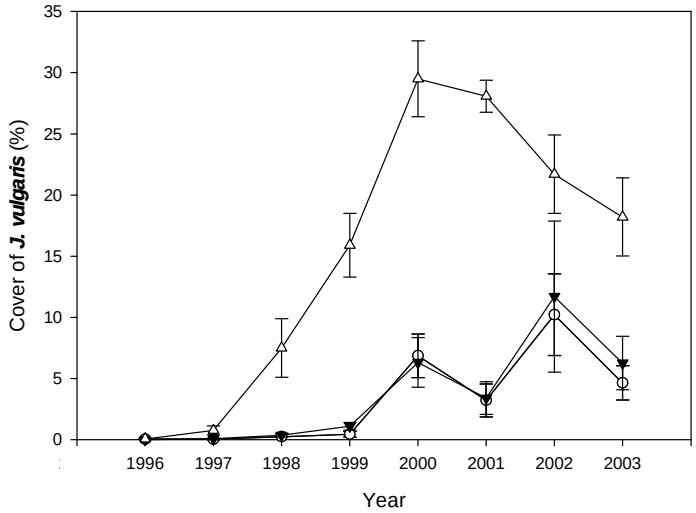

Fig. 1.7 Mean (± SE) percent cover of *Jacobaea vulgaris* from 1996-2003 in plots that were unsown (Δ), sown with 15 (o) or sown with 4 (▼) species (figure adapted from Bezemer *et al.* 2006a).

I will use data from the unsown plots to validate *J. vulgaris* cover data at the chronosequence fields. In addition to studies based on the chronosequence of old-fields, I will also use this experimental field site to determine how plant community composition affects the AMF community colonizing *J. vulgaris* plants (Chapter 6). In that study I will compare the unsown plots (Unsown) with plots that were sown with 15 species (Sown).

J. vulgaris *in the study area*

Since 1996, at the long-term experimental field site the population dynamics of *J. vulgaris* have been recorded annually at peak standing biomass (Bezemer *et al.* 2006a). Although *J. vulgaris* was not one of the species that was sown at the experimental site, *J. vulgaris* invaded all plots of the site in 1997. Since then, *J. vulgaris* cover increased and in 2000 the mean cover peaked at approximately 30% in the unsown plots (Fig. 1.6). From 2000 onwards the abundance of *J. vulgaris* declined again (Fig. 1.6). Bezemer *et al.* (2006a) suggested that this decline was due to negative soil effects, which could be attributed to soil fungal pathogens that build up in presence of *J. vulgaris*. Their study showed that the initial composition of the plant community, *e.g.* sown or unsown, and interactions with the plant and soil community and aboveground invertebrates can influence the dynamics of *J. vulgaris*.

Thesis outline

In **Chapter 2** of this thesis I describe the population dynamics of *J. vulgaris* in a chronosequence of old-fields that were taken out of agricultural production between 2 and 25 years ago. I validated the measured *J. vulgaris* cover of the fields from the chronosequence with a time-series of measurements at a long-term grassland biodiversity experiment in the same area. In order to study the effect of the soil community on *J. vulgaris* performance during old-field succession, I conducted a greenhouse experiment with soil collected from the old-fields. Additionally, in a series of experiments I tested potential other processes that can affect *J. vulgaris* population dynamics, such as germination, seedling establishment and seed availability.

In nature, *J. vulgaris* is part of a diverse plant community. All these plant species can potentially change the abiotic and biotic soil conditions, which may alter plant performance. In **Chapter 3**, I examine if and how plant species that co-occur with *J. vulgaris* influence *J. vulgaris* performance by changing the soil community. In addition, I examined the soil effect of *J. vulgaris* on these co-occurring species. I compared these interspecific plant-soil effects between *J. vulgaris* and co-occurring plants, and *vice versa*, with the intraspecific plant-soil feedback effect of *J. vulgaris* on itself. In addition, I calculated how plant communities surrounding *J. vulgaris* plants can affect, or are affected by, *J. vulgaris* via the soil community.

In order to obtain a better understanding of the plant-soil interactions that *J. vulgaris* is exposed to, I conducted an experiment in which I investigated the effect of different groups of soil organisms on the measured soil effect (**Chapter 4**). I collected soil from two old-fields and conducted a greenhouse experiment with different size fractions of the collected soil. In addition, I examined intraspecific variation in the response of *J. vulgaris* to these altered soil conditions by using *J. vulgaris* seeds that originated from the two fields.

Plants can also affect the performance of other individuals of the same species via plant chemicals. In order to study the effect of autotoxicity on *J. vulgaris* performance, in **Chapter 5**, I conducted a series of laboratory experiments on the inhibitory effects of aqueous extracts made from *J. vulgaris* shoot and root tissues and from soil that was conditioned by *J. vulgaris*. In addition, I studied whether the addition of root fragments affected the performance of *J. vulgaris* seedlings growing in soil or water.

So far I focused on the effect of the soil and plant community on the performance of plants. In **Chapter 6** I focus on the effect of the plant community on soil organisms that colonize individual *J. vulgaris* plants. I determined the composition of arbuscular mycorrhizal fungi in individual *J. vulgaris* plants, using the molecular fingerprinting method terminal restriction fragment length polymorphism (T-RFLP). The plants were collected from an experimental field site, from sown and unsown plant communities. Unsown plant communities were more diverse and spatially heterogeneous than sown ones.

In **Chapter 7** I synthesize the results of all chapters and discuss the implications of these findings for plant community dynamics during old-field succession.

Chapter 2

Plant-soil interactions and the population dynamics of an early successional plant species in an old-field chronosequence

Tess F. J. van de Voorde; Wim H. van der Putten; T. Martijn Bezemer

Submitted

Abstract

Plant-soil interactions can influence the performance of plants, but there are virtually no studies that link plant-soil feedback to long-term plant population dynamics. We hypothesized that long-term changes in the abundance and performance of the plant *Jacobaea vulgaris* are related to plant-soil feedback. Long-term measurements at an experimental field and in a series of ten old-fields representing a chronosequence following land abandonment revealed a remarkably similar hump-shaped temporal pattern of *J. vulgaris* cover, which peaked about 5 years after abandonment. In a plant-soil feedback study, *J. vulgaris* biomass of plants grown in soil from the chronosequence fields was lower than in sterilized control soil and there was a negative relationship between biomass in the feedback study and *J. vulgaris* density in the field. When plants were grown again in the conditioned soil, a much stronger negative plant-soil feedback was observed for all fields. The results indicate that soils from all stages of the chronosequence can develop strong negative soil feedback to *J. vulgaris*, and that there is a positive relationship between *J. vulgaris* density and the level of control by the soil community. In a common-garden experiment with intact vegetation turfs collected from the chronosequence fields in which *J. vulgaris* was seeded, seedling establishment was significantly lower in turfs from older than from young fields. In a seed bank study the number of emerging seedlings declined with time since abandonment of the field. In conclusion, negative plant-soil feedback is an important factor explaining the hump-shaped population development of *J. vulgaris.* However, negative plant-soil feedback is not operating alone, as propagule availability and competition may also be important. Thus, in order to explain its contribution to plant population dynamics, the role of plant-soil feedback along successional gradients needs to be considered from a community perspective.

Key words: Biodiversity, chronosequence, enemy-free space, plant-soil feedback, secondary succession, *Senecio jacobaea*

Introduction

Why and how the composition of plant communities changes over time has been studied frequently and often old-field succession has been used as a model to study the long-term population dynamics of plants (*e.g.* Connel and Slatyer 1977). Many early-successional plant species enter and leave successional sequences according to a boom-bust pattern (Olff and Bakker 1991; Bezemer *et al*. 2006a; Meiners *et al*. 2009), but there is little coherent understanding of what causes these typical temporal processes (Bardgett *et al*. 2005). In many of these studies the focus has been on detecting how changes in abiotic conditions and increased competition with later-successional species influence plant

community composition (*e.g.* Tilman and Wedin 1991; Huston 1994; Olff *et al*. 1994). However, besides changes in abiotic conditions, temporal changes in the performance of individual species can be due to a number of factors, including plant dispersal and recruitment dynamics, resource availability, and control by the surrounding vegetation, as well as by natural enemies and soil biota (Huston and Smith 1987; Olff and Bakker 1991).

Soil biotic interactions play an important role in influencing the population dynamics and performance of plants (Reynolds *et al*. 2003; Kardol *et al*. 2006). For example, pathogenic soil bacteria and fungi, root-feeding nematodes, and mycorrhizal fungi can have strong effects on the performance of individual plants and on plant community composition (*e.g.* van der Heijden *et al*. 1998a; Kardol *et al*. 2007). Even when plants are growing in mixed plant communities, they culture species-specific soil communities which include species that can negatively affect the plant itself (Bezemer *et al*. 2010). Soil-borne enemies are capable of affecting the long-term population dynamics of plant species (Augspurger 1983; Bever 1994), however, relatively little is known about how strongly these plant-soil interactions may influence plant performance and population dynamics in the field.

While the performance of a plant can be greatly influenced by its biotic soil environment, the population size of a plant species is also determined by its seed production and dispersal, and by the subsequent germination and establishment rates (Chapin *et al*. 1994; Anderson 2007). In turn, many of these characteristics are density-dependent, and are under the influence of abiotic conditions of the environment and biotic interactions with aboveground and belowground herbivores, pathogens, symbionts and decomposer organisms (Wardle *et al*. 2004).

We hypothesized that long-term changes in the abundance and performance of an early successional plant species are affected by plant-soil feedback. Specifically, we studied the population dynamics of *Jacobaea vulgaris* (synonym *Senecio jacobaea*) in a long-term experimental field site and in a chronosequence of old-fields. *Jacobaea vulgaris* is a plant species that is typical for old-field succession on relatively dry, sandy soils in northwestern Europe (*e.g.* van der Meijden and van der Waals-Kooi 1979; Crawley and Nachapong 1985; Bezemer *et al*. 2006a). The plant can become very abundant during the first years of old-field succession, but then abundance declines (Bezemer *et al*. 2006a). We hypothesized that the population decline of *J. vulgaris* in the field coincides with the development of a negative plant-soil feedback. In order to determine how soil community composition and soil characteristics influence population dynamics and performance of *J. vulgaris*, we used a series of old-fields representing a chronosequence following land abandonment (Kardol *et al*. 2005). In a greenhouse experiment with soil collected from these fields we tested how plant-soil feedback affects the performance of *J. vulgaris*. We performed a two-phase feedback experiment, as the growth effects in the conditioning phase of the experiment reflect the current net activity of pathogens, symbionts and decomposer organisms in the field, whereas the effects in the feedback phase of the experiment represent the net effects of these groups of soil biota that may develop following plant presence.

Besides studying the effect of plant-soil feedback on *J. vulgaris* performance, in a common garden experiment we investigated how changes in soil characteristics and vegetation influence seedling establishment in turfs with intact vegetation. Finally, in order to relate the experimental findings to patterns observed in the field, we recorded the abundance of *J. vulgaris*, measured the density and size of individual plants, determined seed availability, percentage germination, and we identified soil microorganisms (microbes and nematodes) in the rhizosphere soil of the plants in the old-fields.

Material and Methods

Jacobaea vulgaris ssp. *vulgaris* (synonym *Senecio jacobaea* L.) is a monocarpic perennial weed (Asteraceae) that spends its first year as a rosette. Flowering may take place in the second year, but is often delayed following herbivory (van der Meijden and van der Waals-Kooi 1979). *J. vulgaris* is an early successional plant species native to the Netherlands and Europe, but invasive in other continents. In the Netherlands and in many other countries, *J. vulgaris* is considered a problem weed in abandoned arable fields that are used for nature restoration (Bezemer *et al*. 2006a). This is mainly due to its dominance and because it contains pyrrolizidine alkaloids that are toxic for livestock (Cameron 1935).

Long-term dynamics in a field experiment

In a long-term experimental field experiment, we determined the temporal pattern of *J. vulgaris* abundance in five naturally colonized experimental plots of 10 x 10 m each that had been monitored for 13 years, from 1996 until 2008. These plots had been installed in 1996 as part of a biodiversity experiment (van der Putten *et al*. 2000). All plots were colonized by *J. vulgaris* in 1997. *J. vulgaris* cover has been recorded each year at peak standing biomass (Bezemer *et al*. 2006a; van de Voorde *et al*. 2010). The experimental site is situated in the area of field 12 (see below; Table 2.1).

Chronosequence fields

In order to determine *J. vulgaris* characteristics and the importance of plant-soil feedback for population dynamics in the field, we selected ten fields where agricultural practices were ceased between two and 25 years ago (Table 2.1, Table S2.1). All fields of this chronosequence were at least 1 ha in size, extensively grazed and previously cultivated according to crop rotation schemes. The fields are located on the same parent soil: sandy to sandy loam glacial deposits in the central part of the Netherlands (Veluwe). In July 2007, at each field an imaginary W-shaped transect was laid out in a plot of 50 x 150 m.

Table 2.1 Overview of year of abandonment and soil nutrient content in the chronosequence fields. Means (± SE) nutrient content and P-values of ANOVA testing differences between fields are given. Within columns, means followed by the same letter are not significantly different ($P < 0.05$) based on a Tukey HSD test. Outcome and direction of linear mixed model (REML) with time since abandonment (age) as a continuous factor and field identity as a random factor (df = 8) are also given.

Field code	Year of abandonment	P (mg/kg)	% Org. C	C:N ratio	Min-N (mg/kg)	pH H_2O
2A	2005	86 ± 2 cd	4.5 ± 0.2 ab	21.7 ± 0.5 de	6.5 ± 0.2 a	6.2 ± 0.02 d
2B	2005	83 ± 1 c	5.7 ± 0.1 c	20.9 ± 0.3 d	10.3 ± 0.7 ab	6.0 ± 0.06 cd
5A	2002	124 ± 7 e	4.6 ± 0.2 b	22.8 ± 0.2 e	7.4 ± 0.9 a	5.3 ± 0.02 a
5B	2002	121 ± 13 de	4.2 ± 0.3 ab	22.9 ± 0.6 e	10.7 ± 1.3 ab	5.4 ± 0.15 b
12	1995	90 ± 3 cde	4.2 ± 0.3 ab	16.8 ± 0.1 bc	10.6 ± 0.8 ab	6.1 ± 0.07 d
13	1994	58 ± 4 b	3.6 ± 0.1 a	21.5 ± 0.2 de	6.2 ± 0.3 a	5.4 ± 0.06 a
17	1990	85 ± 10 c	3.9 ± 0.2 ab	15.7 ± 0.3 ab	15.3 ± 3.7 b	5.4 ± 0.12 a
19	1988	84 ± 4 c	4.4 ± 0.1 ab	14.7 ± 0.2 a	9.3 ± 1.3 ab	5.1 ± 0.03a
22	1985	85 ± 5 c	4.7 ± 0.2 bc	18.2 ± 0.4 c	11.4 ± 0.1 ab	5.3 ± 0.01a
25	1982	27 ± 2 a	4.8 ± 0.1 bc	20.9 ± 0.5 d	12.2 ± 2.0 ab	5.8 ± 0.02bc
P (fields)		<0.001	<0.001	<0.001	0.02	<0.001
P (REML~ age)		0.05 (-)	0.60	0.09	0.15	0.26

All soil and vegetation samples were taken at this transect. Vegetation recordings were taken at the five outer-ends of the W-shape. Plant community composition and estimated percent cover of each species including *J. vulgaris* were recorded in five 1 x 1 m quadrats in each field, for a total of 50 observations (10 fields x 5 quadrats per field). Soil samples were taken at ten sampling points at the W-shape in each field, for a total of 100 samples (10 fields x 10 samples per fields).

J. vulgaris *plant characteristics in the chronosequence*

In order to determine if plant characteristics of *J. vulgaris* change during succession, in each field, at the ten positions at the transect that were also used for soil collection (see above), the nearest flowering *J. vulgaris* plant was identified. Plants, including rhizosphere soil, were then excavated, stored individually in plastic bags and brought to the laboratory, where aboveground plant parts and roots were separated. Aboveground plant material was oven-dried (70 ^{o}C for five days) and weighed. In a 3 x 3 m area located in the middle of each chronosequence field, the number of *J. vulgaris* plants was recorded and the reproductive height of all flowering plants and the diameter of all rosettes were measured.

Soil nutrient characteristics in the chronosequence

To determine potential changes in nutrient composition during old-field succession, from each chronosequence field ten soil samples were collected. Three or four individual soil samples (3 cm diameter and 15 cm depth) were combined into three homogenized soil mixtures per field. This resulted in 30 mixtures (10 fields x 3 combined soil samples), which were dried at 40 ^{o}C for 3 days. Soil mineral N was extracted by shaking 10 g (dry weight) soil with 50 ml 1 M KCl for 2 h. NH_4^+-N and NO_3^--N were determined colorimetrically in the KCl extract (Traacs 800 autoanalyzer; TechniCon Systems Inc.) and values were added up to express total mineral N. C:N-ratio was measured on a FlashEA 1112 Series NC soil analyzer (Thermo Scientific). pH was measured in 2:5 dry soil: water suspensions. The percentage organic C was determined according to Nelson and Sommers (1982) and available P according to Olsen *et al.* (1954) and measured at 720 nm.

Soil biotic community

To identify potential agents of the plant-soil feedback that affect abundance and performance of *J. vulgaris* plants in the old-fields, we determined the nematode and fungal community composition. Subsamples of the roots of the ten plants from each field were combined per field. Nematodes were extracted from the combined sample of approximately 2 g dry root mass using a mistifier and an extraction time of 50 hr. Nematodes were heat-killed and fixed (35% formaldehyde diluted to 4%). Plant-feeding nematodes were identified to genus or species level, according to Bongers (1988) and the total number of plant-feeding nematodes per gram dry root material determined. Fungal

community composition of a sub-sample from the pooled and homogenized rhizosphere soil of the ten *J. vulgaris* plants per field was determined using PCR-based denaturing gradient gel electrophoresis (DGGE), using fungal-specific primers (Appendix S1).

Plant-soil feedback

To examine the effect of the soil community in the old-fields on *J. vulgaris* biomass production and to test the potential buildup of a soil feedback, we conducted a plant-soil feedback experiment. This greenhouse experiment consisted of a first phase, in which the effect of the field soil on *J. vulgaris* growth was measured, and a feedback phase, in which the further development of a *J. vulgaris* specific soil feedback effect was tested. For the conditioning phase, seeds were collected from field 12 (Table S2.1), surface sterilized (1 min in 0.1% chloride solution and rinsed) and germinated on glass beads. *J. vulgaris* plants were grown in 0.9 L pots filled with 1.2 kg soil (based on dry weight). The soil was a mixture of 6:1 sterilized bulk soil and field soil. To obtain sterilized bulk soil, soil (approximately 750 kg) was collected from field 12 from 5-20 cm below the soil surface, sieved using a 0.5 cm mesh, homogenized, and sterilized by gamma irradiation (> 25 KGray gamma irradiation, Isotron, Ede, the Netherlands). Field soil was collected by taking approximately 150 soil cores (3 cm diameter and 15 cm depth) randomly from each field in July 2007. Soil of each field was sieved (0.5 cm mesh size) and homogenized. Control plants were grown in sterilized bulk soil inoculated with an autoclaved (3 consecutive days, 20 min at 120 ^{o}C) mixture of the inoculum soil of all ten fields.

In each pot 3 one-week-old seedlings were transplanted. Seedlings that died during the first week of the experiment were replaced. All treatments were replicated five times, which resulted in 55 pots in total (10 fields x 5 replicate pots + 5 sterilized control pots). Pots were placed randomly in a greenhouse at 70% RH, at 16h 21 ^{o}C (day) and 8h 16 ^{o}C (night). Natural day light was supplemented by metal halide lamps (225 µmol s^{-1} m^{-2} photosynthetically active radiation, 1 lamp per 1.5 m^2). Plants were watered every other day and initial soil moisture level (17% at soil dry weight basis) was re-set twice a week by weighing. After 10 weeks all aboveground biomass was harvested, oven-dried (70 ^{o}C for five days) and weighed. The soil, including roots, in each pot was divided into four equal parts. From two parts the roots were gently rinsed and nematodes were extracted from a homogenized sub-sample of these rinsed roots (approximately 1 g based on dry weight), using the same procedure as for the field plants (see above).

The other two parts per pot were used as inoculum for the feedback phase and were mixed in a 1:1-ratio with the sterilized bulk soil (640 g dry weight sterilized soil) to balance for potential nutrient deficiencies after the first growth phase. Also control plants were included. This control treatment is comparable to the control in the conditioning phase and is irradiated bulk soil which is inoculated with an autoclaved (3 consecutive days, 20 min at 120 ^{o}C) mixture of the inoculum soil of all ten fields. This new control was mixed in a 1:1-ratio with irradiated bulk soil, as was done for all pots in the feedback phase. All

treatments were replicated five times, which resulted in 55 pots (10 fields x 5 replicate pots + 5 new sterilized control pots). Three *J. vulgaris* seedlings were planted and seedlings that died during the first week of the experiment were replaced. After one week the seedlings were randomly thinned to two seedlings per pot. Plants were grown under the same conditions as during the conditioning phase. Six weeks after transplanting, aboveground biomass was harvested, oven-dried (70 ^{o}C for five days) and weighed. For both phases, the reduction in aboveground biomass production was calculated relative to the sterile control of that phase.

Common garden experiment

To determine the effect of the plant and soil community in the old-fields on seedling establishment, we conducted a common garden experiment. In the common garden experiment we determined seedling establishment during a whole growth season in intact turfs. In December 2008, 30 x 30 x 30 cm turfs were collected, six turfs each from fields 2A, 5B, 12 and 25. The turfs were placed in a plastic ring of 25 cm diameter, placed on root cloth in a common garden so that the soil surface of the turf leveled with the surrounding soil. The garden was situated at the Netherlands Institute of Ecology, Heteren, the Netherlands. In each turf 9 rows of 9 seeds were placed at 0.5 cm below the surface and 2.5 cm apart. All seeds originated from field 12. For each turf, at the start of the experiment the percentage bare ground was recorded. In September 2009, after one growing season, the percentage established seedlings was determined.

J. vulgaris *seed characteristics*

To determine if reduced seed weight or reduced germination are related to decreased *J. vulgaris* performance and abundance in the older fields, we measured seed weight and germination of plants from each field. Percent germination and weight of *J. vulgaris* seeds from each field was determined for seeds collected from approximately 100 plants in each of the fields in November 2007. Seeds were air-dried and the pappus was removed, per field 100 randomly chosen seeds were weighed individually. To examine germination, seeds were surface sterilized (1 min in 0.1% chloride solution and rinsed) and 25 seeds were placed on filter paper (9 cm diameter) in a Petri-dish with 2 ml demineralised water. There were five replicate Petri-dishes for each field. After 16 days at 16 h 21 ^{o}C (day) and 8 h 16 ^{o}C (night), the number of germinated seeds was determined.

Germination from the soil seed bank was determined for 7 fields (fields 2A, 5B, 12, 13, 17, 22, 25). Soil cores (approximately 50 cores from each field, 3 cm diameter and 7 cm depth) were collected in January 2008. Field soil from each field separately was homogenized and sieved (0.5 cm mesh size). Plastic containers of 12.5 x 17 x 6 cm (l x w x h) were filled with 750 g irradiated bulk soil, which was collected from field 12 (see above). This layer of bulk soil was topped-up with 250 g (both based on dry weight) field

soil. There were five replicate containers for each field. The soil used in each container corresponded with approximately 35 cm^2 of field surface (one core equals 7.1 cm^2 and 51 g of dry weight soil). The containers were placed in a greenhouse at 70% RH and kept at 17% moisture content. Emerged seedlings were removed every four weeks for a total period of 6 months and the number of *J. vulgaris* seedlings was recorded. After the first 3 months, the containers were placed at 4 °C for a period of three weeks to break dormancy of the remaining seeds, and then returned to the greenhouse. For each container, the cumulative number of *J. vulgaris* seedlings was calculated and converted to number of individuals per m^2.

Data analyses

All data were analysed using univariate (GenStat 12; Payne *et al.* 2008) or multivariate statistics (CANOCO 4.55; Ter Braak and Šmilauer 2002).

Univariate analyses: Field, bioassay and plant characteristics and results from the feedback experiment were analysed using analysis of variance (ANOVA), with field identity as a fixed factor. Individual comparisons were based on a Tukey HSD post-hoc test. Before conducting ANOVA, data were checked for homogeneity of variances using Cochran's, Hartley's and Bartlett tests ($P > 0.01$) and for normality by inspection of the normal-probability plot. To fulfill requirements of normality, soil nutrients (P and Mineral-N) and biomass of individual *J. vulgaris* plants in the field were log-transformed, count data were square root-transformed and percentage data were arcsin-transformed prior to statistical analyses. Percentage germination, *J. vulgaris* cover, and seed weight were analysed using a non-parametric Kruskal-Wallis test.

Linear relationships between measurements in each field and field age were analysed using linear mixed models (REML). Regressions were based on all measurements per field, but with field identity as random factor and field age as a continuous factor. Non-linear relationships, i.e. the relationship between time since abandonment and *J. vulgaris* cover in chronosequence fields and in the experimental fields were described by fitting a log-normal curve.

Multivariate analyses: Plant and fungal community compositions in the ten fields were analysed using multivariate statistics. Detrended correspondence analysis (DCA) was used to determine whether linear (principle component analysis (PCA) and redundancy analysis (RDA)) or unimodal (correspondence analysis (CA) and co-correspondence analysis (CCA)) analyses were most appropriate; linear analyses were chosen when the longest gradient was < 3 (Lepš and Šmilauer 2003). Direct analyses were conducted with replicates within each field (*e.g.* quadrats, plant) as split-plots within each whole plot (field). Whole plots were permuted freely and split-plots were not permuted. Significances in multivariate analyses were inferred by Monte Carlo permutation tests (999 permutations). The presence/absence data of the soil fungal banding profiles were analysed using unimodal

multivariate analyses (CA, CCA). DCA was used to analyse plant community cover data (log (n+1) transformed) of the fields, rare species that were present in less than 3 quadrats were excluded from these analyses.

Results

J. vulgaris *population dynamics*

J. vulgaris cover at both the long-term experimental field and the chronosequence showed a hump-shaped temporal pattern and cover peaked at about 5 years after initial colonization or abandonment (Fig. 2.1A+B). Both, the temporal cover of *J. vulgaris* in the experimental field site and *J. vulgaris* cover in the chronosequence fields followed a log-normal pattern ($P < 0.001$, $R^2 = 0.48$, and $P < 0.001$, $R^2 = 0.88$, respectively).

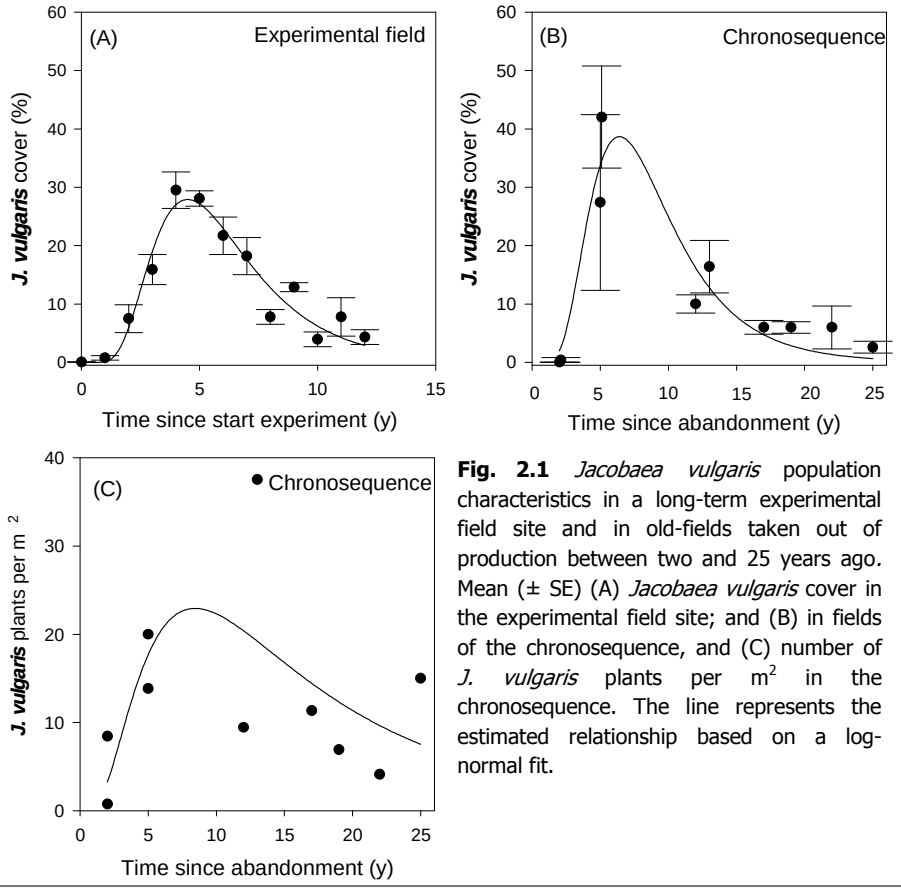

Fig. 2.1 *Jacobaea vulgaris* population characteristics in a long-term experimental field site and in old-fields taken out of production between two and 25 years ago. Mean (± SE) (A) *Jacobaea vulgaris* cover in the experimental field site; and (B) in fields of the chronosequence, and (C) number of *J. vulgaris* plants per m^2 in the chronosequence. The line represents the estimated relationship based on a log-normal fit.

Field soil feedback response

In the conditioning phase of the bioassay feedback experiment, aboveground biomass was on average 20% lower in soils with live field inoculum than in the sterilized control (Fig. 2.2). The fields differed significantly in their magnitude of growth reduction ($F_{9,49}$ = 7.42, P < 0.001). Biomass reduction did neither correlate with field age ($F_{1,8}$ = 0.02, P = 0.88) nor with *J. vulgaris* cover in the fields ($F_{1,8}$ = 0.27, P = 0.61). However, there was a positive relationship between the number of *J. vulgaris* plants in the field and the biomass reduction (relative to control soil) in soil of that field, when we excluded field 13 which had a different history ($F_{1,7}$ = 5.45, P = 0.05, R^2= 0.44; Fig. 2.3A). In the feedback phase, in contrast to the conditioning phase, no difference in biomass reduction between the fields was observed ($F_{9,49}$ = 1.94, P = 0.08; Fig. 2.2). However, aboveground biomass production was on average 70% lower in the conditioned soils than in the sterilized control (Fig. 2.2). Again, there was no correlation with field age or *J. vulgaris* cover in the field ($F_{1,8}$ = 1.26, P = 0.29; $F_{1,8}$ = 2.71, P = 0.14, respectively). However, there was a negative relationship between growth reduction in the feedback experiment and rosette size of the vegetative plants in the field ($F_{1,8}$ = 5.41, P = 0.04, R^2= 0.40; Fig. 2.3B). This relationship was stronger when we excluded field 13 ($F_{1,7}$ = 8.60, P = 0.02, R^2= 0.55).

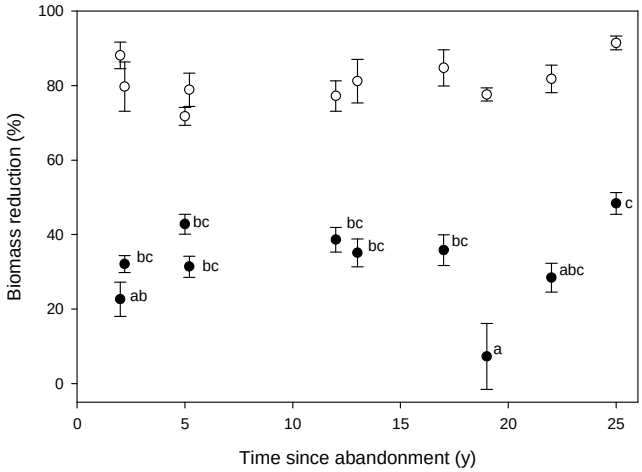

Fig. 2.2 Percentage reduction of aboveground *Jacobaea vulgaris* biomass production in a bioassay feedback experiment, relative to biomass produced in sterile control soil. Means (± SE) are shown for biomass production in phase 1 (closed circles) and phase 2 (open circles). Different letters indicate significant differences (P < 0.05) within phase 1, based on a Tukey's HSD post-hoc test. There were no significant differences in phase 2. To avoid overlapping data points, fields 2B and 5A are moved forward by 0.2 year.

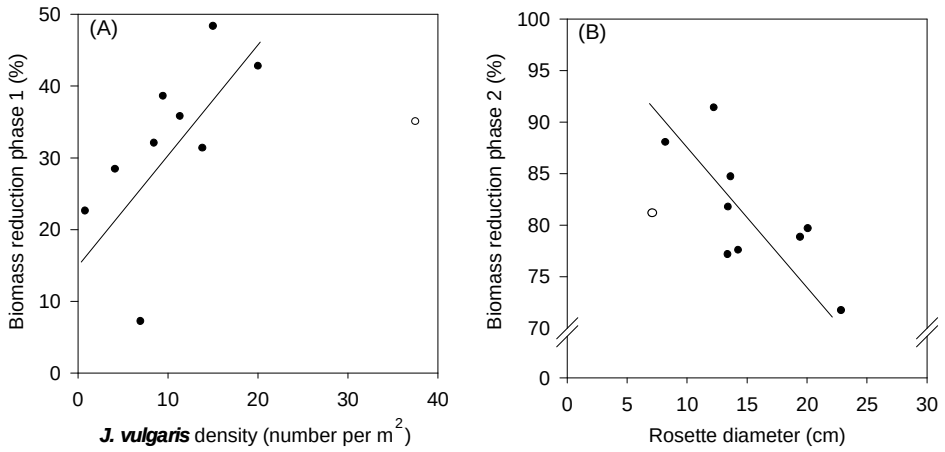

Fig. 2.3 Relationship between (A) the number of *Jacobaea vulgaris* plants per m^2 in each field and aboveground biomass reduction in phase 1 of the feedback experiment ($F_{1,7}$ = 5.45, P = 0.05, R^2= 0.44), and (B) relationship between aboveground biomass production in phase 2 of the feedback experiment and rosette size of vegetative field plants ($F_{1,7}$ = 8.60, P = 0.02, R^2= 0.55). The line is the estimated relationship based on linear regression analysis excluding Field 13 (see results). Values for Field 13 are presented by open circles.

Table 2.2 Plant and seed characteristics of *Jacobaea vulgaris* in the chronosequence fields. Means (± SE) and P-values of ANOVA testing differences between fields are given. Within columns, means followed by the same letter are not significantly different (P < 0.05) based on a Tukey HSD test. Outcome and direction of linear mixed model (REML) with time since abandonment (age) as a continuous factor and field identity as a random factor (df = 8) are also given.

Field code	Aboveground biomass (mg)	Seed weight (mg)	Germination (%)	Reproductive plant height (cm)
2A	8.8 ± 2.3 ab	0.246 ± 0.01 bc	61.2 ± 5.2 ab	86.6 ±7.6 cde
2B	6.1 ± 2.2 ab	0.283 ± 0.01 c	70.4 ± 2.7 ab	100.8 ± 3.2 e
5A	3.4 ± 0.7 ab	0.226 ± 0.01 b	54.0 ± 8.9 a	43.9 ± 1.9 a
5B	19.5 ± 6.1 b	0.196 ± 0.01 ab	68.0 ± 2.5 ab	83.7 ± 6.3 de
12	7.0 ± 3.1 ab	0.249 ± 0.01 bc	60.0 ± 3.1 ab	62.8 ± 2.6 b
13	1.7 ± 0.6 a	0.239 ± 0.01 bc	86.4 ± 1.0 b	45.1 ± 2.2 a
17	9.0 ± 3.5 ab	0.242 ± 0.01 bc	62.8 ± 2.6 ab	67.8 ± 4.2 bcd
19	12.3 ± 4.8 ab	0.210 ± 0.01 ab	51.2 ± 4.8 a	65.6 ± 2.6 bc
22	7.6 ± 3.2 ab	0.178 ± 0.01 a	47.3 ± 6.4 a	64.5 ± 3.2 bc
25	7.9 ± 3.0 ab	0.216 ± 0.01 ab	56.9 ± 2.2 ab	64.5 ± 2.8 bc
P (fields)	0.02	< 0.001	0.002	< 0.001
P (REML ~age)	0.91	0.12	0.28	0.35

Common garden and seed bank experiment

In the common garden experiment, after one growth season the establishment rate of *J. vulgaris* varied between fields ($F_{3,19}$ = 18.2, P < 0.001) and was higher in the two younger fields than in the two older fields (Fig. 2.4A). There was a positive relationship with the percentage of bare ground at the start of the experiment and *J. vulgaris* establishment in each turf ($F_{1,3}$ = 10.1, P = 0.05, R^2 = 0.46).

In the seed bank experiment the average number of emerged *J. vulgaris* seedlings varied between 0.8 to 7.2 individuals per container, corresponding to 230 to 2070 seedlings per m^2. Seedling density followed a log-normal pattern (Fig. 2.4B; P < 0.001, R^2 = 0.56). There was a significant relationship between the number of emerged *J. vulgaris* seedlings and *J. vulgaris* field cover ($F_{1,5}$ = 9.02, P= 0.03, R^2 = 0.64). However, we did not find a relationship between the number of emerged seedlings and *J. vulgaris* density in the field ($F_{1,5}$ = 0.89, P= 0.39).

Identification of soil biota

The fungal community composition in the rhizosphere, as described by DGGE, could be significantly explained by field age (CCA: P = 0.04; 14.4%; Fig S2), but not by *J. vulgaris* cover (CCA: P = 0.55; Fig. S2.1). The number of DGGE fungal bands in the rhizosphere soil of the field plants ranged from 4 (field 12 and 25) to 12 (field 2A and 5A).

In the roots of the field plants, more than 85% of all plant-feeding nematodes were identified as *Pratylenchus crenatus*. Nematode density decreased significantly with field age ($F_{1,8}$ = 14.2, P = 0.005, R^2 = 0.64; Fig. 2.5A). Other plant-feeding nematode species belonged to the genus *Filenchus*. *Pratylenchus crenatus* was also the most abundant plant-feeding nematode in the bioassay plants and represented more than 75% of all plant-feeding nematodes. In the bioassay, nematode density differed significantly between fields (F = 10.2, P < 0.001, Fig. 2.5B), but was not related to field age (P = 0.94, R^2 = 0.12).

Field and nutrient characteristics

A total of 71 plant species were recorded in the 50 quadrats in the ten fields. Plant community composition differed between fields and was related to field age (CCA: P = 0.03, 8.5%; Fig. S2.2). Soil nutrients differed between fields (Table 2.1), however, only available phosphorous decreased significantly over time.

J. vulgaris *characteristics*

The average number of *J. vulgaris* individuals per m^2 ranged from 0.8 to 37.5 (Fig. 2.1c). However, these numbers did not correlate with *J. vulgaris* cover in the field ($F_{1,8}$ = 1.73, P = 0.22, R^2 = 0.18). Individual plant height varied substantially between fields, and plants were tallest in the youngest fields (Table 2.2). The average shoot weight of the ten selected plants was significantly higher in field 5B than in the other fields and significantly

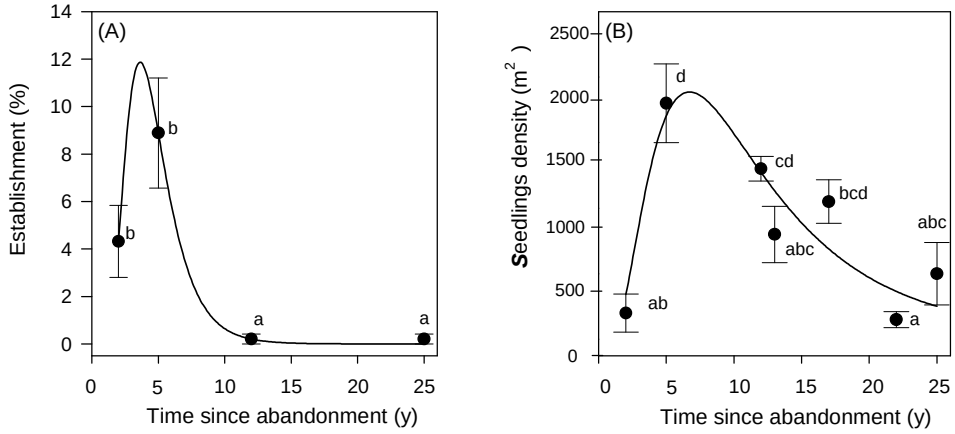

Fig. 2.4 Mean (± SE) (A) *Jacobaea vulgaris* seedling establishment in turfs with intact vegetation in a common garden experiment, and (B) number of *J. vulgaris* seedlings in seed bank in soil from a chronosequence of old-fields taken out of production between two and 25 years ago. Within each panel, different letters indicate significant differences ($P < 0.05$) based on a Tukey's HSD post-hoc test.

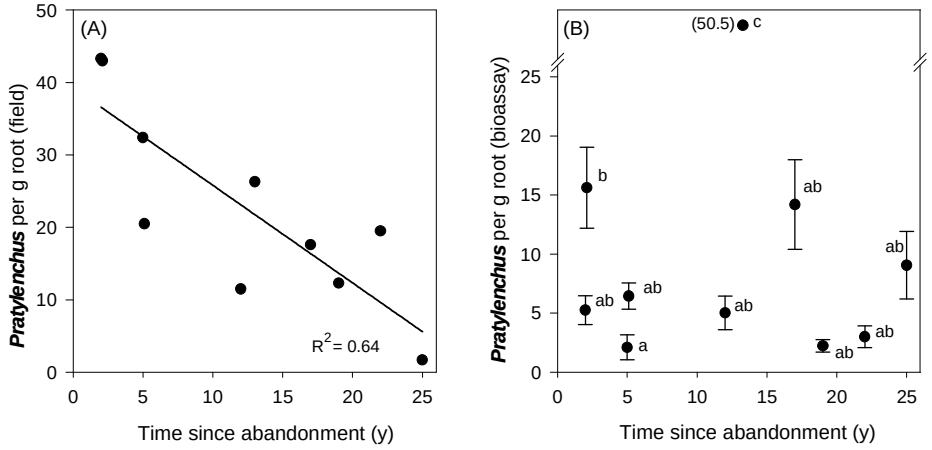

Fig. 2.5 Relationship between time since abandonment and density of *Pratylenchus crenatus* root-feeding nematodes per gram root in plants from (A) the field; and (B) the bioassay. Nematode numbers were determined in plants originated from, or that were grown in soil from a chronosequence of old-fields. Means (± SE) are shown for bioassay plants, different letters indicate significant differences ($P < 0.05$) based on a Tukey's HSD post-hoc test. In (B) plants growing in soil from field 13 had a much higher nematode density than the other fields. The actual density for this field is presented between brackets.

lower in field 13 ($F_{9,89}$ = 2.31, P = 0.02, Table 2.2), but did not correlate with field age ($F_{1,8}$ = 0.01, P = 0.91) or *J. vulgaris* cover ($F_{1,8}$ = 0.01, P = 0.92). Individual seed weight differed significantly between fields (P < 0.001; Table 2.2) and tended to decline with time since abandonment, but this was not significant due to the low seed weights in the two five-year-old fields. Percent germination also differed between fields (P = 0.002; Table 2.2), but did not correlate with field age ($F_{1,8}$ = 1.15, P = 0.31).

Discussion

J. vulgaris cover showed a hump-shaped temporal pattern during old-field succession and cover peaked at about 5 years after lands were abandoned. This pattern was found in the experimental field and in the chronosequence, and thus validates the use of samples collected from the chronosequence fields to disentangle the processes behind this temporal pattern (*e.g.* Johnson and Miyanishi 2008). The hump-shaped pattern of *J. vulgaris* cover can be related to a number of processes that could potentially affect the population dynamics of *J. vulgaris* during old-field succession: a rapid buildup of a negative plant-soil feedback, which may interact with other processes, such as the increased competition with other plant species that suppresses seedling establishment, and diminished seed performance. We found no indications that seed or nutrient availability reduced *J. vulgaris* performance and population dynamics during old-field succession.

The plant-soil feedback experiment revealed a negative effect of the soil community on *J. vulgaris* performance in all fields. This effect became substantially more negative when the soil was conditioned first during one growth period by *J. vulgaris*. As plant-pathogen relationships may follow a density-dependent response (Augspurger 1983), we expected growth reduction (due to control by the soil community) in the first phase with field soil to be strongest in the fields with the highest *J. vulgaris* cover. For the complete dataset however, the growth reduction was not related to *J. vulgaris* cover or density, which was mostly due to one of the fields (field 13) that responded in an idiosyncratic way. This field responded not only idiosyncratic in our study, but also in previous work on this chronosequence (van der Wal *et al*. 2009). This field has a typical history that differs from all other fields in that it has not been used as intensively for agriculture as most other fields (van der Wal *et al*. 2009). All in all, there is a tendency that high *J. vulgaris* density in the field precedes the negative soil feedback-induced decline, but these effects may also depend on environmental context, as the performance in field 13 suggests. This context dependency has not been identifies in previous plant-soil feedback studies in old-field grasslands (Klironomos 2002), tree species in temperate (Packer and Clay 2000) and tropical forests (Mangan *et al*. 2010), but it did appear in cross-system studies in temperate forests (Reinhart *et al*. 2005).

The growth effects in the feedback phase of the experiment represent the net effects of pathogens, symbionts and decomposer organisms in the soil that may develop following plant presence. Thus, our results clearly show that in all fields, even at the youngest succession stages, some latent net soil pathogen activity is present and that severe net soil pathogenic activity can develop in a period shorter than a growth season. The results from the feedback phase suggest that soon after soil has been colonized by *J. vulgaris* it will become less suitable for subsequent plant growth of this species. Once the soil of an entire field has become conditioned, newly establishing plants will become exposed everywhere to the negative soil feedback, provided that the agents causing the negative feedback persist for at least some years in the absence of *J. vulgaris.* When new individuals have to establish in conditioned soil this will reduce their performance and the possibilities for successful establishment.

We did not aim to identify the organisms causing the negative soil feedback, as DGGE only picks up the most dominant microbial taxa in the rhizosphere samples. Nevertheless, the composition of the fungal communities in the rhizosphere soils of fields 5B, 12 and 25 differed from the other fields. *J. vulgaris* biomass production in the conditioning phase was also poorest in these soils, suggesting that soil fungi may be responsible for plant growth reduction (Bezemer *et al*. 2006a). However, the fungal communities of fields 5B, 12 and 25 are also different from each other, indicating that soil fungi are not the only agents affecting *J. vulgaris* growth, or that different fungal taxa may do the same job. Also plant-feeding nematodes can reduce plant performance. In the field the number of plant-feeding nematodes decreased over time, which is probably a direct result of the cessation of crop growing (Korthals *et al*. 2001). In the bioassay, plant-feeding nematodes were almost absent in *J. vulgaris* roots, although diverse nematode communities were found in the soil of these old-fields (Kardol *et al*. 2005). The low nematode abundance in *J. vulgaris* roots could be due to the negative effects of pyrrolizidine alkaloids on plant parasitic nematodes (Thoden *et al*. 2009ab). A strong negative effect of plant-feeding nematodes on *J. vulgaris* performance and establishment is therefore not to be expected, although this needs to be confirmed in inoculation experiments.

The negative feedback effects may be amplified when later succession plant species invade, as they will be disproportionally more competitive when their predecessor is reduced selectively by negative soil feedback (Kardol *et al*. 2007). Indeed, the common garden experiment with intact vegetation turfs, revealed that in older fields soil and vegetation conditions reduced seedling establishment. After one growth season, seeds of *J. vulgaris* had established better in turfs from young than from older fields, and there was a positive relationship between percentage bare ground in the beginning of the experiment and *J. vulgaris* establishment. This shows that besides the effect of the soil, the structure and openness of the surrounding vegetation are important characteristics that determine germination and seedling success of *J. vulgaris*, for example, by influencing light availability and micro-climate (van der Meijden and van der Waals-Kooi 1979; Olff *et al*.

1994). Therefore, the reduced availability of bare ground and the increased competition with other plants in later stages of old-field succession can change establishment success. Thus, changes in bare ground availability and competition, together with the developing negative plant-soil feedback all appear to play an important role in explaining the pattern of population development of *J. vulgaris* during old-field succession by changing establishment success.

The number of emerging *J. vulgaris* seedlings from the seed bank corresponded with *J. vulgaris* cover in the fields, but was substantially higher than the number of *J. vulgaris* plants recorded in the field. This will be due to ongoing soil disturbance during the seed bank experiment and absence of aboveground herbivores. However, the relatively high number of seedlings that emerged from the seed bank suggests that in the field the availability of viable propagules is not limiting (*e.g.* Tilman 1997; Ozinga *et al*. 2005), but that the absence of disturbances (van der Meijden and van der Waals-Kooi 1979) or the presence of aboveground herbivores may be more limiting (Wilby and Brown 2001). Thus, our results suggest that the seed bank follows *J. vulgaris* plant population dynamics and that only in the youngest fields, the number of seedlings may be limited by seed availability. While seed germination and seed weight did not appear to strongly contribute to the general trend in *J. vulgaris* population development during old-field succession, seed weight in both fields where *J. vulgaris* cover peaked was reduced compared to other fields. This may reduce plant fitness and the competitive ability of these seedlings (Crawley and Nachapong 1985), thereby negatively affecting fitness of the next generation. Also, nutrient availability can influence plant performance and plant community composition during succession (*e.g.* Huston 1994). However, in our study nutrient availability was relatively high in all fields. Therefore, it is unlikely that limited nutrient availability is a direct cause of decline of *J. vulgaris* populations.

In conclusion, we show that negative plant-soil feedback effects can be induced in all stages of a chronosequence representing secondary succession on old-fields. The growth reduction in soils directly collected from field sites tends to relate positively to the density of *J. vulgaris* in the fields. Therefore, negative plant-soil feedback may be an important factor explaining the hump-shaped population development of *J. vulgaris.* However, negative plant-soil feedback is not operating alone. Initially, plant population development is limited by propagule availability, which is independent of plant-soil feedback. During later succession stages, the effects of plant-soil feedback may be enforced by competition from later succession plant species. Thus, the role that plant-soil feedback plays in determining the dynamics of plant populations along successional gradients needs to be considered from a community perspective.

Acknowledgements

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Supporting Information

Appendix S2.1 Supplementary information about DGGE analysis

Fungal community composition in each field was determined using PCR-based denaturing gradient gel electrophoresis (DGGE; Muyzer *et al.* 1993) of a sub-sample from the pooled and homogenized rhizosphere soil of ten *J. vulgaris* plants. DNA was extracted using the PowerSoil® DNA Isolation Kit (Mobio, Carlsbad, USA) according to the manufacturer's protocol. DNA quantity and quality were assessed using NanoDrop® ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Delaware, USA). The extracted DNA was amplified using the fungal-specific primers ITS1f and ITS4 (White *et al.* 1990). All amplification reactions were performed a PTC200 thermal cycler (Bio-Rad Laboratories B.V., Veenendaal, the Netherlands) in a volume of 25 µL and consisted of 15 µmol of each primer, approximately 50 µg of environmental template DNA, 2 U FastStart High Fidelity DNA polymerase (Roche, Basel, Switzerland), and the manufacturer's recommended nucleotide concentrations and buffer conditions. PCR amplification conditions were 5 min at 95 °C, followed by 32 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, and an extension step of 72 °C for 10 min, followed by 10 °C until further use. PCR product size and quantity were verified on a 1.5% agarose gel. PCR product (20 µL) was used for DGGE analyses, which were performed in 6% acrylamide gels with a gradient of 20 – 60% denaturant (100% denaturant = 7 mol/l urea with 40% formamide) using the DGene system (Bio-Rad Laboratories B.V., Veenendaal, the Netherlands). Gradient gels were topped with 10 ml of acrylamide containing no denaturant. Electrophoresis was carried out at 60 °C for 10 min at 200V, followed by 17 h at 80 V. Gels were stained with ethidium bromide for 20 min followed by destaining them for 20 min, prior to UV transillumination and digital photography using the ImaGo system (B & L, Maarssen, the Netherlands). DGGE banding patterns were analysed using Imagemaster elite v4.20 (Amersham Pharmacia Biotech, USA) with rolling ball background subtraction, normalization, and band detection.

Table S2.1 Characteristics of chronosequence fields
Field properties (name, year of abandonment and location) and plant community characteristics (diversity and species richness) of the fields in a chronosequence of old fields that were abandoned two to 25 years ago. Means (± SE) are shown for diversity and species richness, different letters indicate significant differences ($P < 0.05$) based on a Tukey's HSD post-hoc test.

Field code (age)	Field name	Year of abandonment	Latitide (^{o}N)	Longitude (^{o}E)	Diversity (H')	Species richness
2A	Oud Reemst	2005	52.02	5.48	0.64 ± 0.13 a	3.0 ± 0.0 a
2B	Reyerskamp	2005	52.01	5.47	0.93 ± 0.08 ab	3.6 ± 0.4 ab
5A	Telefoonweg	2002	52.00	5.45	1.00 ± 0.19 ab	4.4 ± 0.4 b
5B	Assel	2002	52.12	5.49	1.21 ± 0.13 ab	8.6 ± 0.9 cde
12	Mossel	1995	52.03	5.45	1.38 ± 0.27 b	9.8 ± 0.7 e
13	Plantage W III	1994	51.59	5.31	1.35 ± 0.11 b	7.6 ± 0.7 cde
17	Nieuw Reemst	1990	52.04	5.47	1.09 ± 0.08 ab	7.0 ± 0.9 cd
19	Wolfhezerveld	1988	51.6	5.47	1.19 ± 0.10 ab	6.6 ± 0.7 c
22	Mosselse veld	1985	52.04	5.44	1.45 ± 0.13 b	9.4 ± 1.2 de
25	Dennenkamp	1982	52.02	5.48	1.30 ± 0.09 b	7.4 ± 1.3 cd

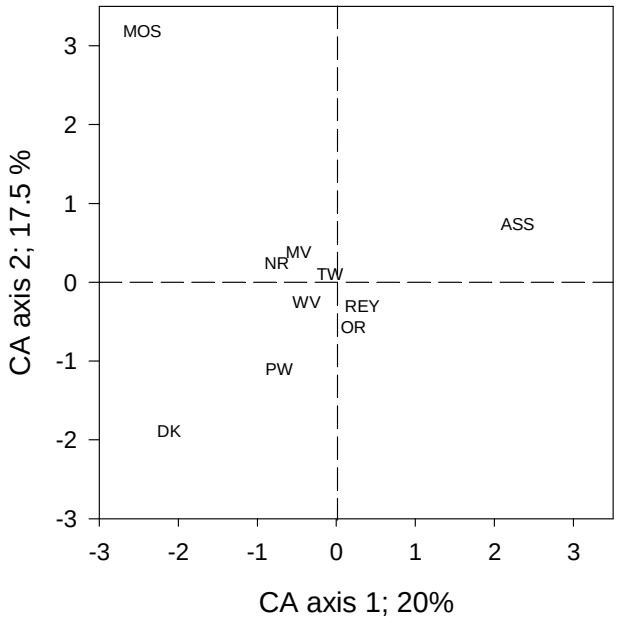

Fig. S2.1 Soil fungal community composition in the chronosequence fields, based on DGGE analyses. Unconstrained unimodal canonical analysis (CA) of soil fungal community composition in field soil inoculum from the chronosequence fields based on presence-absence data of DGGE bands. The different fields are indicated with their abbreviated field name.

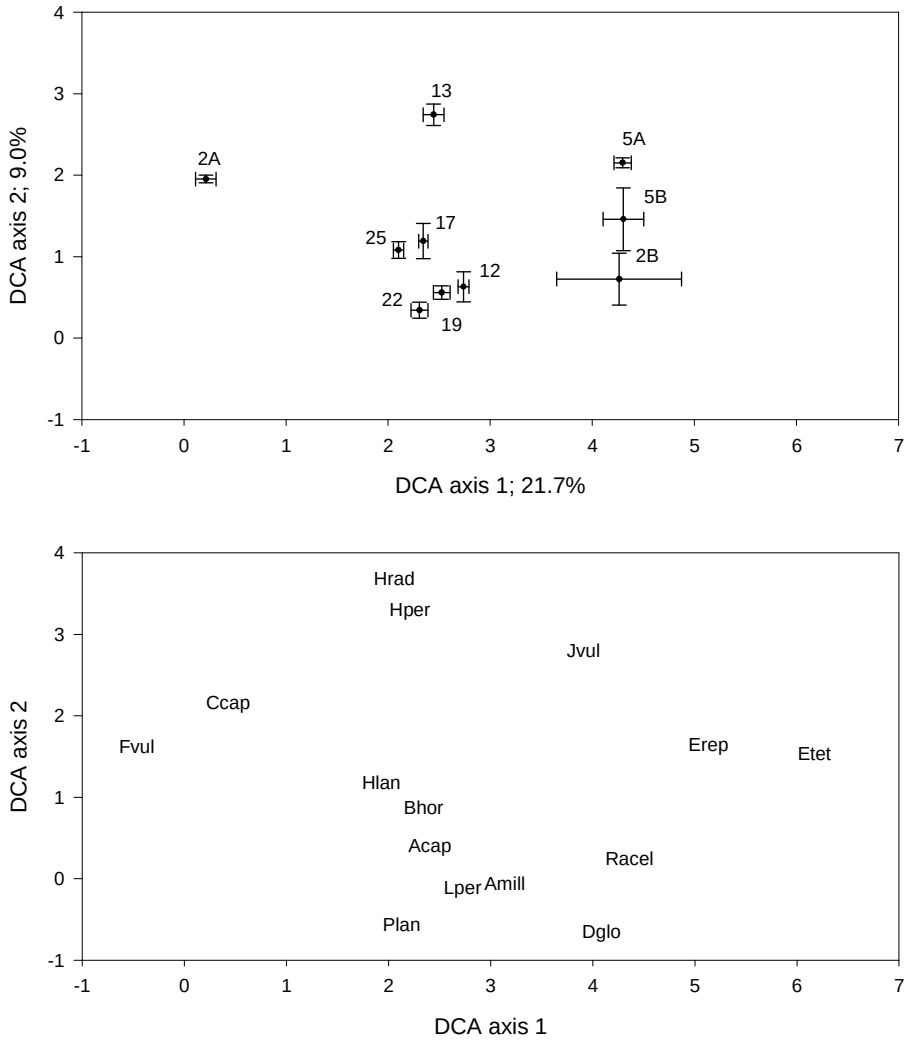

Fig. S2.2. Plant community composition in the chronosequence fields.
Detrended correspondence analysis of mean sample scores (± SE) of the plant community composition in 5 quadrats in each field. Species cover data were log (n+1) transformed and amount of explained variation by the first two DCA axes are given in parentheses. Names of the 15 most contributing plant species are: Amill = *Achillea millefolium*, Acap = *Agrostis capillaris*, Bhor = *Bromus hordeaceus* ssp. *Hordeaceus*, Ccap = *Crepis capillaris*, Dglo = *Dactylis glomerata*, Erep = *Elytrigia repens*, Etet = *Epilobium tetragonum*, Fvul = *Filago vulgaris*, Hlan = *Holcus lanatus*, Hper = *Hypericum perforatum*, Hrad = *Hypochaeris radicata*, Jvul = *Jacobaea vulgaris*, Lper = *Lolium perenne*, Plan = *Plantago lanceolata* and Racel = *Rumex acetosella.*

Chapter 3

Intra- and interspecific plant-soil interactions, soil legacies and priority effects during old-field succession

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Abstract

Legacy effects of plant influences on abiotic and biotic soil properties can result in priority effects that influence the structure and composition of plant communities. In order to better understand the role of these plant-soil interactions, here we expand the concept of plant-soil feedbacks from a within species approach (intraspecific plant-soil feedback) to a between species approach (interspecific plant-soil interactions).

In a greenhouse experiment we tested how the early-successional plant *Jacobaea vulgaris* affects its own performance and the performance of 30 co-occurring plant species via changes in abiotic and biotic soil conditions. In addition, we examined the reciprocal effect of the co-occurring species on *J. vulgaris*.

Our study had three important results. First, *J. vulgaris* exhibits strong negative plant-soil feedback. Second, there were large differences among the co-occurring species in interspecific plant-soil effects on *J. vulgaris* growth. Approximately half the species reduced *J. vulgaris* performance, whereas the other half had no effect. Third, soil conditioned by *J. vulgaris* had a positive or neutral effect on the growth of the co-occurring species.

To test the soil effects of entire plant communities, in ten old-fields that differed in time since abandonment we recorded the identity of all plants surrounding *J. vulgaris* individuals. We calculated the weighted soil effect of this community on *J. vulgaris* and the reciprocal effect of *J. vulgaris* on the community. There was a positive linear relationship between time since abandonment and the weighted feedback effect of *J. vulgaris* on the plant community.

We suggest three mechanisms how the legacy of plant-soil interactions may enhance the rate of succession through priority effects: early successional plant species exert negative plant-soil feedback; co-occurring plant species cause negative interspecific plant-soil effects to the early successional species; and the early successional species have overall positive interspecific plant-soil effects on the co-occurring plant species.

Synthesis: the performance of an early-successional species can be reduced directly by the legacy effects of intraspecific plant-soil feedback, as well as indirectly by the legacy effects of both intra- and interspecific plant-soil interactions. The legacies of these plant soil-interactions can prioritize transitions of plant species in plant communities.

Key words: Legacy effects, plant-soil feedback, ragwort, secondary succession, *Senecio jacobaea*, soil community.

Introduction

The performance of plants can be greatly influenced by interactions with the abiotic and biotic soil environment (Wardle *et al*. 2004). While the importance of abiotic soil conditions has been relatively well established, the role of soil biota in influencing the population dynamics and performance of plants has become acknowledged more recently (Reynolds *et al.* 2003; Wardle *et al*. 2004; Kulmatiski *et al*. 2008). Plants, in turn, also affect soil biota and the soil abiotic environment, for example, via living or decaying roots and root exudates, and these effects can greatly differ among plant species (Bever 1994; Bardgett *et al*. 1999; Wardle 2002; Ehrenfeld *et al*. 2005, Kulmatiski *et al*. 2008; Berg and Smalla 2009). The interactions between the soil community and plants of the same species are called plant-soil feedback (Bever *et al*. 1997). Plant-soil feedback has been recognized increasingly as an important mechanism by which plants can affect their own performance, that of other specimens of the same species (Kulmatiski *et al.* 2008). Plant-soil feedback effects, for example, can determine the success of exotic invasive species (Reinhart and Callaway 2006), or speed up species replacement during old-field succession (Kardol *et al*. 2006).

In nature, plants typically grow in mixed communities and thus also interact with individuals from other species. Similar to the plant-soil feedback mechanism described above, plants can also facilitate (van der Heijden & Horton, 2009; van der Putten 2009) or inhibit (Bonamoni et al. 2005; Casper & 73 Castelli, 2007) the performance of other species via their effects on soil biota or on abiotic conditions of the soil. Depending on the temporal and spatial dimension of these plant-soil interactions, such effects can be termed 'associative resistance or susceptibility' when two plants physically grow together (Barbosa *et al*. 2009), or 'priority effects' (Young *et al*. 2001; Grman and Suding 2010) when one plant follows after the other. Priority effects depend on the strength of plant-soil interactions and their time of endurance, also called the legacy effects (Kardol *et al*. 2007). Here, we name the effects of one plant species on another plant species through soil legacies 'interspecific plant-soil interactions' in order to distinguish them from the commonly studied 'intraspecific plant-soil feedback' effects.

Recent work by Harrison and Bardgett (2010) has shown that the effects of intraspecific plant-soil feedback also play an important role in mixed plant communities. In general, intraspecific plant-soil feedback studies point to negative effects, because antagonistic interactions in the soil overrule those of mutualists (Bever 2003; Kulmatiski *et al.* 2008). In mixed plant communities, negative intraspecific effects can play a role in the replacement of plant species during succession, because they weaken the competitiveness of the plant, or its offspring (van der Putten 2003). Although less well studied, there is also evidence pointing to the importance of interspecific plant-soil interactions for plant species replacement (*e.g.* Kardol *et al.* 2007). However, conclusions about the role of interspecific plant-soil interactions in the field are typically derived from experiments with soil collected

from monocultures of plants (*e.g.* Bezemer *et al.* 2006b; Peterman *et al.* 2008; Markham *et al*. 2009). Therefore, the debate is still open whether or not in mixed plant communities interspecific plant-soil interactions play a significant role and what the strength is of these interspecific interactions as compared to intraspecific plant-soil feedback (Kulmatiski *et al.* 2008). Further, if plant-soil feedback plays a role in the replacement of plant species during succession, such plant-soil interactions should be asymmetric, rather than symmetric. So far, the symmetry of plant-soil interactions among successional plant species has received little attention (van der Putten *et al*. 1993).

In the present study, we examine the intra- and interspecific plant-soil interactions of a plant species that dominates the early stages of secondary succession on old-fields and of 30 co-occurring plant species. We test the hypothesis that the sign and strength of plant-soil interactions depends on the plant species combination tested and determine how intra - and interspecific plant-soil interactions may contribute to species replacement during secondary succession. Moreover, we measured small scale plant co-occurrences in the field and calculated the weighted plant-soil effect of a community of co-occurring plants. We also tested whether the strength and direction of the community effect changes during secondary succession, thus with time since land abandonment. We used a weighted soil feedback approach, accounting for the possibility that plant species with a higher percentage cover in the community contribute more to the calculated community plant-soil effect. The reason is that more abundant plant species are more likely to have a stronger effect on soil biochemistry, soil biota, or both than plant species with lesser abundance (Wardle *et al.* 1998).

Central in this study is the plant (tansy) ragwort, *Jacobaea vulgaris* ssp. *vulgaris* (synonym *Senecio jacobaea*), which is considered an early successional plant. In old fields that were used previously for agriculture, after an initial short period of high abundance, the cover of *J. vulgaris* typically declines with time since abandonment (Bezemer *et al.* 2006a). Because of its prominent initial abundance, we assumed that *J. vulgaris* influences the soil conditions for many later successional plant species. In order to test the strength of these inter- and intraspecific plant-soil interactions, we conducted a greenhouse experiment. In this experiment we tested how *J. vulgaris* affects the performance of other *J. vulgaris* plants, as well as of 30 co-occurring plant species via changes in the soil community and abiotic soil conditions. We performed the experiment reciprocally, by also examining the plant-soil effect of each of the 30 species on the performance of *J. vulgaris*.

Based on *J. vulgaris* dynamics in the field, we expected that the plant-soil interactions between *J. vulgaris* and other species may contribute to its decline in abundance. Therefore, we hypothesized that other plant species would have a negative effect on *J. vulgaris* and that this effect is more negative than the effect of *J. vulgaris*, through influencing the soil, on the other plant species. In order to test if the plant-soil effect of a plant species on *J. vulgaris* and *vice versa* is related to specific types of plant species, we included forbs, legumes and grasses. *J. vulgaris* is considered an early successional plant.

Material and Methods

Jacobaea vulgaris ssp. *vulgaris* (synonym *Senecio jacobaea* L.) is a monocarpic perennial weed (Asteraceae) that spends its first year as a rosette. Flowering takes place in the second year, but is often delayed following herbivory (van der Meijden and van der Waals-Kooi 1979). *J. vulgaris* is an early successional plant species native to the Netherlands and Europe, but invasive on other continents (Wardle *et al*. 1995). In many countries *J. vulgaris* is considered a problem weed in natural and agricultural areas (Wardle 1987; Bezemer *et al.* 2006a) due to its dominance and because it contains pyrrolizidine alkaloids (PAs) that are toxic for livestock (Cameron 1935).

Greenhouse experiment

In order to test the soil effect of a range of grassland species on the growth of *J. vulgaris* and *vice versa*, we conducted a greenhouse experiment. We selected 27 species that are typical for old-fields on sandy soils in the Netherlands and that co-occur with *J. vulgaris* in these fields (Table 3.1). Moreover, we included three species that could potentially co-occur with *J. vulgaris*, although we did not record these species in our fields. The selected species include forbs, grasses and legumes, and plant species that are indicative for early, mid- and later-successional stages (Table 3.1). The experiment consisted of two phases: a conditioning phase, in which the soil was conditioned by growing species individually in that soil, and a feedback phase, in which the effect of the conditioned soil on plant performance was measured. In the conditioning phase all plant species, including *J. vulgaris*, were grown in monocultures in soil collected from an old-field in the Netherlands (see below). In the feedback phase *J. vulgaris* was grown in the soils that were conditioned by these species and in a control soil, which was a mixture of soil conditioned by all 30 species, so except *J. vulgaris* (Fig. 3.1A; see below). In the feedback phase, we also grew each of the grassland species in soil conditioned by *J. vulgaris* (Fig. 3.1B) and in the multispecies control soil.

In general, for the experiment *c*. 1000 kg soil was collected (5-20 cm deep) in November 2008 from an old-field that was taken out of agricultural production in 1995 (field 12, Table S3.1 in Supporting Information). In this field *J. vulgaris* is present. The collected field soil was homogenized, and sieved (< 0.5 cm) to remove coarse fragments. Approximately half the amount of soil was stored at 4 ^{o}C, while the other half was sterilized by gamma irradiation (> 25 KGray gamma irradiation, Isotron, Ede, The Netherlands). *J. vulgaris* seeds were collected in 2006 from approximately 30 *J. vulgaris* plants in the same field where the soil was collected. Seeds of the 30 other plant species were obtained from specialized suppliers that provide seeds collected from wild plants (Cruydt-hoeck, Assen, The Netherlands; Rieger-Hofmann, Blaufelden-Raboldshausen,

Table 3.1 List of plant species used in feedback experiment, their abbreviation, the functional group of each species (F = forbs, G = grasses, L = legumes), successional stage of the species, and a measure of presence around *Jacobaea vulgaris* or in the old-fields for each species (present around *J. vulgaris* (++), present in the fields (+) or not present in the fields (-)).

	Abbreviation	Func. group	Succ. stage	Presence
Achillea millefolium	AchMil	F	mid	++
Agrostis capillaris	AgrCap	G	mid	++
Anthoxanthum odoratum	AntOdo	G	mid	++
Briza media	BriMed	G	late	-
Bromus hordeaceus	BroHor	G	mid	++
Campanula rotundifolia	CamRot	F	late	-
Crepis capillaris	CreCap	F	early	++
Elytrigia repens	ElyRep	G	early	++
Festuca rubra	FesRub	G	mid	++
Holcus lanatus	HolLan	G	mid	++
Hypochaeris radicata	HypRad	F	early	++
Leucanthemum vulgare	LeuVul	F	mid	+
Lolium perenne	LolPer	G	early	++
Lotus corniculatus	LotCor	L	mid	+
Myosotis arvensis	MyoArv	F	early	+
Nardus stricta	NarStri	G	late	-
Phleum pratense subsp***. pratense***	PhlPra	G	mid	++
Plantago lanceolata	PlaLan	F	mid	++
Plantago major subsp***. major***	PlaMaj	F	mid	++
Rumex acetosa	RumAsa	F	early	++
Rumex acetosella	RumAla	F	early	++
Tanacetum vulgare	TanVul	F	mid	+
Taraxacum officinale	TarOff	F	mid	++
Trifolium dubium	TriDub	L	mid	++
Trifolium pratense	TriPra	L	early	+
Trifolium repens	TriRep	L	early	++
Tripleurospermum maritimum	TripMar	F	mid	++
Vicia cracca	VicCra	L	early	++
Vicia hirsuta	VicHir	L	mid	++
Vicia sativa subsp***. nigra***	VicSat	L	early	++
Jacobaea vulgaris	JacVul	F	early	++

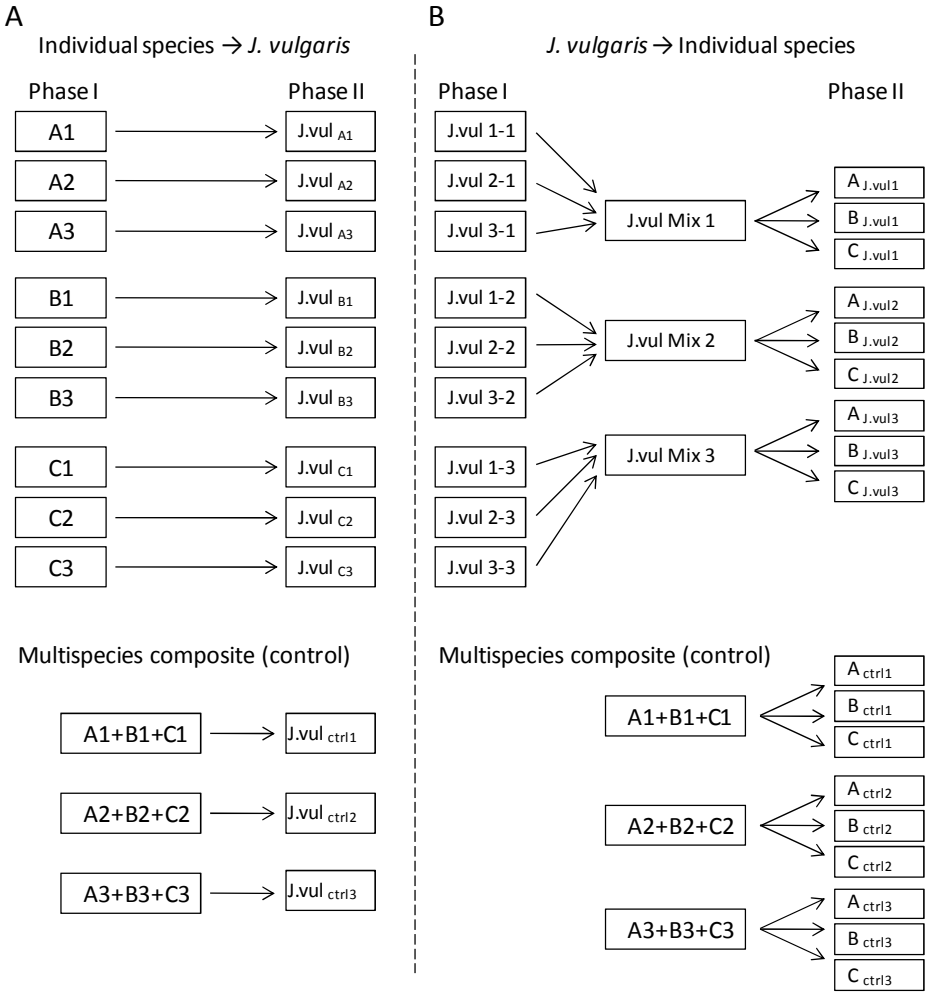

Fig. 3.1 Schematic overview of the greenhouse experiment. Part A shows the set-up to test the effect of soil conditioning by the 30 species and *Jacobaea vulgaris* on *J. vulgaris*. Part B shows the set-up to test the effect of soil conditioning by *J. vulgaris* on the 30 species and on *J. vulgaris*. For clarity only 3 species (A,B,C) and replicates (1,2,3) are shown. In the experiment there were 5 replicates and 31 species (including *J. vulgaris*).

Germany). All seeds were surface sterilized (1 min in 0.1% chloride solution), rinsed and germinated on glass beads.

In total, the conditioning phase comprised 155 pots with monocultures of each of the 30 species and *J. vulgaris* (31 species x 5 replicate pots), and 155 pots with monocultures of *J. vulgaris* to be used in the feedback phase. One-week-old seedlings were placed at 4 ^{o}C until transplanting to ensure that all species were of comparable size at the start of the experiment. Pots (1 L each) were filled with 1 kg field soil (based on dry weight) and left for two weeks to enable seedlings to emerge from the seed bank. These seedlings were removed. Hereafter, five one-week-old seedlings were transplanted in monocultures in each pot. Seedlings that died during the first week of the experiment were replaced. The pots were placed randomly in a greenhouse at 70% RH, at 16 h 21 ^{o}C (day) and 8 h 16 ^{o}C (night). Natural day light was supplemented by metal halide lamps (225 µmol s^{-1} m^{-2} photosynthetically active radiation, 1 lamp per 1.5 m^2). The pots were watered every other day and initial soil moisture level (17% at soil dry weight basis) was re-set twice a week by weighing. After eight weeks, all aboveground biomass was harvested, oven-dried (70 ^{o}C for three days) and weighed. The soil in these pots was used as substrate in the feedback phase.

In order to carry out the feedback phase of the experiment, at the end of the conditioning phase, the soil in each pot was homogenized and the largest roots were removed as they may act as a source for re-growing plants. All the finer roots were left in the soil as their rhizosphere can be a major source of inoculum for the microbial rhizosphere community, ensuring that the soil of each pot was kept separately. To avoid nutrient deficiency and to obtain ample soil, each 1 L pot was filled with a mixture of 0.5 kg (dry weight) of conditioned soil mixed with 0.5 kg (dry weight) irradiated bulk soil. In each pot five one-week-old seedlings were transplanted and seedlings that died during the first week of the experiment were replaced. In addition, one part (0.5 kg dry weight) of the conditioned soil of each pot in the conditioning phase was used to make control soil. The control soil consisted of a mixture of soil of all 30 species, excluding soil conditioned by *J. vulgaris*. In order to prepare the control soil mixtures, equal amounts of soil conditioned by the 30 plant species were homogenized (Fig. 3.1A). To examine the response of the 30 species on *J. vulgaris* soil we made five *J. vulgaris* soil mixtures. Each mixture was made from the soil of 50 pots (Fig. 3.1B). The *J. vulgaris* and control mixtures were also mixed in a 1:1 ratio with irradiated bulk soil.

During the feedback phase there were 470 pots in total ; 310 for the effects of *J. vulgaris* on all 31 species (31 species x 5 replicate *J. vulgaris* soil mixtures + 31 species x 5 replicate control soils), and 160 for the effects of all 31 species on subsequent *J. vulgaris* growth (soil conditioned by 31 species x 5 replicate soils + 5 control soils). All pots in the feedback phase were kept under the same growth conditions as described for the conditioning phase. Eight weeks after transplanting, for each pot, all aboveground biomass

was harvested, oven-dried (70 °C for three days) and weighed. Roots were rinsed, oven-dried (70 °C for three days) and weighed.

The plant-soil effect of soil conditioning by a species on *J. vulgaris* performance was calculated as the natural logarithm of *J. vulgaris* biomass on soil conditioned by that species minus the natural logarithm of the average *J. vulgaris* biomass on control soil. The effect of soil conditioned by *J. vulgaris* on the biomass production of a species was calculated as the natural logarithm of biomass of that species on soil conditioned by *J. vulgaris* minus the natural logarithm of the average biomass of that species on control soil. These calculations are symmetrical around the no-effect point, which means that the effect sizes of positive and negative values are fully comparable (Petermann *et al*. 2008; Brinkman *et al*. 2010).

Field observations and community plant-soil effects

To investigate how the plant-soil effect of a plant community relates to *J. vulgaris* performance in the field, we selected ten old-fields in a central part of the Netherlands that were abandoned between 2 and 25 years ago (Table S3.1). In July 2007, in each field the *J. vulgaris* cover per m^2 was estimated at ten locations that were 25 m apart. At each of those 10 locations, we then selected the nearest flowering *J. vulgaris* individual in each field. In a 10 cm radius around each of these *J. vulgaris* plants, the estimated percent cover was recorded for all species, including other *J. vulgaris* individuals. Aboveground material of the ten focal *J. vulgaris* plants in each field was harvested, oven-dried (70 °C for three days) and weighed.

For each focal *J. vulgaris* plant the weighted plant-soil effect of the surrounding plant community on the *J. vulgaris* plant was then calculated as the proportional cover of species *i* surrounding the *J. vulgaris* plant multiplied by its plant-soil effect on *J. vulgaris* (based on the greenhouse experiment), summed over all species in the surrounding plant community. In order to assess how the surrounding community is affected by soil conditioning by *J. vulgaris*, we also calculated the weighted plant-soil effect of *J. vulgaris* on the surrounding community. This was calculated as the proportional cover of a species surrounding the *J. vulgaris* individual multiplied by the soil effect of *J. vulgaris* on this species (based on the greenhouse experiment), summed for the entire surrounding community. We then analyzed whether the calculated weighted community plant-soil effects were related to time since abandonment, or plant size of the focal *J. vulgaris* plant. For 17 *J. vulgaris* plants (out of a total of 100 plants) that occurred in six fields, less than 75% cover of the surrounding plant community was made up by species tested in the greenhouse experiment. These plants were excluded from further analyses. The number of replicates per field varied between 5 and 10 (Table S3.1).

Statistical analyses

The difference in plant-soil interactions between species was analysed using analysis of variance (ANOVA, Genstat 12; Payne *et al.* 2008). Individual comparisons between species were based on least significant differences (l.s.d.; $P < 0.05$). Before conducting ANOVA, data were checked for homogeneity of variances and for normality by inspection of the normal-probability plot. For each individual species we also tested whether the plant-soil effect differed from zero using a one-sample T-test. ANOVA was also used to compare differences in plant-soil effects on *J. vulgaris* between functional groups (forbs, grasses and legumes). To test whether the feedback responses differed between successional stages of the species we used a nonparametric Kruskal-Wallis test, as requirements of ANOVA were still not met for these parameters after transformation. To test for symmetry in plant-soil interactions between species during succession, the relationship between the response of the 30 species on soil conditioning by *J. vulgaris* and the effect of each of the species on *J. vulgaris* growth were analysed using linear regression. For the field data, the relationship between the weighted plant-soil effect and the biomass of the focal *J. vulgaris* or with field age were analysed using linear mixed models (REML), using data from all focal plants, but with field identity as random factor and field age or plant size as continuous factors.

Differences in the composition of the plant communities surrounding the ten *J. vulgaris* plants between fields were tested using multivariate correspondence analysis (CA, CANOCO 4.55; Ter Braak and Šmilauer 2002) and co-correspondence analysis (CCA). Detrended correspondence analysis (DCA) indicated that unimodal analyses were most appropriate; the longest gradient was > 3 (Lepš and Šmilauer 2003). Direct analyses were conducted with replicates within each field (i.e. plant communities surrounding the ten *J. vulgaris* plants per field) as split-plots nested within each whole plot (field). Whole plots were permuted freely and split-plots were not permuted. Significances in multivariate analyses were inferred by Monte Carlo permutation tests (999 permutations). Plant community cover data was log-transformed prior to analyses. Species that were recorded on less than 3 occasions were excluded from these analyses.

Results

Plant-soil effects on J. vulgaris

We observed a strong negative intraspecific plant-soil feedback effect (Fig. 3.2A). Soil conditioning by *J. vulgaris* reduced *J. vulgaris* biomass by 37% as compared to *J. vulgaris* biomass production in the control soil (Fig. S3.1A). When effects of *J. vulgaris* on itself were excluded, interspecific plant-soil effects reduced *J. vulgaris* biomass production by on average 10% relative to control soil. The plant-soil effects of the individual species on *J. vulgaris* differed significantly among species (all species including *J. vulgaris*: $F_{30,153}$ =

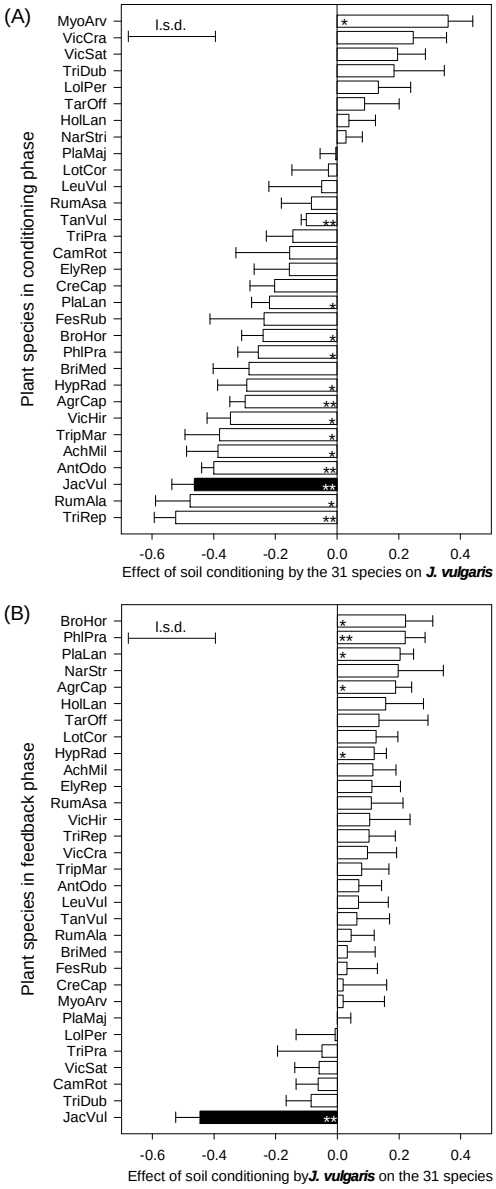

Fig. 3.2 Plant-soil effects of 30 species and *Jacobaea vulgaris* on *J. vulgaris* performance (A), and the plant-soil effects of *J. vulgaris* on *J. vulgaris* and 30 other species (B). Mean (± SE) plant-soil effects per species are shown. Differences in response of individual species can be compared by the least significant difference (l.s.d., $P < 0.05$). Strength of the plant-soil effect was also tested against zero using a one sample t-test for each species separately. An asterisk (*) indicates that the response differs significantly from zero: * $P < 0.05$, ** $P < 0.01$. Species abbreviations are given in Table 3.1.

4.98, $P < 0.001$; excluding *J. vulgaris*: $F_{29,148} = 4.73$, $P < 0.001$; Fig. 3.2A). Significant negative effects were found for soil conditioned by thirteen species. *J. vulgaris* produced significantly more biomass in soil conditioned by *M. arvensis*, while soil from seventeen other species did not significantly influence biomass production of *J. vulgaris* compared to the control soil (Fig. 3.2A). The soil effect on *J. vulgaris* did not differ among forbs, grasses and legumes ($F_{2,27} = 0.50$, $P = 0.61$) nor between successional stages ($H = 0.71$, $P = 0.70$).

Plant-soil effects of J. vulgaris

Soil conditioned by *J. vulgaris* significantly enhanced the biomass of five species (*A. capillaris*, *B. hordeaceus*, *H. radicata*, *P. pratense* and *P. lanceolata*). The only significant negative effect we observed was the effect of *J. vulgaris* on itself (Fig. 3.2B). On average, soil conditioning by *J. vulgaris* increased biomass production of the 30 other species by 9%, as compared to biomass production in control soil (Fig. S3.1B). The effect of *J. vulgaris* on the other 30 species via soil conditioning did not differ among species ($F_{29,147} = 1.14$, $P = 0.30$), or between grasses, forbs or legumes ($F_{2,27} = 1.39$, $P = 0.27$) or successional stages ($H = 3.52$, $P = 0.17$). There was no significant relationship between the effects of the 30 species on *J. vulgaris* and the reciprocal response of the 30 species to conditioning by *J. vulgaris* ($F_{1,28} = 2.59$, $P = 0.12$). The majority of species were situated below the 1:1-line and thus had a stronger negative effect on *J. vulgaris* than the reciprocal effect of *J. vulgaris* on the other species (Fig. S3.2). Thus, interspecific plant-soil interactions were asymmetric and were most negative towards *J. vulgaris*.

Field

The composition of the plants surrounding the individual *J. vulgaris* plants differed significantly among fields (CCA: $F = 5.12$, $P = 0.04$; Fig. S3.3). The variation in plant community composition was significantly explained by time since abandonment (CCA: $F = 6.39$, $P = 0.002$). Although the composition of the plant communities surrounding *J. vulgaris* changed with time since land abandonment, there was no relationship between the weighted plant-soil effect of the surrounding plant community on *J. vulgaris* and time since abandonment ($F_{1,8} = 0.62$, $P = 0.45$; Fig. 3.3). Moreover, there was no significant relationship between the weighted effect of the community and aboveground biomass of the focal *J. vulgaris* plants ($F_{1,8} = 0.17$, $P = 0.67$). On the other hand, the weighted plant-soil effect of *J. vulgaris* on the surrounding plant community changed from negative/ neutral in early successional fields to positive in later successional ones, and increased significantly with time since abandonment ($F_{1,8} = 12.47$, $P = 0.008$, $R^2 = 0.62$; Fig. 3.3).

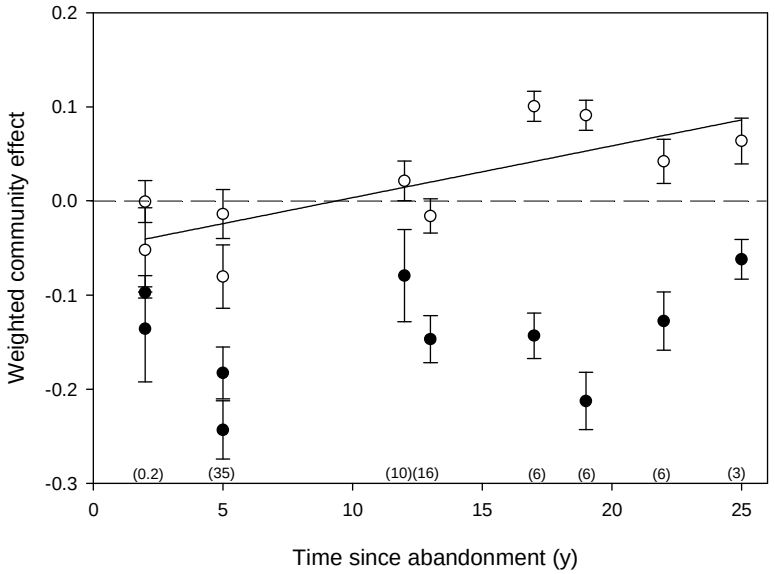

Fig. 3.3 Mean weighted plant-soil effect (± SE) of the plants surrounding *Jacobaea vulgaris* individuals in the field on *J. vulgaris* (closed symbols), in fields that differ in time since abandonment, and the weighted plant-soil effect of *J. vulgaris* on the surrounding plants (open symbols). The line is the estimated linear relationship between time since abandonment and the effect of *J. vulgaris* on the plant community ($F_{1,8}$= 12.47, *P* = 0.008, R^2 = 0.62). The average *J. vulgaris* cover (%) in the fields is presented between brackets.

Discussion

Our study on the importance of intra- and interspecific plant-soil effects of *J. vulgaris* revealed three important points. First, there were large differences in the effects of the tested plant species on *J. vulgaris* biomass production. Approximately half the species reduced *J. vulgaris* performance, whereas the other half had no effect, while only one species had a positive effect on *J. vulgaris*. Second, soil conditioned by *J. vulgaris* had a neutral or positive effect on other species; no significant negative interspecific soil effects of *J. vulgaris* were recorded. Third, *J. vulgaris* exhibits a strong negative intraspecific feedback effect. Such a negative intraspecific plant-soil feedback is common for grassland species that typically peak during early secondary succession following land abandonment (Kardol *et al*. 2006). However, thus far little information existed on the importance of interspecific plant-soil effects of individual species. In line with recent studies on priority effects (Grman and Suding 2009) our study shows that plants, via the legacy effects on the abiotic and, especially, the biotic component of the soil, can facilitate and inhibit the

growth of other plants. This can affect the rate and direction of succession. Specifically, we suggest three mechanisms how the legacy of plant-soil interactions may enhance the rate of early secondary succession through priority effects: (1) negative intraspecific plant-soil feedback by the early successional species, (2) negative interspecific plant-soil effects by co-occurring plant species on the early successional *J. vulgaris*, and (3) overall positive interspecific plant-soil effects by *J. vulgaris* on co-occurring plant species. All these effects may influence plant performance directly by reducing growth, or indirectly through altering the outcome of plant competition.

Positive plant-soil effects of early successional species on other plant species may reduce the competitive ability of the early successional species, for example, via increased asymmetry in competition for resources (Weiner 1990). Differences between species in their responses to soil conditioning by the early successional species can further drive changes in the composition of the plant community and may replace early successional species by later-successional ones (Kardol *et al*. 2006). The soil effect of *J. vulgaris* on other species and *vice versa* was asymmetric; the majority of the tested plant species had a stronger (negative) soil effect on *J. vulgaris* than its effect on these plant species. These results indicate that interspecific plant-soil interactions indeed can play a role in the successional replacement of *J. vulgaris* by other species via priority effects: *J. vulgaris* can better precede than succeed the other plant species. This shows that the soil-borne contribution to secondary succession in old-fields may have conceptual similarity with that in primary succession in coastal foredunes (van der Putten *et al*. 1993). It is important to note that during succession following land abandonment, not only plant community composition changes, but also that soil abiotic conditions change considerably (van der Wal *et al*. 2006). Other studies have shown that plant-soil feedback can vary greatly between different parent soils (Bezemer *et al*. 2006b; Joosten *et al*. 2009; Vandegehuchte *et al*. 2010). We did not test to what extent the observed plant-soil effects can be sensitive to changes in abiotic soil conditions. However, there was no relationship between the abundance of *J. vulgaris* in each of ten fields and the nutrient availability or soil chemistry of that field (TFJ van de Voorde, unpublished data). Thus, other factors, including interactions between plants and soil biota, appear to play an important role in the temporal changes in *J. vulgaris* abundance.

Besides individual plant-soil interactions, we also studied the combined plant-soil interactions in mixed plant communities and how this can affect subsequent plant community dynamics. Therefore, we calculated a 'weighted' plant-soil effect of the communities directly surrounding *J. vulgaris* plants based on the soil effects of individual plant species multiplied by their contribution to vegetation cover. Our calculation showed that with increasing successional time, the effect of *J. vulgaris* via the soil on the surrounding plant community shifted from no effect towards a positive effect. This could occur when, with increasing time since abandonment, species that are better able to cope with soil conditioned by *J. vulgaris* start to appear in the community, or when species that

are already present in the plant community and that can grow well in soil conditioned by *J. vulgaris* become more abundant. The negative effect of *J. vulgaris* on the surrounding plant community immediately following abandonment suggests that the abundance of this species should initially increase during succession, and this is indeed the case (Bezemer *et al.* 2006a; van de Voorde *et al.* 2010; Fig 3.3). Clearly this initial increase cannot be explained by the strong negative intraspecific feedback that we found in the current study. Therefore, we suggest that early during succession other processes, such as dispersal and establishment, are more important than plant-soil interactions.

The strong intraspecific plant-soil feedback effect of *J. vulgaris* can be due to changes in soil chemistry or other abiotic effects, or due to changes in the composition or abundance of soil biota. For example, tissue extracts of *J. vulgaris* have been reported to be phytotoxic (Ahmed and Wardle 1994). Therefore, the negative effect of *J. vulgaris* on itself could have been caused by autotoxicity. However, such phytochemical effects are generally not very species-specific and in our study we did not observe significant negative plant-soil effects of *J. vulgaris* on any of the other species. Rather, as the other plants grew generally better in soil conditioned by *J. vulgaris* than in a mixture consisting of soil conditioned by all other species, our results provide no support for interspecific phytotoxic effects. Pyrrolizidine alkaloids (PAs), the group of phytochemicals that is best studied in *J. vulgaris*, can also affect plant growth indirectly by affecting soil organisms, such as fungi, bacteria and plant parasitic nematodes (Hol and van Veen 2002; Kowalchuk *et al*. 2006; Thoden *et al*. 2009b). In addition, Hol and van Veen (2002) showed that PAs extracted from *J. vulgaris* roots stimulate fungi specific to *J. vulgaris* roots, whereas PAs inhibit generalistic pathogenic soil fungi. This provides support for the hypothesis that the strong negative feedback response of *J. vulgaris* is due to the accumulation of host-specific pathogens adapted to the high PA levels. Moreover, it suggests that other plant species benefit from growing in soil conditioned by *J. vulgaris*, because their specific pathogens do not survive the high PA conditions of the soil.

There is an ongoing discussion about the question whether the composition of the plant community or its diversity determine the composition of the soil community (Bardgett and Wardle 2010). Although some studies suggest that the diversity of the plant community is an important determinant, other studies have shown that the presence of specific plant species is more important in determining the soil community than plant diversity per se (De Deyn *et al*. 2004; Wardle *et al*. 2006; Viketoft *et al.* 2009; Ladygina and Hedlund 2010). A few years after cessation of agriculture, *J. vulgaris* typically becomes locally very abundant and is the most dominant plant species in old-field plant communities in The Netherlands (Bezemer *et al.* 2006a; van de Voorde *et al.* 2010). High abundance usually implies that such a species contributes substantially to ecosystem processes (Grime 1998). Our work on plant-soil feedback and interspecific plant-soil interactions shows that *J. vulgaris* with its characteristic boom-bust pattern of population development during early secondary succession may as well be a keystone species in shaping plant community

composition via changes in soil properties from which other plant species can benefit. Via these soil effects, *J. vulgaris* can potentially influence the successional replacement of itself by other species. More general, these interspecific interactions can have important consequences for restoration ecology through soil legacy and priority effects (Hausmann and Hawkes 2010; Grman and Suding 2010).

Kardol *et al.* (2006) found negative plant-soil feedback in communities of early successional species, neutral for mid-successional and positive feedback for communities of late-successional species. We expected similar results for interspecific plant-soil interactions between individual plant species, so that early successional species would have a negative effect on the early successional *J. vulgaris*, and *vice versa*. However, we found no relationship between the successional stage of the investigated plant species and its effect on *J. vulgaris*, or *vice versa*. Also, we found no differences between grasses, forbs and legumes, in their effect on *J. vulgaris* performance, or *vice versa*. In addition we found large differences within genera, for example, between the species of the genus *Trifolium*. These results together indicate that the direction and strength of interspecific plant-soil interactions is species-specific, rather than that it is related to genus or type of plant species.

We conclude that the performance of an early successional species can be reduced by legacy effects that may act directly through intraspecific plant-soil feedback when reducing plant growth and indirectly by intra- and interspecific plant-soil interactions when reducing plant competitiveness. These legacy effects of plant-soil interactions can play a role in prioritizing transitions of plant species in plant communities. We suggest three mechanisms: (1) negative intraspecific plant-soil feedback of early successional species enhance the rate of succession, because of directly reducing its own performance and that of its offspring, thereby indirectly enhancing competitiveness of other early successional plant species; (2) overall positive interspecific plant-soil effects of the early successional species on the co-occurring plant species may enhance the rate of succession by promoting the performance of the co-occurring species, and (3) negative interspecific plant -soil effects of co-occurring plant species on the early successional species indirectly enhance the performance of the other species.

Acknowledgements

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Supporting Information

Table S3.1 Field properties (name, year of abandonment and location) of the ten old-fields, and the number of replicates that were used to calculate the weighted plant-soil feedback of the plants surrounding a focal *Jacobaea vulgaris* plant.

Field code (age)	Field name	Year of abandonment	Latitude (^{o}N)	Longitude (^{o}E)	Number of replicates
2A	Oud Reemst	2005	52.02	5.48	6
2B	Reyerskamp	2005	52.01	5.47	5
5A	Telefoonweg	2002	52.00	5.45	10
5B	Assel	2002	52.12	5.49	6
12	Mossel	1995	52.03	5.45	9
13	Plantage Willem III	1994	51.59	5.31	8
17	Nieuw Reemst	1990	52.04	5.47	10
19	Wolfhezerveld	1988	51.60	5.47	10
22	Mosselse veld	1985	52.04	5.44	9
25	Dennenkamp	1982	52.02	5.48	10

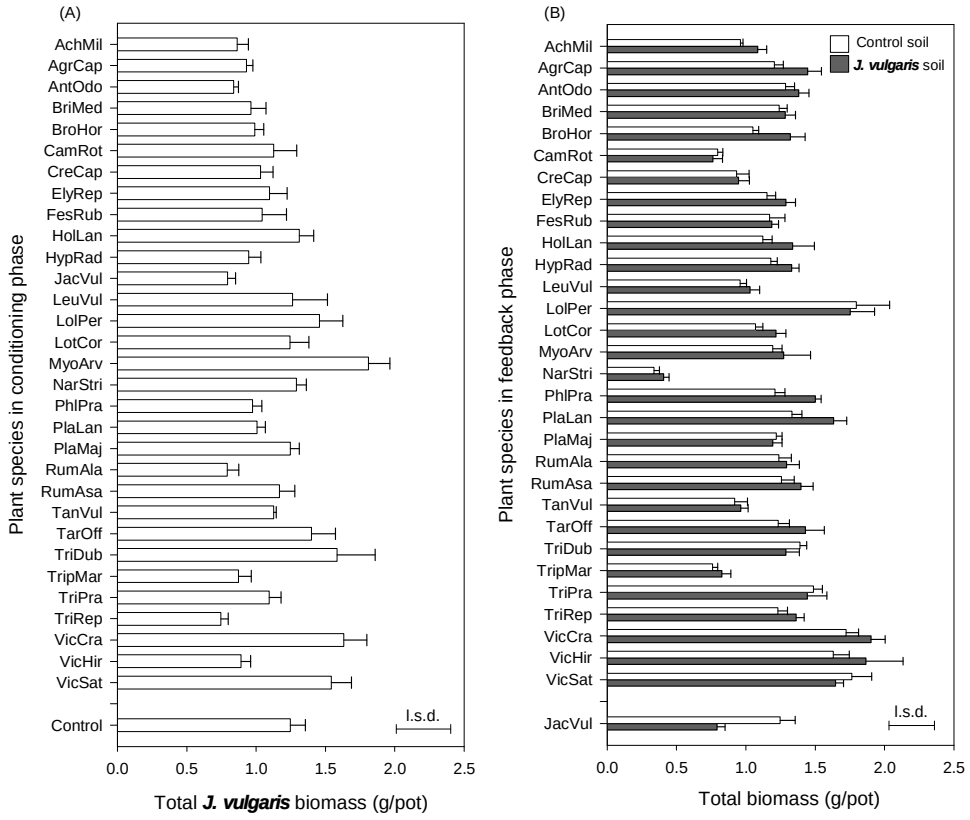


Fig. S3.1 Total plant biomass (g pot^{-1}) of (a) *Jacobaea vulgaris* in soil conditioned by the 31 species and in the multispecies composite (control) soil, and (b) biomass of the 31 species in soil conditioned by *J. vulgaris* (grey bars) and in the multispecies composite (control) soil (white bars). Mean (± SE) total biomass per species is shown. Differences in biomass of individual species can be compared by the least significant difference (l.s.d., $P < 0.05$). Species abbreviations are given in Table 3.1.

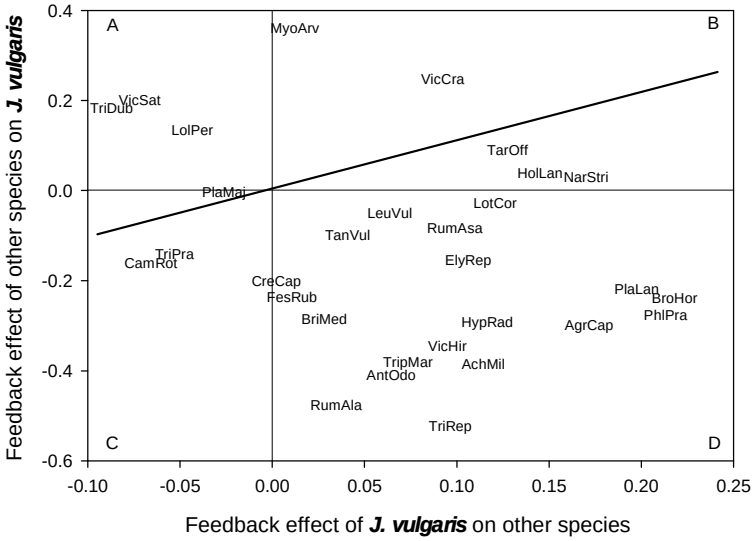
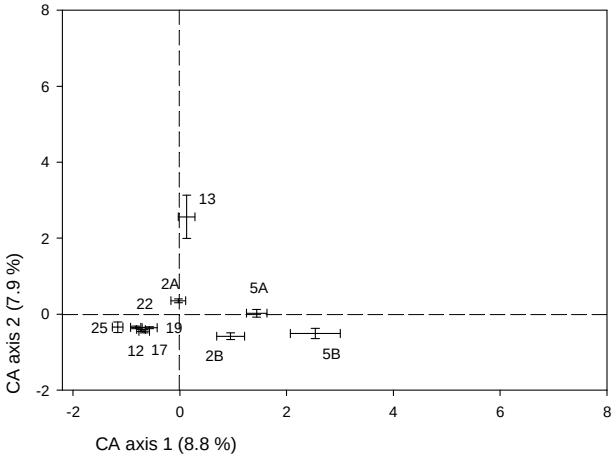
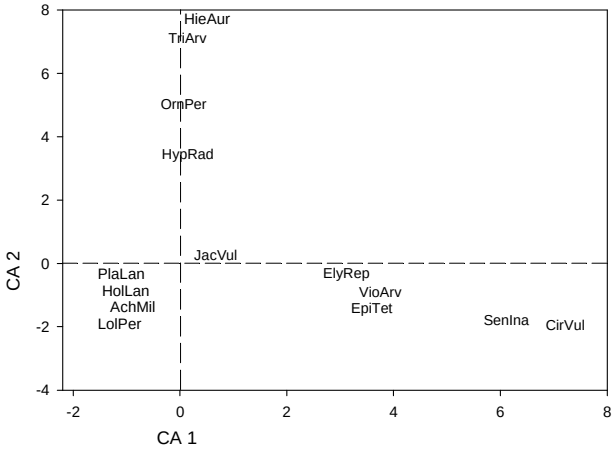


Fig. S3.2 Relationship between feedback effect of *Jacobaea vulgaris* on each of the 30 species and the reciprocal effect of each of the 30 species on *J. vulgaris*. Most species fall in quadrant (D) and have a negative effect on *J. vulgaris* but respond positively on *J. vulgaris* soil. The line is the 1:1-line of equal feedback strength. Species abbreviations are given in Table 3.1.

Fig. S3.3 Multivariate analyses of the plant community composition around *Jacobaea vulgaris* plants in ten old-fields

Correspondence analysis (CA) of mean sample scores (± SE) of the plant community composition in an area with 10 cm diameter around 10 *J. vulgaris* plants in each field. Species cover data were log (n+1) transformed and amount of explained variation by the first two CA axes are given in parentheses. Species scores of the 14 plant species with the highest scores present in the plant communities surrounding the *J. vulgaris* individuals in the ten fields are given. Full names of the plant species are: AchMil = *Achillea millefolium*, CirVul = *Cirsium vulgare*, ElyRep = *Elytrigia repens*, EpiTet = *Epilobium tetragonum*, HieAur = *Hieracium aurantiacum*, HolLan = *Holcus lanatus*, HypRad = *Hypochaeris radicata*, JacVul = *Jacobaea vulgaris*, LolPer = *Lolium perenne*, OrnPer = *Ornithopus perpusillus*, PlaLan = *Plantago lanceolata,* SenIna = *Senecio inaequidens*, TriArv = *Trifolium arvense*, and VioArv = *Viola arvensis*.





Chapter 4

Sieving out negative soil effects: Inoculation method determines soil feedback strength in *Jacobaea vulgaris*

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Submitted

Abstract

There is increasing evidence that feedback interactions between plants and biotic components of the soil influence plant productivity and plant community composition. Many plant-soil feedback experiments start from inoculating relatively small amounts of live soil to sterilized soil. These soil inocula may include a variety of size classes of soil biota, each having a different role in the observed soil feedback effects. In order to examine what may be the effect of various size classes of soil biota we compared inoculation with live soil sieved through a 1 mm mesh, a soil suspension also sieved through a 1 mm mesh, and a microbial suspension sieved through a 20 µm mesh. We tested these effects for different populations of the same plant species and for different soil origins.

Biomass was greatest in pots inoculated with the microbial suspension and smallest in pots inoculated with sieved soil, both in the first and second growth phase, and there was no significant population or soil origin effect. Plant-feeding nematodes were almost exclusively found in the sieved soil treatment. By sieving the soil to obtain a microbial suspension we reduced the strength of the negative soil effect in both the first and second growth phase. We also show that the results obtained with inoculating sieved soil or with a soil suspension are not comparable. In conclusion, when designing plant-soil feedback experiments, it is crucial to consider that soil inoculum preparation can strongly influence the observed effect.

Key words: ragwort, soil community, intraspecific variation

Introduction

Plants influence the community composition of soil organisms around their roots, which, in turn, influence the plant's performance, either directly through antagonistic or mutualistic effects, or through their effects on nutrient availability. This process is called plant-soil feedback (Bever *et al*. 1997) and is considered a key process that connects belowground and aboveground compartments in terrestrial ecosystems (Bardgett and Wardle 2010). Despite the importance of plant-soil feedback, it remains difficult to disentangle the various roles soil biota play in this feedback process. Plant-soil feedbacks can be caused by soil micro-organisms, such as bacteria and mycorrhizal or pathogenic fungi (Packer and Clay 2000; Klironomos 2002; Kardol *et al*. 2007), but also by larger soil fauna, such as nematodes, protozoa, or collembolans (Bradford *et al*. 2002; De Deyn *et al*. 2003; Bonkowski *et al*. 2009). Therefore, the effects of soil organisms on plant performance may depend on the size class of the organisms involved (Bradford *et al*. 2002), and on the co-occurrence with other groups of soil organisms (Ladygina *et al*. 2010).

Experiments that study plant-soil feedback can be set up in a variety of ways, depending on the research question, or on the type of soil studied. Frequently, in order to avoid the confounding effect of nutrient availability, sterilized (field) soil is inoculated with a live field soil inoculum or with an aqueous suspension of the field soil (*e.g.* Troelstra *et al*. 2001; Klironomos 2002; Callaway *et al*. 2004; Bezemer *et al*. 2006a,b; Kardol *et al*. 2006; 2007; Brinkman *et al*. 2010). Clearly, the experimental method that is used can affect the abundance and size classes of the organisms that will be inoculated. Kardol *et al*. (2007) showed that adding live soil or an aqueous suspension of the live soil resulted in a qualitatively similar soil effect on plant growth, but they did not compare effect sizes. As hardly any study has compared various soil inoculation methods within a single study, it remains largely unclear whether and how the method of inoculation influences plant-soil feedback results.

Plant-soil feedback effects might also depend on the abiotic conditions of the soil (Bezemer *et al*. 2006b; Casper *et al*. 2008; Manning *et al*. 2008; Joosten *et al*. 2009; Harrison and Bardgett 2010). For example, abiotic soil conditions can change the chemical composition of *Jacobaea vulgaris* plants (Hol *et al*. 2003; Joosten *et al*. 2009), which can subsequently influence soil fungal growth rate and soil fungal community composition (Hol *et al*. 2002; Kowalchuk *et al*. 2006). In addition, co-occurring plant species can change the abiotic and biotic conditions in the soil, which can then feed back to the focal plant (Aerts and Chapin 2000; Klironomos 2003). Therefore, besides being influenced by the type of soil biota present, plant-soil feedback effects can also differ among soil origins.

While most studies have considered feedback effects of a single plant species or compared responses among plant species, plant responses to soil conditions can also vary between genotypes or varieties of the same species. There is ample evidence that microbial community composition and root herbivore performance varies among genotypes or cultivars of the host plant (Kowalchuk *et al*. 2006; Schweitzer *et al*. 2008; Crutsinger *et al*. 2008; Johnson *et al*. 2010; Vandegehuchte *et al*. 2010b) and that plant genotypes differ in their effects on belowground processes, such as decomposition (Schweitzer *et al*. 2004). However, whether plant genotypes differ in their response to soil biota has been less well studied. Recently, Vandegehuchte *et al*. (2010a) showed that genotypes of the dune grass *Ammophila arenaria* indeed respond differently to inoculation of soil with soil biota. Moreover, other studies have shown that arbuscular mycorrhizal fungi differentially affect the performance of plant genotypes of the plant *Allium vineale* (Ronsheim and Anderson 2001) or *Ruellia nudiflora* (Ramos-Zapata *et al*. 2010).

In the present study, we examine how the method of inoculum preparation influences soil effects on the ruderal plant *Jacobaea vulgaris*. We study these effects using seeds and soils originating from two different fields, to examine intraspecific plant variation and the influence of soil conditions. In a greenhouse experiment we inoculated sterilized soil with live soil that was sieved through a 1 mm mesh, with an aqueous soil suspension also sieved through a 1mm mesh, or with an aqueous microbial suspension, sieved through a

20 µm mesh. We hypothesized that inoculation with the sieved soil and the soil suspension will have the same effect on plant performance, as they contain soil organisms of the same size fraction. In addition, we questioned whether the microbial suspension would have a less or, instead, a stronger negative effect on plant performance, as this suspension only contains bacteria and fungi, and not the larger organisms, among which plant-feeding nematodes and arbuscular mycorrhizal fungi.

Material and Methods

We performed our study using the plant (tansy) ragwort, *Jacobaea vulgaris* ssp. *vulgaris* (synonym *Senecio jacobaea* L.). *J. vulgaris* is an early-successional plant species native to the Europe, but invasive on other continents. In many countries *J. vulgaris* is considered a problem weed in pastures, agricultural and natural areas (Bezemer *et al.* 2006a). This is mainly due to its high abundance and because it contains PAs that are toxic for livestock (Cameron 1935). We selected this species because it is strongly affected by plant-soil interactions (Bezemer *et al*. 2006a; Joosten *et al*. 2009). It is also one of the most abundant early secondary successional plant species in the study area in the Netherlands (Bezemer *et al*. 2006a).

Plant and seed characteristics

In July 2007, at each field an imaginary W-shaped transect was laid out in a plot of 50 x 150 m. At the five outer ends of the W-shape *J. vulgaris* cover was recorded in 1 x 1 m quadrats. In a 3 x 3 m area located in the middle of each field, the flowering height of each *J. vulgaris* plant was recorded.

In November 2007, *J. vulgaris* seeds were collected from fields A and B from approximately 100 *J. vulgaris* plants. The seeds were surface sterilized during 1 min in 0.1% chloride solution, rinsed, and germinated on sterilized glass beads. Seeds were air-dried and the pappus was removed, per field 100 randomly chosen seeds were weighed individually. To examine germination, 25 surface sterilized seeds were placed on filter paper (9 cm diameter) in a Petri-dish with 2 ml demineralised water. There were five replicate Petri-dishes for each field. After 16 days at 16 h 21 ^{o}C (day) and 8 h 16 ^{o}C (night), the number of germinated seeds was determined.

Soil collection

In July 2007, from soil for the inocula was collected by taking 150 soil cores of 3 cm diameter and 15 cm depth at two ex-arable old-fields. The two fields are situated at the Veluwe, the Netherlands (Field A and B, Table 4.1). The soil samples were lumped for each field, sieved using a 5 mm mesh, and homogenized. The dry weight of a sub-sample was determined gravimetrically after 24 hours at 105 ^{o}C. Ratios and weights hereafter are

based on dry weights. In addition, approximately 500 kg of soil was collected from field B at 5-20 cm below the soil surface, sieved using a 0.5 cm mesh, homogenized, and sterilized by gamma irradiation at a dosage > 25 KGray gamma irradiation by Isotron, Ede, the Netherlands. This served as sterilized bulk soil, to which the soil inocula were added.

Soil chemistry

In each field ten soil samples (3 cm diameter and 15 cm depth) were collected. Three or four individual soil samples were combined into three homogenized soil mixtures per field. The mixtures were dried at 40 ^{o}C for 3 days. Soil mineral N was extracted by shaking 10 g (dry weight) soil with 50 ml 1 M KCl for 2 h. NH_4^+-N and NO_3^--N were determined colorimetrically in the KCl extract (Traacs 800 autoanalyzer; TechniCon Systems Inc.) and values were added up to express total mineral N. pH was measured in 2:5 dry soil: water suspensions. Available P was analysed according to Olsen *et al.* (1954) and measured at 720 nm.

Inocula preparation

We prepared three types of soil inocula from both field soils: sieved soil inoculum, a soil suspension, and a microbial suspension. The soil was prepared by sieving 2 kg of the collected field soil through a 1 mm mesh. The soil and microbial suspensions were prepared by gently stirring 2 kg field soil with 1.5 L demineralised water for 2 min. The suspension was set aside for 15 min, stirred again for 2 minutes and left to settle for 15 min. The supernatant was then sieved. In order to prepare the soil suspension, half of the supernatant was sieved through a 1 mm mesh. The other half of the supernatant was

Table 4.1 Field characteristics and mean (± SE) soil chemistry, and *Jacobaea vulgaris* characteristics of fields A and B.

	Field A	Field B
Field characteristics		
Location	52.12^{o}N 5.49^{o}E	52.04^{o}N 5.45^{o}E
Soil texture	Coarse sand	Coarse sand
Soil chemistry		
Soil pH	5.4 ± 0.15 b	6.1 ± 0.07 a
Mineral N (mg/kg)	10.7 ± 1.3 a	10.6 ± 0.8 a
P (mg/kg)	121 ± 13 a	90 ± 3 b
J. vulgaris ***characteristics***		
Abundance (%)	27.4 ± 15.0 a	10.0 ± 1.6 a
Flowering height (cm)	84 ± 6 a	63 ± 3 b
Seed weight (µg)	0.196 ± 0.01 b	0.249 ± 0.01 a
Germination (%)	54.0 ± 8.9 a	60.0 ± 3.1 a

used to prepare the microbial suspension and was sieved through sieves with mesh sizes of subsequently 1 mm, 180 µm, 75 µm, twice 45 µm, and 20 µm. Therefore, this microbial suspension does not contain micro-arthropods, nematodes, or mycorrhizal fungi, whereas it should contain soil bacteria and fungi (Swift *et al*. 1979, Ames *et al*. 1987, Klironomos *et al*. 1993; Bardgett 2005). Two subsamples (1 mL of the suspensions and 1 g of the soil) of each inoculum were frozen at -80 ^{o}C for molecular analyses (see below).

Pots of 0.9 L were filled with 1.2 kg of soil. In the case of the soil treatment this was a 6:1 mixture of sterilized soil and sieved field soil inoculum. In the case of the soil and microbial suspension treatments, pots were filled with 1.2 kg sterilized soil and inoculated with 75 ml suspension extracted from 100 g of field soil. Pots in the soil treatment received 75 ml of demineralised water in order to obtain equal levels of soil moisture for all treatments.

Greenhouse experiment

The plant-soil feedback experiment consisted of two growth phases. In the first phase, *J. vulgaris* plants were grown in soils prepared as described in the previous section. Prior to planting, the pots were incubated for 3 days. Then, into each pot three one-week-old *J. vulgaris* seedlings from either population A or B were planted. Seedlings that died during the first week of the experiment were replaced, because this may have been due to the transplanting. All treatments were replicated five times, which resulted in 60 pots in total: 2 fields x 3 inoculum types x 2 seed origins x 5 replicates. Pots were positioned randomly in a greenhouse at 70% RH, at 16 h 21 ^{o}C (day) and 8 h 16 ^{o}C (night) and soil moisture was set at 17% based on dry weight. Natural day light was supplemented by metal halide lamps (225 µmol s^{-1} m^{-2} photosynthetically active radiation, 1 lamp per 1.5 m^2). After 10 weeks all aboveground biomass was clipped, oven-dried for five days at 70 ^{o}C and weighed. The soil and roots of each pot were divided into four equal parts. From two parts the roots were gently rinsed and nematodes were extracted from a homogenized sub-sample of these rinsed roots (see below). The remaining roots were washed, dried for five days at 70 ^{o}C, and weighed.

The soil of the two remaining parts was used in the second growth phase. The two remaining parts were homogenized and soils from the individual pots were kept separate. Large roots were removed, because they may re-sprout. All the finer roots were left in the soil, so that their rhizosphere can serve as a source of inoculum for the microbial rhizosphere community. These soils from the first phase were mixed in a 1:1-ratio with 640 g of sterilized bulk soil to balance for potential nutrient variability that may have occurred during the first growth phase. Pots were incubated for 3 days, after which three one-week-old *J. vulgaris* seedlings from either population A or B were planted, in such a way that each soil received seedlings from the same population as during the first phase. Seedlings that died during the first week of the experiment were replaced. After one week the number of seedlings per pot was randomly thinned to two seedlings per pot. Plants were grown under the same conditions as during the first phase. Six weeks after

transplanting, shoots were harvested and roots were separated from the soil and rinsed. Shoots and roots were oven-dried for five days at 70 ^{o}C, and weighed.

Soil community composition

We determined the number of plant-feeding nematodes in the roots and soil from each pot at the end of the first growth phase. A sub-sample of approximately 2 g dry root mass was used to extract nematodes using a mistifier and an extraction time of 48 hr. Plant-feeding nematodes were heat-killed and fixed (35% formaldehyde diluted to 4%), after which a minimum of 150 nematodes were identified to genus or species level, according to Bongers (1988). Numbers of nematodes were expressed per 1 g dry root.

Inocula conditions

To test whether the three inocula types differ in abiotic conditions, the three inocula (mixed between soils from different fields) were autoclaved for 20 min at 120 ^{o}C on 3 consecutive days in order to kill of all soil biota. Sterilized bulk soil was then inoculated with the sterilized inocula as described above. After inoculation, the pots were incubated for 3 days, after which three one-week-old *J. vulgaris* seedlings from either population A or B were transplanted, using 5 replicate pots per treatment. Seedlings that died during the first week of the experiment were replaced. Pots were randomly positioned in a greenhouse at 70% RH, at 16 h 21 ^{o}C (day) and 8 h 16 ^{o}C (night) and soil moisture was set at 17% based on dry weight. Natural day light was supplemented by metal halide lamps (225 µmol s^{-1} m^{-2} photosynthetically active radiation, 1 lamp per 1.5 m^2). After 10 weeks all aboveground biomass was clipped and the roots were separated from the soil and washed. Plant biomass was dried at70 ^{o}C for five days, and weighed.

Data analyses

Biomass data from the feedback experiment were analysed using three-way analysis of variance (ANOVA), with field origin, treatment and seed origin as fixed factors using Genstat 12 (Payne *et al.* 2008). Treatments were compared using a Tukey HSD post hoc test. Field characteristics, soil chemistry, and *J. vulgaris* characteristics of fields A and B were compared using a T-test. Biomass production in pots with live and sterilized inocula was compared using a two-way ANOVA, separately for inoculation type and seed origin. All data were checked for homogeneity of variances Levene's tests ($P > 0.05$) and the assumption of normality was tested with Kolmogorov–Smirnov procedures. Biomass data were log-transformed and count data were square root-transformed prior to analysis to meet the assumptions of ANOVA.

Results

In sterilized soil inoculated with sterile inocula, *J. vulgaris* biomass did not differ significantly differ between the inoculum treatments (Fig. 4.1, Table 4.2). However, plants grown from seeds collected from Field A were significantly larger than plants originating from seeds from Field B (Fig. 4.1, Table 4.2). Flowering plants from Field A were significantly larger and seed weight significantly lower than of plants from Field B (Table 4.1)

In the first phase of the feedback experiment, *J. vulgaris* biomass was significantly lower in pots inoculated with sieved soil than in pots inoculated with the suspensions ($F_{1,48}$ = 43.3, P < 0.001; Fig. 4.2A). Plants growing in the soil suspension treatment, in turn, produced less biomass than plants growing in pots with the microbial suspensions ($F_{1,48}$ = 16.3, P < 0.001; Fig. 4.2A).

J. vulgaris biomass in pots inoculated with soil was significantly lower than in pots inoculated with sterilized soil (Table 4.3). Also, biomass in pots inoculated with the soil suspension from Field A was significantly lower than in pots inoculated with the sterilized soil suspension. However, biomass in pots that were inoculated with the microbial

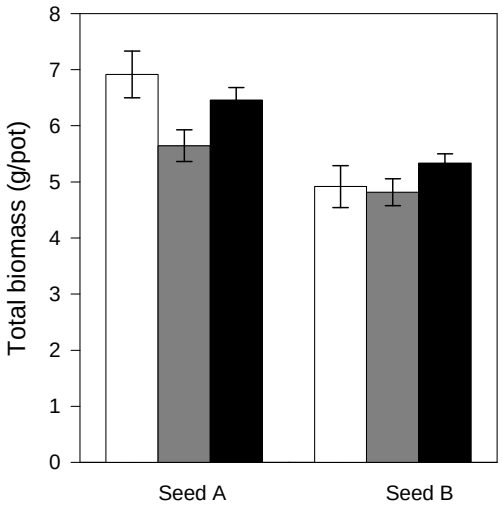

Fig. 4.1 Mean (± SE) total biomass of *Jacobaea vulgaris* in the first phase, grown in sterilized soil inoculated with sterilized field soil (white bars), sterilized soil suspension (grey bars) or sterilized microbial suspension (black bars). Seeds originated from Fields A and B.

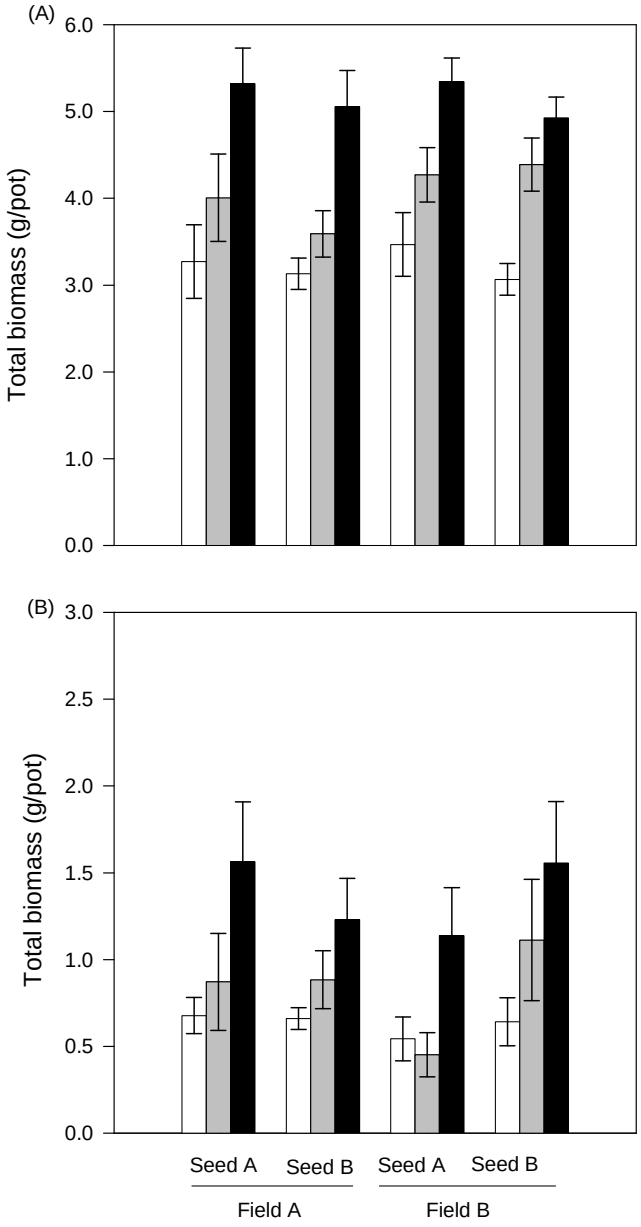

Fig. 4.2 Plant-soil feedback experiment. Mean (± SE) total biomass of *Jacobaea vulgaris* plants in the first phase (a), and in the second growth phase (b). *J. vulgaris* plants were grown in sterilized soil inoculated with sieved field soil (white bars), a soil suspension (grey bars) or a microbial suspension (black bars). Soil inocula and seeds originated from fields A and B.

Table 4.2 Results of a two-way ANOVA for the effects of sterilized inoculation treatments (soil, soil suspension or microbial suspension) and seed origin (Field A or B) on total *Jacobaea vulgaris* biomass.

	d.f.	F	***P***
Treatment	2	3.27	0.07
Seed origin	1	29.8	<0.001
Treatment*Seed origin	2	1.84	0.18
Error (mean squares)	24		

Table 4.3 Results of two-way ANOVA's comparing biomass production in the first phase in pots inoculated with sterilized inoculum, with biomass production in pots inoculated with live inoculum from Field A or Field B. Statistical analyses were performed separately per inoculation treatment (soil, soil suspension or microbial suspension) and seed origin (Field A or B). Within a row different letters indicate significant differences ($P < 0.05$) based on a Tukey HSD test.

				Inoculum source		
		$F_{2,12}$	***P***	Sterile	Field A	Field B
Soil	Seed A	17.33	< 0.01	a	b	b
	Seed B	15.60	< 0.01	a	b	b
Soil suspension	Seed A	3.99	0.04	a	b	ab
	Seed B	4.74	0.03	a	b	ab
Microbial suspension	Seed A	3.93	0.06	a	a	a
	Seed B	0.58	0.57	a	a	a

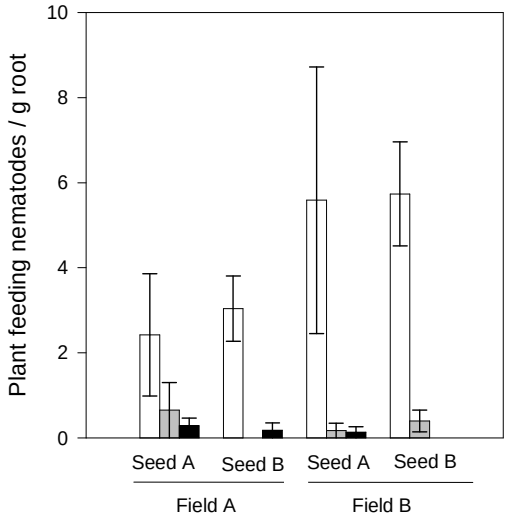

Fig. 4.3 Mean (± SE) total number of plant-feeding nematodes per gram of roots. *Jacobaea vulgaris* plants were grown in sterilized soil inoculated with sieved field soil (white bars), a soil suspension (grey bars) or a microbial suspension (black bars). Soil inocula and seeds originated from Fields A and B.

Table 4.4 Results of a three-way ANOVA for the effects of soil origin (Field A or B), inoculation treatment (soil, soil suspension or microbial suspension) and seed origin (Field A or B) on total *Jacobaea vulgaris* biomass in the first and second growth phase, and on the total number of plant-feeding nematodes (*Pratylenchus* and *Meloidogynidae*) per gram root in the first phase.

		Total biomass				Nematodes	
		First phase		Second phase		First phase	
	d.f.	F	***P***	F	***P***	F	***P***
Soil origin (Soil)	1	1.20	0.28	1.57	0.22	3.92	0.05
Inoculum	2	29.54	<0.001	6.92	0.002	18.3	<0.001
Seed origin (Seed)	1	1.08	0.30	2.67	0.11	0.12	0.74
Soil*Inoculum	2	0.82	0.45	0.06	0.94	3.41	0.04
Soil*Seed	1	0.00	1.00	3.02	0.09	0.06	0.81
Inoculum*Seed	2	0.06	0.94	0.90	0.41	0.22	0.80
Soil*Inoculum*Seed	2	0.36	0.70	0.44	0.65	0.06	0.94
Error	48						

suspension did not significantly differ from pots inoculated with the sterilized microbial suspension.

In the second phase, biomass of *J. vulgaris* was significantly smaller in pots of the sieved soil treatment than in pots inoculated with the microbial suspension ($F_{1,48}$ =12.0, P = 0.001; Fig. 4.2B). Biomass in the pots inoculated with the microbial suspension was significantly larger than in the pots inoculated with the soil suspension ($F_{1,48}$ = 8.5, P = 0.005). Biomass did not differ between the soil and soil suspension treatment ($F_{1,48}$ = 0.3, P = 0.58). Total biomass per pot in the second phase was substantially lower than in the first phase, but there was a significant positive relationship between biomass of both phases ($F_{1,59}$ = 11.7, P = 0.001, R^2 = 0.17), indicating that nutrient availability was not limiting. During both growth phases, there were no significant effects of either soil or seed origin (Table 4.4).

At the end of the first phase, plant-feeding nematodes belonging to the genera *Pratylenchus* and *Meloidogyne* were found. Nematode densities differed significantly between inoculation treatments (Table 4.4) and were largest in pots inoculated with sieved soil (Fig. 4.3). In most pots that were inoculated with a suspension no plant-feeding nematodes were detected. In addition to the treatment effect, there was also a significant soil origin effect, as there were more nematodes in pots inoculated with soil from Field B than Field A (Table 4.4). There was a significant negative relationship between plant biomass in the first phase and nematode density ($F_{1,58}$ = 21.7, P < 0.001). However, there was no significant relationship between nematode density and biomass in the second phase ($F_{1,58}$ = 0.29, P = 0.59).

Discussion

Our study clearly shows that a negative soil effect can be sieved out when the soil inoculum is sieved with a mesh of 20 µm. In addition, the type of inocula that is used can greatly influence the effect of inoculation on plant performance and the observed soil effect. In both the first and the second growth phase, plant biomass was highest in soil that was inoculated only with the microbial community, and biomass did not differ from the plants that were grown in the sterilized inocula. These results suggest that negative soil effects can be partly sieved away and that this effect is robust, as it was found in both phases. Hence, our results show that choices made on what soil inoculation method to use in feedback experiments can have large consequences on the size of the effect that the soil community has on plant performance. Below we discuss a number of possible mechanisms that may explain how the inoculation method can influence plant-soil effects. In addition, we discuss how intraspecific variation in plants and variation in soil abiotic and biotic components can affect plant-soil effects.

The higher biomass production in pots inoculated only with the microbial community indicates that the main growth reducing agents were partly sieved out and thus that these agents did not pass the 20 µm sieve. The most obvious explanation is that the main growth reducing agents are larger than 20 µm. This corresponds with the observation that plants with a higher number of plant-feeding nematodes were smaller. This observation points at plant-feeding nematodes as a cause of reduced *J. vulgaris* growth in pots inoculated with sieved field soil. However, there are also many pathogenic soil bacteria and fungi present in soils and it could be that some of them were filtered out by the 20 µm sieve as well. For example, because soil bacteria and fungi can be associated to larger soil aggregates (Six *et al*. 2004), which are filtered out by the 20 µm sieve. It is also possible that the differences are due to a dilution effect. Recent work showed that rare soil microbes are not redundant, as they can have strong negative growth effects (Hol *et al*. 2010). By sieving out part of the soil (micro-)organisms from the microbial suspension, it is possible that rare species are sieved out as well. Even though we did not examine arbuscular mycorrhizal colonization, they were probably not present in the microbial suspension as their spores are larger than 20 µm (Klironomos *et al*. 1993). As plants had more biomass in pots inoculated with the microbial suspension than in pots inoculated with the soil suspension this allows us to conclude that growth stimulating arbuscular mycorrhizal fungi were of less importance for plant performance than the growth reducing agents.

J. vulgaris biomass in the treatment inoculated with sieved soil was significantly lower than when inoculated with the soil suspension. However, both treatments did not differ when the inocula were sterilized, which suggests that nutrient availability in both inocula was similar. Even though both the sieved soil inoculum and the soil suspension were sieved through 1 mm mesh there was a clear difference in the number of plant-feeding nematodes between these treatments. Nematodes are sensitive to soil processing (de Rooij-van der Goes *et al*. 1997) and the additional sieving and stirring to create the suspensions may have reduced their number. Our results emphasize that soil suspensions should not be used as a direct substitute of soil inoculation, as their effect on plant performance is not the same.

While frequently ignored, recent studies have shown that plant-soil feedback effects can differ greatly between soils (Manning *et al*. 2008; Joosten *et al*. 2009; Harrison *et al*. 2010). In our study, *J. vulgaris* performance did not differ between the two soil origins for none of the three inocula. The soils that we tested originate both from old-field grasslands on the same soil substrate. Studies that reported effects of soil abiotic conditions on plant-soil feedback effects often compare soils that are more different from each other, for example, clay versus sandy loam soils (Bezemer *et al*. 2006b), or soils that originate from more different ecosystems, such as a dune system versus an experimental grassland (Joosten *et al*. 2009). However, the fact that we did find effects of the inoculation method

but no soil origin effect indicates that *J. vulgaris* is more sensitive to small differences created between soil inocula from the same soil, than to differences between soils.

In the field, flowering plants collected from Field A were much larger than plants originating from Field B, and also in the experiment with sterilized inocula these plants were significantly larger. Nevertheless, in the greenhouse experiment with live inocula, there was no intraspecific variation in plant size between both populations. The absence of a population effect when soil biota are present shows that the inoculation method has a stronger effect on plant performance than intraspecific variation. Joosten *et al.* (2009) found that *J. vulgaris* performance differed significantly between clones that differed in PA-levels. Here, we used seeds that were collected from multiple plants within a population, and it is inevitable there was considerable genetic variation within each population. Moreover, these populations may not have such strong differences in PA content and composition as in the study of Joosten *et al.* (2009). In an experiment with white cabbage cultivars (*Brassica oleracea* var. *capitata*) that differed in glucosinolate levels, Kabouw *et al.* (2010) found an effect of intraspecific variation on plant-feeding nematodes, but not on other parts of the soil food web. Again, as in the case of Joosten *et al.* (2009), these effects of cabbage were mainly due to differences in secondary chemistry. Therefore, the strong effects of inoculation preparation on plant-feeding nematodes in our experiment could have overruled intra-specific variation as well.

In conclusion, we show that the method of inoculation can strongly influence the soil effect on plant performance. The strongest negative soil effect was observed when using sieved field soil. By sieving the soil to obtain a microbial suspension we removed the negative soil effect, which was observed during both growth phases. In addition, we show that the results obtained with inoculating sieved field soil or with an aqueous soil suspension are not comparable in terms of effect strength. Thus, when designing plant-soil feedback experiments, it is crucial to consider that soil inoculum preparation can strongly influence the effect size.

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Chapter 5

Can negative plant-soil feedback of *Jacobaea vulgaris* be explained by autotoxicity?

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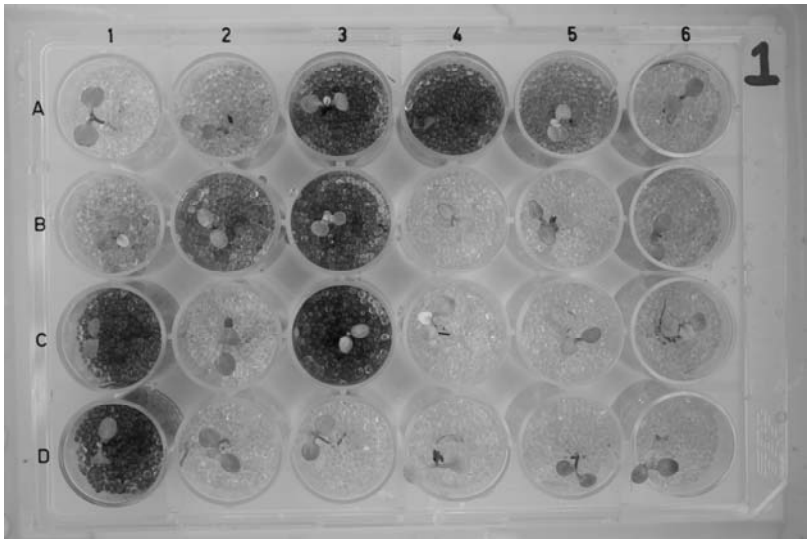
Submitted



Abstract

Many wild plant species show temporal boom-collapse patterns of population development and the question is what causes these patterns? Field and bioassay studies with *Jacobaea vulgaris* have shown that plants grow poorly in soil from areas where it dominates. We hypothesized that this poor growth is due to autotoxicity.

We tested this hypothesis by examining the inhibitory effects of aqueous extracts made from root and shoot tissues on seed germination and seedling performance of *J. vulgaris*. We also examined the effects of extracts taken from soil conditioned by *J. vulgaris*, as well as the effect of root fragments on seedling performance.

Performance of seedlings growing on glass beads was significantly reduced by the high and medium strength root and shoot extracts. Extracts made from soil did not differ significantly in their effect from the control, and seedlings growing in sterilized soil were also not affected by *J. vulgaris* extracts. Incorporating root fragments reduced maximum root length significantly, also when seedlings were growing in sterilized soil. Only the high strength shoot extract reduced germination.

We conclude that under laboratory conditions *J. vulgaris* can be autotoxic and we discuss the role that autotoxicity may play in the decline of *J. vulgaris* abundance in old-fields.

Key words: allelochemicals, allelopathy, phytotoxicity, *Senecio jacobaea*, soil sickness, succession

Introduction

Jacobaea vulgaris is an early successional species that is highly dominant in old-fields early after cessation of agricultural practices. However, after a few years of dominance, its abundance sharply declines. This boom-collapse pattern is characteristic for many wild plant species occurring in succession gradients (Olff and Bakker 1991; Halpern *et al*. 1997; Meiners *et al*. 2009). Bioassay studies have shown that *J. vulgaris* plants grow poorly in soil collected from areas where it is dominant (Bezemer *et al*. 2006a; van de Voorde *et al*. 2010). These negative soil effects have been attributed to soil fungal pathogens that build up in presence of this plant species (Bezemer *et al*. 2006a). However, it is also possible that the negative growth responses are due to autotoxic allelopathic effects (Rice 1984; Wilson and Rice 1968).

Allelopathy is the harmful effect of one plant on another, through the production of chemical compounds that are released into the environment (Rice 1984; Lambers *et al*. 2008). A special form of allelopathy is autotoxicity, which occurs when the released

chemical compounds inhibit the growth of plants of the same species (Miller 1996; Kumari and Kohli 1987; Lambers *et al*. 2008). Through autotoxic effects seed germination and seedling establishment near full-grown plants can be reduced, which minimizes intraspecific competition for resources (Singh *et al. 1*999; Liu *et al.* 2008) or can enhance population viability (Canals *et al*. 2005). Although allelopathy and autotoxicity are predominantly studied within an agricultural context (Miller 1996; Liu *et al*. 2008), they can also play an important role in natural systems (Singh *et al*. 1999), for example, during succession (Wilson and Rice 1968; Quinn 1974) or exotic plant invasions (Callaway and Aschehoug 2000; Bais *et al*. 2003; Hierro and Callaway 2003).

In nature, plant chemical compounds can enter the soil through foliar leaching, root exudation, decomposition of plant tissues or volatilization (Rice 1984; Inderjit and Nilsen 2003; Lipinska and Harkot 2007). However, these plant chemical compounds are not equally distributed amongst plant organs and the concentration and composition can differ considerably between aboveground and belowground parts (Hol *et al*. 2003; van Dam 2009). Typically, leaf extracts are a more consistent source of allelochemicals than root extracts (Rice 1984; Lipinska and Harkot 2007). On the other hand, plant responses to allelochemicals are generally stronger in roots than in aboveground tissues (Blum *et al*. 1999; Tawaha and Turk 2003). Moreover, in experiments allelopathic effects often become stronger with increasing concentrations of the extract, while at very low concentrations allelochemicals might even have stimulating effects on plant growth (Rice 1984; Zeng *et al.* 2001).

A well studied group of chemical compounds that are known to exhibit allelopathic effects are alkaloids (Wink *et al*. 1999). *J. vulgaris* (synonym *Senecio jacobaea*) and other species in the *Senecio* family produce a variety of pyrrolizidine alkaloids (PAs) that can act as toxins and deterrents against other plants (Fujieda *et al*. 1988) and plant antagonists, such as insects and soil fungi (Macel *et al*. 2005; Hol and van Veen 2002; Kowalchuk *et al*. 2006). Ahmed and Wardle (1994) tested the effects of extracts of *J. vulgaris* tissues on other plant species and showed that these extracts inhibit seed germination, seedling emergence and growth of co-occurring pasture species. They also showed that extracts made from leaf material inhibit growth of other plant species more than root extracts (Ahmed and Wardle 1994). Although PAs are important for the defense of *J. vulgaris* (Macel *et al*. 2005), it is not known whether they are the only compounds in *J. vulgaris* causing allelopathy.

While allelopathic effects can be attributed directly to plant chemicals (Bais *et al*. 2003), soil microorganisms can also play an important role in allelopathy (Rice 1984; Inderjit and Weiner 2001, Inderjit and Nilsen 2003). Soil organisms can (de-)activate toxic plant compounds or release phytotoxic compounds when they decompose plant tissues (Rice 1984; Inderjit and Nilsen 2003). The properties of plant chemicals can also be influenced directly by the physical and chemical properties of the soil, for example, due to adsorption to soil particles (Wardle *et al*. 1998).

We carried out a plant-soil feedback study and show that *J. vulgaris* performs poorly in soil preconditioned by the same species. Subsequently, we test the hypothesis that reduced *J. vulgaris* performance in soil in which it has been growing previously is due to autotoxicity. In order to test this hypothesis, we examined the autotoxic potential of *J. vulgaris* in a series of laboratory experiments. We tested the inhibitory effects of aqueous extracts made from root and shoot tissues on seed germination and seedling performance of *J. vulgaris*. We also examined the effects of extracts taken from soil conditioned by *J. vulgaris*, as well as the effect of root fragments on seedling performance.

Material and Methods

In November 2007, *J. vulgaris* seeds were collected from approximately 100 plants in an old field (see below) where *J. vulgaris* is abundant. Pappus was carefully removed and the seeds were surface sterilized for 2 min in 0.4% chloride solution and rinsed. Seeds were germinated in demineralised water on glass beads of 1 mm diameter in a growth cabinet at 16 h 25 ^{o}C light and 8 h 20 ^{o}C dark, in order to mimic natural day/night conditions. One-week-old seedlings were used for all experiments.

Plant-soil feedback experiment

To examine the effect of *J. vulgaris* on subsequent *J. vulgaris* performance via changes in the soil, we carried out a plant-soil feedback experiment. The feedback experiment consisted of two growth phases: in the first phase the soil was conditioned by growing *J. vulgaris*. In the second phase the feedback of the conditioned soil on *J. vulgaris* performance was measured. In the first phase *J. vulgaris* plants were grown in live or sterilized field soil. All field soil had been collected from a depth of 5-20 cm below the soil surface in the old-field that was taken out of agricultural production in 1995 and where *J. vulgaris* is abundant (10% cover). The soil type is sandy loam (van der Putten *et al*. 2000). The field is located in a nature reserve at the Veluwe, the Netherlands at 52^{o}04'N, 5^{o}45'E. Pots (0.9 L) were filled with sterilized field soil (> 25 KGray gamma irradiation, Isotron, Ede, the Netherlands), which was inoculated in a 6:1 ratio with field soil. A second set of pots was inoculated with autoclaved (3 consecutive days, 20 min at 120 ^{o}C) field soil. Each pot contained 1.2 kg of soil, based on dry weight. Three one-week-old seedlings were planted per pot and both treatments were replicated five times. All pots were placed randomly in a greenhouse at 70% RH, at 21 ^{o}C during the day (16 h) and 16 ^{o}C during the night (8 h) at 17% soil moisture content (based on dry weight). Natural day light was supplemented by metal halide lamps (225 µmol s^{-1} m^{-2} photosynthetically active radiation, 1 lamp per 1.5 m^2). After 10 weeks aboveground biomass was harvested. The soil and roots of each pot were subdivided into four equal parts. From two parts the roots were rinsed. Shoots and roots were oven-dried for 5 days at 70 ^{o}C and weighed. The other two parts of soil and roots were used as inoculum for the second growth phase. Large roots were removed as they may re-sprout. The two parts were homogenized in a 1:1 ratio with

640 g of sterilized soil, on a dry weight basis, ensuring that soil and roots from each pot were kept separate. As during the previous growth phase, we included a new sterilized soil treatment, which was autoclaved field soil homogenized in a 1:1 ratio with sterilized soil. Three one-week-old *J. vulgaris* seedlings were planted in each pot and after one week the seedlings were randomly thinned to two seedlings per pot. Growing conditions were as in the first phase. After six weeks, aboveground biomass was harvested and the roots were rinsed. Shoots and roots were oven-dried for 5 days at 70 °C and weighed.

Preparation of plant and soil extracts

To obtain shoot, root, and soil material for the extract preparation, three seedlings were planted in a pot of 1 L filled with 950 g sterilized field soil on a dry weight basis. There were three replicate pots. Pots were kept separate and from each pot a root, a shoot and a soil extract was made. After 12 weeks, all leaf material was clipped and cut into pieces of approximately 1 cm. The soil of each pot was sieved through a mesh of 2 mm in order to separate roots from soil. The roots were collected from the mesh and rinsed in demineralised water for 20 sec. A subset of approximately 8 g of roots from each pot was kept separate to test the autotoxic effect of root fragments (see below). The remaining roots were cut into 1 cm pieces to be used as inoculum (see below). Shoot, root, and soil extracts were then made from each replicate pot separately. Twelve g of fresh leaf or root material (corresponding with 3 g dry weight (dw)) or soil (corresponding with 10.5 g dw) was soaked in 40 ml demineralised water, stirred for 20 sec and left in the dark for 18 hours. Twelve grams of shoot tissue corresponds with roughly the total foliar biomass from a pot; twelve grams of roots corresponds roughly with one fourth of the total root biomass per pot. The solutions were filtered (125 µm mesh size, Omnilabo, Breda, the Netherlands). Solutions were used pure (high strength), diluted 1:1 with demineralized water (medium strength) or diluted 1:19 with demineralized water (low strength). The extracts were filter-sterilized (0.2 µm, Whatman, Puradisc FP 30) and placed at 4 °C until further use.

Autotoxicity experiments

Growing conditions: Experiments were conducted with seedlings growing on glass beads or sterilized soil. In the experiments testing autotoxicity on glass beads and testing the autotoxic effect of root fragments, seedlings were grown individually in 24–well microplates in 3.3 ml wells (16.2 mm diameter). Each well was filled with 3 g soil that had been sterilized for 20 min at 110 ° C on 2 consecutive days, or on glass beads of 1 mm diameter that had been sterilized for 48 hr at 110 °C. In the experiment testing autotoxicity in sterilized soil, the seedlings were grown individually in 10 ml glass vials (22 mm diameter), filled with 12 g soil that had been sterilized for 20 min at 110 ° C on 2 consecutive days. Microplates and vials were placed in plastic boxes (13 x 18 x 6 cm) with transparent lids to prevent evaporation. The boxes were placed in a greenhouse at conditions as described above, to mimic day/night conditions. At the end of each

experiment, maximum root length was measured. Seedlings were then dried for two days at 50 ° C and weighed.

Autotoxic effects in glass beads

At the start of the experiment, every well received 0.7 ml high, medium or low strength shoot, root or soil extract. Control seedlings received 0.7 ml demineralised water. All treatments were replicated 8 times, so that there were 224 seedlings (3 replicate pots x 3 extract types x 3 concentrations x 8 seedlings + 8 control seedlings). All seedlings received 0.1 ml half-strength Hoagland solution (Hoagland and Arnon 1950) in order to enable growth. Seedlings received five times 0.2 ml demineralised water during the experiment and were harvested after 19 days.

Methodological checks

In order to identify possible side-effects of the solutions used for applying the extracts, we compared seedling growth in demineralised water, acidified demineralised water, and a half-strength Hoagland solution. The pH of the acidified control (pH = 5.4) equals the pH of the root extract. The nutrient solution was a 7:1 mixture of demineralised water: half-strength Hoagland solution. In order to test the possible side effects, 0.8 ml of the solutions was added to individual seedlings growing on glass beads. All treatments were replicated 7 times. All seedlings received five times 0.2 ml demineralised water during the experiment. After 19 days the seedlings were harvested.

Autotoxic effects in sterilized soil: Each vial containing one seedling received 1 ml low or high strength shoot or root extract. Control plants received 1 ml demineralised water. There were 8 replicates for each treatment resulting in 104 vials (3 replicate pots x 2 extract types x 2 concentrations x 8 seedlings + 8 control seedlings). During the experiment the seedlings received three times 0.2 ml demineralised water. After 21 days all seedlings were harvested.

Autotoxic effect of root fragments

Root fragments were obtained from a subset of the roots that were used for extract preparation (see above). Seedlings on glass beads or sterilized soil grew individually in 24–well microplates in 3.3 ml wells of 16.2 mm diameter. Each well was filled with 3 g glass beads (1 mm diameter) or with 2.65 g dw soil that had been sterilized for 20 min at110 ° C on 2 consecutive days. Soil or glass beads were homogenized with root fragments, corresponding with 0.06 g dw root material per well. Seedlings growing in soil received 0.5 ml of demineralised water and seedlings on glass beads 1.2 ml, resulting in equal water content of both substrates. Control seedlings on glass beads received 0.5 ml water. All treatments were replicated 10 times, which resulted in 70 seedlings in total (3 replicate pots x 2 substrates x 10 seedlings + 10 control seedlings). During the experiment seedlings received four times 0.2 ml water. After 12 days seedlings were harvested.

Autotoxic effects on seed germination

Twenty surface sterilized *J. vulgaris* seeds were placed on a filter paper (diameter 8.5 cm, ref. 0/971510) in a Petri-dish. Each dish received 4.5 ml high or low strength extract made from shoots, or roots, whereas controls received 4.5 ml demineralized water. For both extract types and concentrations there were five Petri-dishes. In total the experiment comprised 65 Petri-dishes (3 replicate pots x 2 extract types x 2 concentrations x 5 dishes + 5 control dishes). Petri-dishes were placed in a germination cabinet at 20 ^{o}C in the light (16 h) and 15 ^{o}C in the dark (8 h). During the experiment seeds were given an additional dosage of 0.25 ml of extract and 1.5 ml demineralised water, whereas the controls received 1.75 ml of demineralised water. Germination was checked daily for 19 days and total percentage germination per Petri-dish was calculated.

Data analyses

Data were analyzed using analysis of variance (ANOVA, Genstat 12; Payne *et al.* 2008). Data were checked for homogeneity of variances using Levene's tests ($P > 0.05$ in all cases) and for normality using Shapiro-Wilk's test ($P > 0.05$ in all cases). Results from the first and second growth phase of the plant-soil feedback experiment were analysed separately, and individual comparisons were based on a Tukey HSD post-hoc test. For all autotoxicity experiments, replicates for treatments originating from the same extract (from the same pot) are strictly speaking pseudoreplicates and were therefore averaged prior to analyses, so that there were three replicates for each extract type and concentration. To fulfill requirements of normality, biomass and root length data were log-transformed and percentage data were arcsin-transformed prior to statistical analyses. Treatments were compared to the control using a post-hoc Dunnet's test. When treatment effects were significant, tissue or substrate types were compared using planned comparisons.

Results

Plant-soil feedback experiment

Total biomass in the first growth phase of the greenhouse experiment was significantly lower in the treatment inoculated with live field soil than in the sterilized control treatment (38% reduction; $F_{1,8} = 33.1$, $P < 0.001$; Fig. 5.1). Biomass in the second phase was significantly lower in both soils that originated from the first phase than in the new sterile soil ($F_{1,8} = 9.3$, $P = 0.008$; Fig. 5.1). *J. vulgaris* biomass in the field and the sterile soil that had been conditioned by *J. vulgaris* was reduced by 70% and 63% respectively, as compared to biomass production in the new sterile soil.

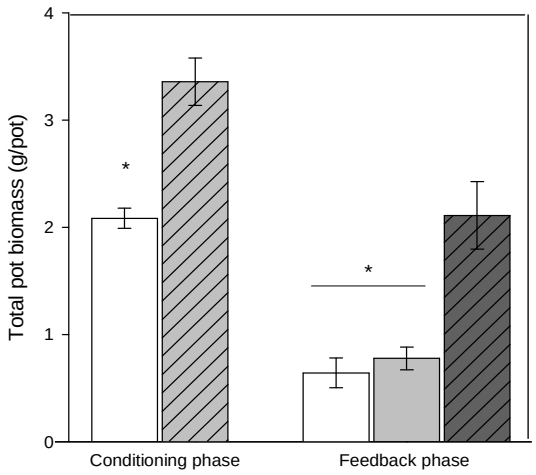


Fig. 5.1 Mean (± SE) total pot biomass of *Jacobaea vulgaris* plants growing in the conditioning phase in sterilized soil (light gray bar) or in sterilized soil inoculated with live field soil (white bar); and biomass of plants in the feedback phase growing in field soil that was conditioned by *J. vulgaris* (white bar) or in sterile soil that was conditioned by *J. vulgaris* (light gray bar), or not in sterilized soil that was not conditioned (dark gray bar). The dashed bars indicate the sterilized control for the conditioning and feedback phase. For the conditioning and the feedback phase separately, significant differences ($P < 0.05$) between control and treatment are indicated with an asterisk.

Autotoxic effects in glass beads

Maximum root length and total biomass differed significantly between treatments (root length (RL): $F_{9,24} = 246.1$, $P < 0.01$; biomass (BM): $F_{9,24} = 35.0$, $P < 0.01$; Fig. 5.2). As compared to the control, root length was significantly reduced in the treatments with the high and medium strength of both root and shoot extracts. Shoot extracts reduced root length more than root extracts ($F_{1,18} = 95.7$, $P < 0.01$). Seedling biomass was significantly reduced in the high root, and medium and high shoot extract (Fig. 5.2). Root extracts reduced biomass more than leaf extracts ($F_{1,18} = 6.0$, $P = 0.03$). Extracts made from soil did not differ significantly from the control.

Methodological checks

Total seedling biomass did not significantly differ between the control treatment with water only and the treatments with the lowered pH or additional nutrients, nor did root length (Table 5.1). Thus, this minimizes the possibility that the assumed autotoxic effects were purely due to pH changes or nutrient availability brought about by the plant extracts.

Table 5.1 Mean (± SE) root length and biomass of *Jacobaea vulgaris* seedlings that received the control treatments: water, Hoagland nutrient solution (Nutrients) or an acidified water solution (pH). F and *P* values of an ANOVA are also presented.

	Root length (cm)	Total biomass (g)
Water	6.8 ± 0.8	1.9 ± 0.1
Nutrients	6.7 ± 0.7	1.6 ± 0.1
pH	5.6 ± 0.5	1.6 ± 0.1
$F_{2,18}$	1.18	0.60
P	0.33	0.56

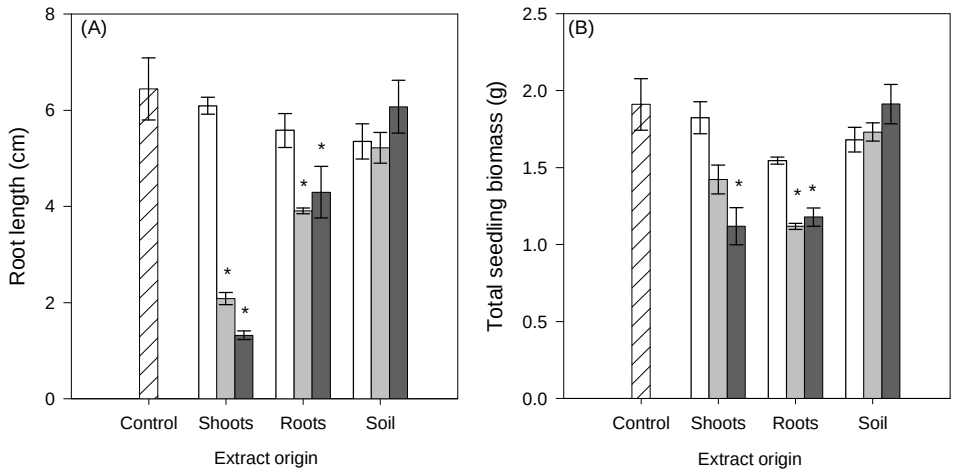


Fig. 5.2 Mean (± SE) maximum root length (A) and total biomass (B) of *Jacobaea vulgaris* seedlings growing on glass beads inoculated with demineralised water (control, dashed bar), or with low (white bars), medium (gray bars) or high (black bars) concentrations of shoot, root or soil extracts. Significant differences ($P < 0.05$) from the control treatment are indicated with an asterisk.

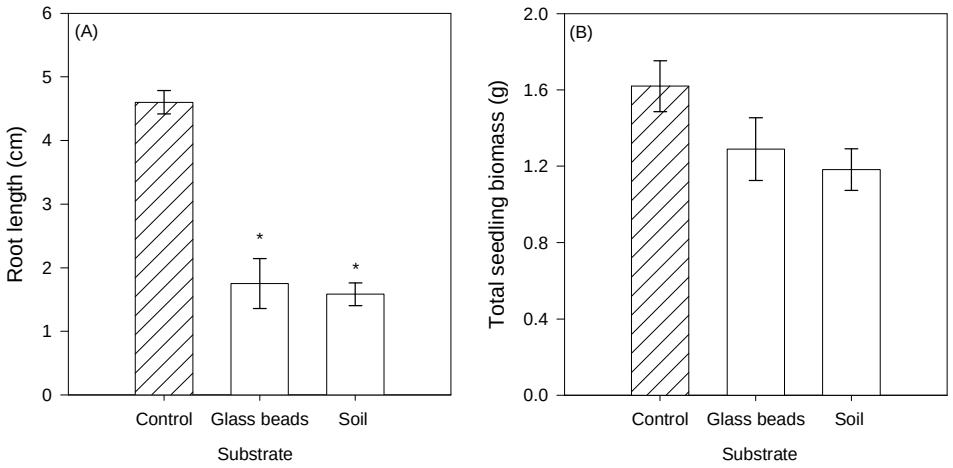


Fig. 5.3 Mean (± SE) maximum root length (A) and total biomass (B) of *Jacobaea vulgaris* seedlings growing on glass beads without root fragments (control), and of seedlings growing on glass beads or sterilized soil with root fragments incorporated. Significant differences ($P < 0.05$) with the control treatment are indicated with an asterisk.

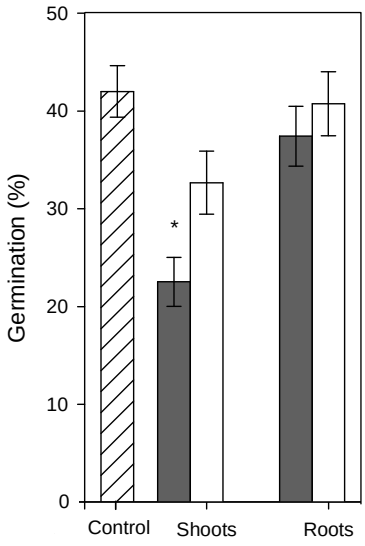


Fig. 5.4 Mean (± SE) percentage germination of seeds that received demineralized water (control, dashed bar), or of seeds that received low (white bars) or high (black bars) strength leaf or root extracts. Significant differences ($P < 0.05$) with the control treatment are indicated with an asterisk.

Autotoxic effects in sterilized soil: In sterilized soil, there were no significant autotoxic effect of root or shoot extracts on root length or biomass (RL: $F_{4,14} = 0.72$, $P = 0.59$; BM: $F_{4,14} = 0.68$, $P = 0.61$).

Autotoxic effect of root fragments: In both treatments with root fragments, maximum root length was significantly reduced as compared to the control ($F_{2,13} = 46.85$, $P < 0.01$; Fig. 5.3). Moreover, root fragment addition resulted in shorter and thicker roots than in the control treatment (TFJ van de Voorde, personal observation). For the seedlings exposed to root fragments, root length did not differ between seedlings growing in soil or on glass beads (Fig. 5.3).Total biomass was not significantly influenced by addition of root fragments ($F_{2,13} = 1.88$, $P = 0.19$).

Autotoxic effects on germination: High strength shoot extracts reduced germination significantly ($F_{4,12} = 3.23$, $P = 0.05$; Fig. 5.4). The reduction was 53% as compared to the control, pointing at strong autotoxic effects of shoot extracts on germination. Germination in the other treatments did not significantly differ from the control treatment.

Discussion

In a series of laboratory experiments we tested the autotoxic potential of *J. vulgaris.* Our results show that *J. vulgaris* can be autotoxic, but that the substrate in which the plant grows, the type of plant extract used, and the extract concentration are important determinants of the autotoxic effect. We will discuss the possible consequences of autotoxicity for *J. vulgaris* performance under greenhouse conditions and in the field.

In our study, extracts made from *J. vulgaris* tissues reduced germination and seedling growth. Seedling performance was not different when seedlings received the acidified control solution or extra nutrients, which strongly reduces the possibility that side effects, such as acidity or nutrient deficiency, may have caused the observed growth reducing capacity of the plant extracts when applied to the seedlings. Three conclusions can be drawn from these results. First, extracts made from shoots inhibit germination and root growth more than root extracts. Ahmed and Wardle (1995), who studied the allelopathic effects of *J. vulgaris* on other species, also found that extracts from shoots had the strongest allelopathic effects on other pasture species, and this seems quite a general observation in studies on allelochemical effects (Rice 1984; Lipinska and Harkot 2007). Second, the autotoxic effects of *J. vulgaris* are dosage dependent, being strongest for the most concentrated extracts. This is in line with studies on allelopathic effects of other plant species (Chon and Kim 2002; Tawaha and Turk 2003; Chon *et al.* 2005; Dorning and Cipollini 2006).

Third, extracts do not exhibit autotoxic effects when applied to soil. Seedling growth on glass beads was considerably reduced when the seedlings were exposed to plant extracts, but this was not the case when seedlings were growing in sterilized soil. The absence of growth reduction in soil could be because the chemical compounds that cause the autotoxic effect were adsorped to the soil particles, which reduces their mobility and buffers their negative effect (Krogmeier and Bremner 1989; Wardle *et al*. 1989). This is in line with our observation that addition of extracts made from soil did not reduce seedling performance. Another explanation is that nutrient availability was higher in soil than in glass beads, however, this is unlikely, because we corrected for nutrient differences by adding Hoagland solution. Moreover, biomass in the root fragment addition experiment in wells filled with soil was comparable to that in wells filled with glass beads. Other studies have shown that increasing nutrient availability can reduce the negative effects of allelopathy (Rice 1984; Inderjit and Weiner 2001). In contrast to our study, Ahmed and Wardle (1995) found relatively strong effects of extracts from soil in which *J. vulgaris* had been growing on other plant species. However, they provided these soil extracts more regularly and for a longer period of time than we did and this could explain why they found growth inhibition. Alternatively, *J. vulgaris* could be less sensitive to soil extracts than the pasture species that were studied by Ahmed and Wardle (1995).

While our study shows that extracts from *J. vulgaris* tissues potentially exhibit autotoxic effects, it is unlikely that these autotoxic effects are the cause of growth reduction in soil where the plant has previously been grown. In our study, we used extracts from damaged plant tissues, which typically have stronger allelopathic effects than extracts made from intact tissues (Orr *et al*. 2005). In addition, we included concentrations that are higher than what would occur under natural conditions. Still we did not find autotoxic effects when these extracts were applied to seedlings growing in soil. Therefore, our results suggest that under field conditions, with probably much lower concentrations of allelochemicals, tissue extracts are not likely to have a strong effect on seedling performance. However, shoot extracts did reduce *J. vulgaris* germination and this could consequently affect *J. vulgaris* establishment and population dynamics on a longer time scale. However, whether leachate concentrations in the field, for example, due to rain, are strong enough to cause negative effects on germination should be tested under field conditions.

In contrast to the addition of extractions, when we incorporated entire root pieces into the soil, we found strong growth reduction effects on *J. vulgaris*. Roots of those seedlings were also much shorter and thicker than the roots from control seedlings (TFJ van de Voorde, personal observation). Altered root morphology is a commonly observed allelopathic effect (Chon and Kim 2002; Gatti *et al*. 2010). Eventually, these changes in morphology can limit nutrient uptake and reduce seedling performance and fitness (van der Putten *et al*. 1989). Therefore, in the field, such root deformations may have large consequences, especially when plants that co-occur with *J. vulgaris* are not affected by

presence of these root fragments. It is important to note that while it is possible that the growth effects that we observed after addition of root pieces are due to allelochemical effects, it is also possible that the effects were caused by micro-organisms that are present on or in the root fragments, which can also explain the morphological changes (van der Putten *et al*. 1989). Pathogens might be transferred via the root fragments. Alternatively, soil-microorganisms might have released or activated certain chemicals from the decomposing root fragments (Rice 1984; Blum and Shafer 1988; Inderjit and Nilsen 2003). These chemicals can have different autotoxic effects than the allelochemicals present in the sterile plant extracts. It is also possible that, as root fragments decompose slowly, the allelochemicals were released over a longer time period. Incorporating plant material into soil can also have indirect inhibitory effects by influencing, for example, nutrient mobilization, pH or microbial activity (Facelli and Pickett 1991). Future studies should focus on disentangling the mechanisms through which root fragments cause this strong reduction in growth of *J. vulgaris*.

In conclusion, our experiments show that *J. vulgaris* can be autotoxic under laboratory conditions. However, the effects of root and shoot extracts were dependent on the concentration applied, and autotoxicity was not observed when seedlings were growing in sterilized soil. In contrast, incorporation of root fragments into soil reduced *J. vulgaris* performance considerably, although the role of soil (micro-)organisms should be investigated in more detail. These results suggest that autotoxicity does not play an important role in the decline of *J. vulgaris* abundance in old-fields and growth reduction in greenhouse experiments. Future studies should address the role of autotoxicity in influencing population dynamics of *J. vulgaris* in the field and to what extend autotoxic effects of other co-occurring species affect the performance of this plant in the field.

Chapter 6

Comparing arbuscular mycorrhizal communities of individual plants in a grassland biodiversity experiment

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Abstract

Plants differ greatly in the soil organisms colonizing their roots. However, how soil organism assemblages of individual plant roots can be influenced by plant community properties remains poorly understood.

We determined the composition of arbuscular mycorrhizal fungi (AMF) in *Jacobaea vulgaris* plants, using terminal restriction fragment length polymorphism (T-RFLP). The plants were collected from an experimental field site with sown and unsown plant communities. Natural colonization was allowed for 10 yr in sown and unsown plots. Unsown plant communities were more diverse and spatially heterogeneous than sown ones.

Arbuscular mycorrhizal fungi diversity did not differ between sown and unsown plant communities, but there was higher AMF assemblage dissimilarity between individual plants in the unsown plant communities. When we grew *J. vulgaris* in field soil that was homogenized after collection in order to rule out spatial variation, no differences in AMF dissimilarity between sown and unsown plots were found.

Our study shows that experimental manipulation of plant communities in the field, and hence plant community assembly history, can influence the AMF communities of individual plants growing in those plant communities. This awareness is important when interpreting results from field surveys and experimental ecological studies in relation to plant–symbiont interactions.

Key words: Arbuscular mycorrhizal fungi (AMF), *Jacobaea vulgaris* / *Senecio jacobaea*, plant community composition, soil biodiversity, spatial heterogeneity, terminal restriction fragment length polymorphism (T-RFLP).

Introduction

Understanding the factors that lead to the enormous diversity in communities of soil biota and the impacts on ecosystem functioning is one of the major challenges in soil ecology (Fitter *et al.* 2005). A well studied group of soil organisms are the arbuscular mycorrhizal fungi (AMF; Glomeromycota), forming symbiotic associations with most terrestrial plant families. AMF have important functions in ecosystems, as they influence nutrient cycling, plant productivity and diversity (Van der Heijden *et al.* 1998a; Klironomos *et al.* 2000; Klironomos and Hart 2002; Smith and Read 2008). Less is known about the factors that determine the composition of the AMF community that colonizes plant roots (Johnson *et al.* 2004; Börstler *et al.* 2006). Clearly, AMF propagules in the soil are important sources for the AMF community inside roots, but propagule composition does not necessarily reflect the community composition of AMF that colonize plant roots (Clapp *et al.* 1995; Merryweather and Fitter 1998; Rodríguez-Echeverría *et al.* 2008). Also, host plants can be

associated with very distinct mycorrhizal taxa (Vandenkoornhuyse *et al.* 2003; Gollotte *et al.* 2004; Scheublin *et al.* 2004). Recently, Hausmann and Hawkes (2009) showed that the identity of surrounding plants can influence the composition of AMF in a focal plant. That study was performed in pots under glasshouse conditions. In the field, soil organism assemblages of individual plant roots could also be influenced by additional properties, such as the assembly history and heterogeneity of the plant community. The new question addressed in the present study is how assembly history and heterogeneity of the plant community may affect the AMF community colonizing roots of individual plants.

The AMF spore community within the soil can be influenced by soil characteristics, such as disturbance history, soil type and chemistry (Helgason *et al.* 1998; Egerton- Warburton *et al.* 2007; Fitzsimons *et al.* 2008). Other studies have shown that the composition of neighbouring plant species can affect AMF communities (Johnson *et al.* 1992; 2004; Mummey *et al.* 2005; Öpik *et al.* 2006). Individual plant species can show preferences for specific AMF taxa (Scheublin *et al.* 2004), so that changes in plant community composition may alter the relative proportion of AMF propagules in the soil, leading to shifts in AMF assemblages (Bever *et al.* 1996; Burrows and Pfleger 2002). Teasing apart the relative effects of these environmental influences on AMF community composition in plant roots would require experimental conditions where single factors are varied one by one. However, the number of such examples is limited, as in field studies where the plant community has been experimentally changed, other environmental parameters often co-vary or *vice versa* (Vandenkoornhuyse *et al.* 2003; Börstler *et al.* 2006; Egerton-Warburton *et al.* 2007). As a consequence, the importance of individual parameters as determinants of AMF community composition in roots remains poorly understood.

We analysed the AMF community composition in individual *Jacobaea vulgaris* L. (synonym *Senecio jacobaea*; Pelser *et al.* 2006) plants growing in plots with different assembly histories, using terminal restriction fragment length polymorphism (T-RFLP, Liu *et al.* 1997; Mummey and Rillig 2007) and cloning and sequencing. In early secondary successional stages on relatively dry sandy soils, *J. vulgaris* is a characteristic plant species (Bezemer *et al.* 2006a). We collected *J. vulgaris* plants from a long-term field experiment initiated in 1996 on ex-arable land. In this field, half the plots were sown with mid-succession grassland plant species, whereas the other plots were left to become colonized naturally (van der Putten *et al.* 2000). Natural colonization was allowed in all plots, but the sowing treatment has resulted in a plant community composition that diverged from unsown plant communities (Fukami *et al.* 2005), and sown plant communities possessed higher temporal stability and lower diversity than unsown plant communities (Bezemer and van der Putten 2007). One year after starting the experiment, *J. vulgaris* established spontaneously in all plots (Bezemer *et al.* 2006a). This enables us to analyse the influence of plant community composition and assembly history on AMF community composition using a single plant species that has been present for almost a decade in both the sown and unsown plant communities.

Here, we test the effects of plant community assembly history and spatial heterogeneity on the AMF assemblages colonizing the roots of individual *J. vulgaris* plants from sown and unsown plant communities 10 yr after their establishment. Our hypothesis was that individual plants growing in the two types of plant communities (sown and unsown) will have different AMF communities. In addition to our field study approach, we conducted a glasshouse bioassay in which we grew *J. vulgaris* plants in homogenized soils collected from the field plots. Homogenization of the field soil enabled us to reduce spatial variation that may have influenced the AMF community composition in the plants collected directly from the field. We hypothesized that in the glasshouse the community composition of AMF in individual plants grown in homogenized field soil would not differ between the two treatments. In this comparison, as glasshouse conditions can yield different AMF assemblages in plant roots, we considered the full AMF community composition as well as that part of the community that overlapped with the community observed in the field plants.

Materials and Methods

Study plant

Tansy or common ragwort, *Jacobaea vulgaris* ssp. *vulgaris* (synonym *Senecio jacobaea* L.; Pelser *et al.* 2006), is a monocarpic perennial weed (Asteraceae) that spends its first year as a rosette. Flowering may take place in the second year, but is often delayed because of herbivory (van der Meijden and van der Waals-Kooi 1979). *J. vulgaris* is an early successional plant species native to the Netherlands and Europe, but invasive in other continents. In the Netherlands, it is considered a problem weed in abandoned arable fields that are used for nature restoration (Bezemer *et al.* 2006a), because the plant contains pyrrolizidine alkaloids that are toxic for livestock (Cameron 1935).

Field experiment

To study the influence of the surrounding plant community and biotic conditions on the AMF composition within individual *J. vulgaris* plants, we collected plants from experimental grassland field plots that differed in plant community composition. The experimental field is located near Ede, the Netherlands (52°04′ N, 05°45′E), in a nature restoration area on arable land, which was abandoned in 1996. In 1996, the 0.5 ha field experiment was set up by ploughing and sowing 0 or 15 mid-successional grassland species in plots of 10 x 10 m. There are five replicate plots for both treatments, arranged in five blocks. After sowing, plots were left to be colonized by plant species from the seed bank and the surrounding area. Once a year, at the end of the growing season, above-ground biomass was removed from all plots (see van der Putten *et al.* 2000 and Bezemer *et al.* 2006a for further details). The plant community characteristics of the plots sown with 15 species (sown) and naturally colonized (unsown) treatments differed significantly and consistently over the

years (Fukami *et al.* 2005; Bezemer and van der Putten 2007; Lepš *et al.* 2007). *J. vulgaris* was not sown, but since 1997 this species has been present in varying densities in all plots (Bezemer *et al.* 2006a).

Plant community

In July 2006, 10 yr after establishing the experiment, in all 10 plots we recorded the cover of all plant species, including *J. vulgaris*, in 12 permanent quadrats of 1 x 1 m. Plant productivity was estimated by clipping all above-ground biomass at 2 cm above the soil surface for an area of 0.25 x 0.25 m adjacent to each permanent quadrat. Above-ground biomass was dried at 70 °C and weighed. Shoots of *J. vulgaris* were dried and weighed separately, and values of total and *J. vulgaris* above-ground biomass per m^2 were calculated. In all 10 plots, we also measured the plant height of 10 randomly chosen flowering *J. vulgaris* plants. Vegetation recordings in the 12 permanent quadrats were used to calculate mean species richness per m^2, Shannon diversity (H') and spatial heterogeneity for each plot. Spatial heterogeneity of the plant community was determined by calculating the dissimilarity (based on Bray–Curtis distance) among the 12 vegetation surveys per plot. Heterogeneity calculations were performed using Poptools version 3.06 (Hood 2008) in Excel.

Soil chemistry

In July 2006, from each plot we randomly collected 24 soil samples (2.5 cm diameter and 15 cm depth). The 24 samples from each plot were homogenized and sieved (< 0.5 cm). A subsample was dried for 3 d at 40 °C. In this subsample, pH, plant available P and K were analysed in 1:10 (w / v) 0.01 M $CaCl_2$. Concentrations of available NH_4^+-N and NO_3^-)-N were determined colorimetrically in the $CaCl_2$-extract using a Traacs 800 autoanalyzer (TechniCon Systems Inc.).

Experimental J. vulgaris *plants*

In July 2006, two flowering *J. vulgaris* plants were randomly selected in each plot. All plants were located at least 2 m apart. Plants were removed together with the soil underneath them (15 x 15 x 15 cm) and transported to the laboratory in individual plastic bags. Plant height was recorded, all adhering soil and roots of other plants were removed from the soil, and individual *J. vulgaris* roots were rinsed with tap water to remove adhering soil. Approximately 100 mg of fine root material was then collected, frozen immediately at -80 °C and used for DNA extraction (Supporting Information, Methods S6.1). Shoots were oven-dried at 70 °C and weighed.

Glasshouse bioassay

Soil collection In July 2006, field soil was gathered by randomly collecting 24 soil cores from each plot (2.5 cm diameter, 15 cm deep). Soil samples from each plot were homogenized to omit spatial variation present in the field and sieved (< 0.5 cm). To obtain

sterilized soil, adjacent to the experimental field site soil from a depth of 5 – 20 cm was collected, sieved (< 0.5 cm), homogenized and gamma-sterilized (> 25 kGray gamma irradiation; Isotron, Ede, the Netherlands). The dry weight of each soil sample was determined gravimetrically (24 h at 105 °C). *J. vulgaris* plants were grown in a 7:1 mixture (on a dry weight basis) of sterilized soil and field soil inoculum. Control plants were grown in a 7:1 mixture of sterilized soil and autoclaved field inoculum (autoclaved on three consecutive days, 20 min at 121 °C) from sown and unsown plots of each block combined.

Growing conditions

Plants were grown in pots of 0.9 L with 1.16 kg soil mixture (on a dry weight basis). There were three pots used for each field plot and those data were averaged. Therefore, we had two treatments (sown and unsown) x five replicate field plots x three pots per field plot + five pots with sterile soil serving as a control = 35 pots.

In order to carry out the experiment, seeds of *J. vulgaris* plants growing in the area adjacent to the experimental field site were surface-sterilized (30 s in 0.1% chloride solution), rinsed and germinated on glass beads. In each pot, three 1-wk-old seedlings were planted. Seedlings that died during the first week of the experiment were replaced. Pots were placed randomly in the glasshouse with 70% relative air humidity, temperatures of 21 °C (day) and 16 °C (night), and a 16:8 h day:night light cycle. Natural daylight was supplemented by metal halide lamps (225 µmol s^{-1} m^{-2} photosynthetically active radiation, one lamp per 1.5 m^2). Plants were watered every other day and initial soil moisture content (17% soil mass) was reset twice a week by watering to the original weight.

After 8 weeks, all plants were harvested. Above-ground and below-ground plant material was separated for each pot, roots were rinsed with tap water and 0.5 g of fresh root material was stored in 50% EtOH at 4 °C to determine mycorrhizal colonization. Also, *c.* 100 mg of fine root material was collected and frozen immediately at -80 °C. For two of the three replicates this was used for DNA extraction (Methods S6.1). The remaining plant material was then oven-dried at 70 °C and weighed.

AMF composition in J. vulgaris *roots*

To determine the AMF colonization of *J. vulgaris* bioassay plants, roots from the bioassay plants were cleared for 1 h in 2.5% KOH at 90 °C in a water bath, rinsed with water and left overnight in 1% HCl. Thereafter, roots were stained for 30 min at 60 °C with 1% Parker Ink solution, destained and stored in lactic acid:glycerol:water (14:1:1) solution. Percentage mycorrhizal colonization was scored using gridline intersection with 100 intersections (McGonigle *et al.* 1990).

Molecular characterization of AMF communities

The AMF community composition in roots of *J. vulgaris* was determined by T-RFLP analyses of the FLR3/FLR4 fragments of the LSU rRNA gene (Liu *et al.* 1997; Gollotte *et al.*

2004; for AMF specificity see Mummey and Rillig 2007; Krüger *et al.* 2009). This method involves dual end-labeling of PCR amplicons and enzyme digestion of these fragments with the restriction enzymes AluI, MboI and TaqI. Multiple enzymes were chosen to improve the discrimination of T-RFLP and these three enzymes have been used successfully in AMF T-RFLP analyses before (Mummey and Rillig 2007). Digestion with restriction endonucleases yielded terminal restriction fragments (TRF) of different sizes, caused by sequence variation. The fragments are electrophoretically separated according to size and their presence ⁄ absence is scored. To identify the dominant community members, clone libraries were constructed and sequenced. Twelve clone libraries from the AMF-specific PCR amplicons were prepared for the root samples from the two plants from the sown and unsown plots of the experimental field blocks 2, 4 and 5, using the pGEM-T vector (Promega, Leiden, the Netherlands) and Escherichia coli JM109 High Efficiency Competent cells (Promega). Twelve to 30 clones per library (i.e. plant root system) were randomly selected for sequencing with the SP6 and T7 vector primers. Electropherograms of 143 successfully sequenced clones were checked in Chromas (version 1.45, Technelysium, Australia), before the sequences were compared against those in the public databases by BLASTN searches (http://www.ncbi.nlm.nih.gov; Altschul *et al.* 1997). All nonredundant sequences were deposited in Gen-Bank under the accession numbers FJ820857–FJ820960 (Fig S6.1). A more detailed description of the molecular analysis is given in the Supporting Information (Methods S6.1).

Statistical analyses

All data were analysed using univariate (GenStat version 11.1; VSN International Ltd, Hempstead, UK) or multivariate statistics (CANOCO version 4.55; Ter Braak and Šmilauer 2002). Plot characteristics, biomass, bioassay data and number of TRFs were analysed using linear mixed models (residual maximum likelihood, REML) with treatment (sown or unsown) as fixed factor and block as random factor. For the bioassay, data from the three pots with soil from the same plot were averaged before univariate analyses. Compositions of AMF and plant community were analysed using multivariate analyses. Detrended correspondence analysis (DCA) was used to determine whether linear (principal components analysis (PCA), redundancy analysis (RDA)) or unimodal (correspondence analysis (CA), canonical correspondence analysis (CCA)) analyses were most appropriate for multivariate analyses (Lepš and Šmilauer 2003). Significances in multivariate analyses were inferred by Monte Carlo permutation tests (999 permutations). Plant community composition (log (n + 1) transformed) in sown and unsown plots was compared using multivariate linear unconstrained (PCA) and linear constrained analyses (RDA).

Terminal restriction fragment incidence data of all enzyme–dye combinations together were analysed using unimodal multivariate analyses. Statistical analyses and results for the separate enzymes and dyes are given in the Supporting Information (Tables S6.1 and S6.2). Unimodal constrained analyses (CCA) were used to test for differences between mycorrhizal communities in sown and unsown plots, and to compare mycorrhizal

communities originating from bioassay and field. For both glasshouse and field conditions, the two plants within each plot were analysed as split plots (not permuted) within each whole plot (field plot; permuted freely). There was one missing value, as the AMF community of one of the plants could not be successfully fingerprinted with the MboI enzyme. This plant was not included in the analyses in which all enzymes were combined.

To determine the dissimilarity in AMF communities between the two plants originating from the same plot (or growing in the same soil in the bioassay), we submitted the data, field and glasshouse samples separately to a unimodal unconstrained analysis (CA) and calculated the Euclidian distance between the two samples, based on the first three axes. The effect of sowing treatment on AMF community dissimilarity was then analysed using linear mixed models (REML). Finally, variance partitioning (Lepš and Šmilauer 2003) was carried out using CANOCO to determine if AMF community composition of the field plants could be significantly explained by characteristics of the plant community, *J. vulgaris*, or soil chemistry.

Results

Field experiment

Plant community composition in 2006 differed significantly (RDA; F = 4.951, P = 0.003, 38% explained variation) between sown and unsown plots (Fig. 6.1). Nevertheless, during the 10 yr following establishment, in plots that were not sown, annually 91 ± 2% (mean ± SE) of the plant cover was made up by species that were also found in the sown plots (individual plant species cover data not shown). Plant community heterogeneity Shannon diversity and species richness were all significantly higher in unsown than in sown plots, whereas aboveground productivity was significantly higher in sown plots (Table 6.1). In 2006, soil chemistry and the number of *J. vulgaris* individuals, above-ground *J. vulgaris* biomass per m^2 and abundance did not differ between sown and unsown plots (Table 6.1).

AMF communities of field plants

Above-ground biomass of the sampled *J. vulgaris* plants did not differ significantly between the two types of plant communities (sown, 3.53 ± 0.76 g per plant; unsown, 3.18 ± 0.49 g per plant; $F_{1,4}$ = 0.15, P = 0.70). For all enzyme–dye combinations the number of TRFs did not differ significantly between plants growing in sown and unsown plots (Table S6.1). There was also no significant difference in the composition of TRFs between plants from sown and unsown plots (CCA, F = 0.67, P = 0.89; see Table S6.2 for individual enzyme–dye combinations). Of the TRFs found, 97% were found both in plants originating from the sown and the unsown plots; 3% were found only in plants from the unsown plots. However, the AMF communities of the two individual plants originating from the same plot were three times more dissimilar in unsown than in sown plots ($F_{1,4}$ = 22.94, P = 0.008; Fig. 6.2A) and there was a positive relationship between plant community heterogeneity

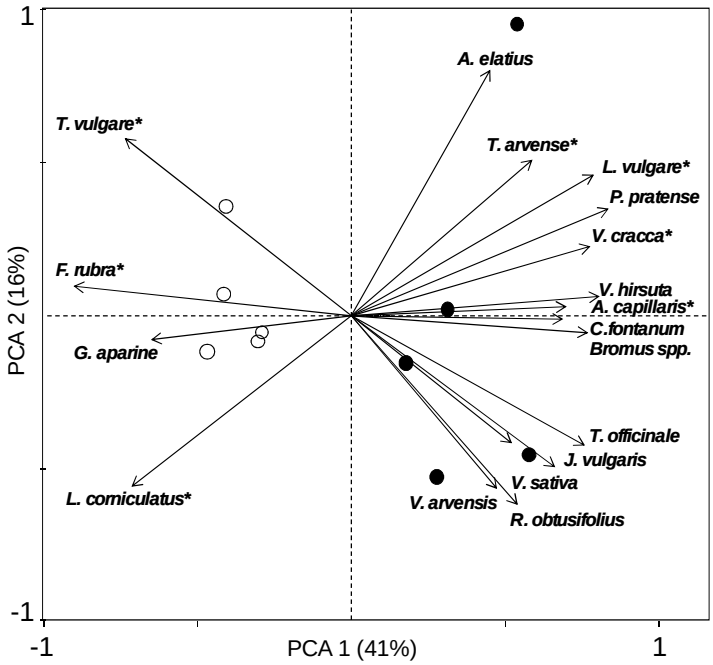


Fig. 6.1 Principal components analysis (PCA) of species scores of the 18 plant species with the highest scores present in the plant communities and PCA sample scores of sown (open) and unsown (closed) field plots. Amounts of explained variation by the first two PCA axes are given in parentheses. Species names are: *Agrostis capillaris, Arrhenatherum elatius, Cerastium fontanum, Festuca rubra, Galium aparine, Jacobaea vulgaris, Leucanthemum vulgare, Lotus corniculatus, Phleum pratense, Rumex obtusifolius, Taraxacum officinale, Tanacetum vulgare, Trifolium arvense, Veronica arvensis, Vicia cracca, Vicia hirsuta* and *Vicia sativa*. An asterisk (*) indicates species that were sown at the start of the field experiment in 1996.

and AMF dissimilarity ($F_{1,7}$ = 14.02, *P* = 0.007, R^2 = 0.67). Variance partitioning showed that AMF community composition could not be explained significantly by plant community characteristics (F = 1.07, *P* = 0.32), *J. vulgaris* field measurements (F = 1.24, *P* = 0.11) or soil chemistry (F = 1.12, *P* = 0.28). Analysis of partial LSU rDNA sequences from clone libraries containing 143 AMF clones of the field-collected plants demonstrated the specificity of the used PCR amplicons and revealed eight different clades (Schüßler *et al.* 2001; Fig. S6.1). All plants revealed sequences from multiple clades (Table S6.3).

Bioassay

In the glasshouse, plants grown in soil from sown plots had significantly more below-ground biomass than plants grown in soil from unsown plots (Table S6.4). The percentage

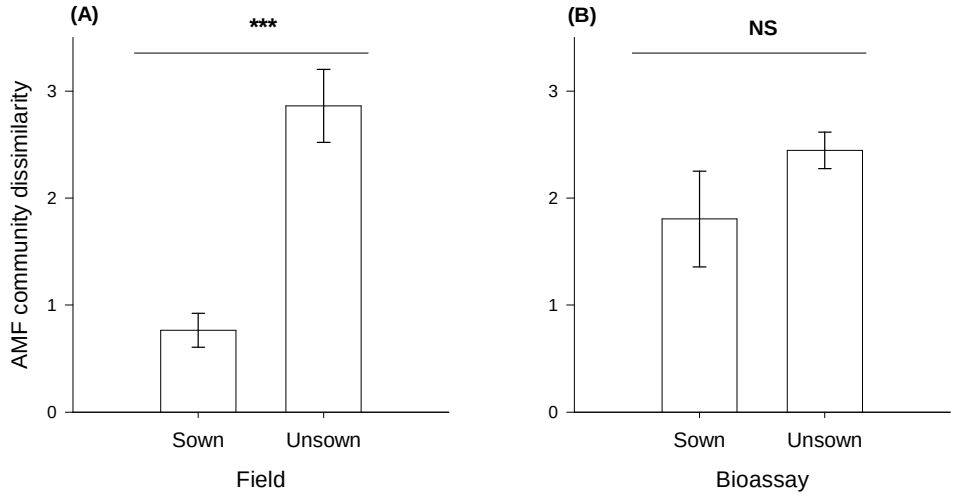


Fig. 6.2 Average dissimilarity (Euclidean distance) between the arbuscular mycorrhizal fungal (AMF) communities of two plants originating from the same field plot (a) or growing in soil obtained from the same field plot (b). Means (±SE; n = 5) are shown. ***, Significant difference between sown and unsown plots at $P < 0.01$ based on linear mixed models (residual maximum likelihood, REML), with treatment as fixed factor and block as random factor; ns, no significant difference.

Table 6.1 Effect of sowing treatment on plant community, chemical and *Jacobaea vulgaris* characteristics in 2006. Means (± SE) are shown for sown and unsown plots (n = 5) and results of mixed model (REML) analyses, with treatment as a fixed, and block as a random factor.

Measurement	Sown	Unsown	$F_{1,4}$	***P***
Plant community				
Spatial heterogeneity of plant community	0.37 ± 0.04	0.56 ± 0.02	14.80	0.02
Species richness (m^2)	10.8 ± 0.75	14.0 ± 0.77	10.35	0.03
Aboveground productivity (g m^{-2})	563 ± 80.6	298 ± 53.3	28.66	0.01
Diversity (Shannon H)	1.32 ± 0.04	1.87 ± 0.12	21.66	0.01
Jacobaea vulgaris				
Abundance (%)	0.65 ± 0.30	3.95 ± 1.28	6.72	0.06
Aboveground biomass (g m^{-2})	5.80 ± 3.96	21.4 ± 6.79	2.48	0.19
Number of plants per plot	43.4 ± 14.4	318 ± 109	5.36	0.08
Height (cm)	59.4 ± 3.69	62.8 ± 1.39	0.52	0.51
Soil chemistry				
P (mg kg^{-1})	4.77 ± 0.37	4.01 ± 0.33	3.90	0.12
K (mg kg^{-1})	42.3 ± 5.65	60.5 ± 5.16	8.31	0.05
NO_3 (mg kg^{-1})	1.57 ± 0.50	1.56 ± 0.12	0.00	0.99
NH_4 (mg kg^{-1})	0.17 ± 0.08.	1.17 ± 0.78	1.66	0.27
pH $CaCl_2$	5.19 ± 0.07	5.26 ± 0.03	0.75	0.44

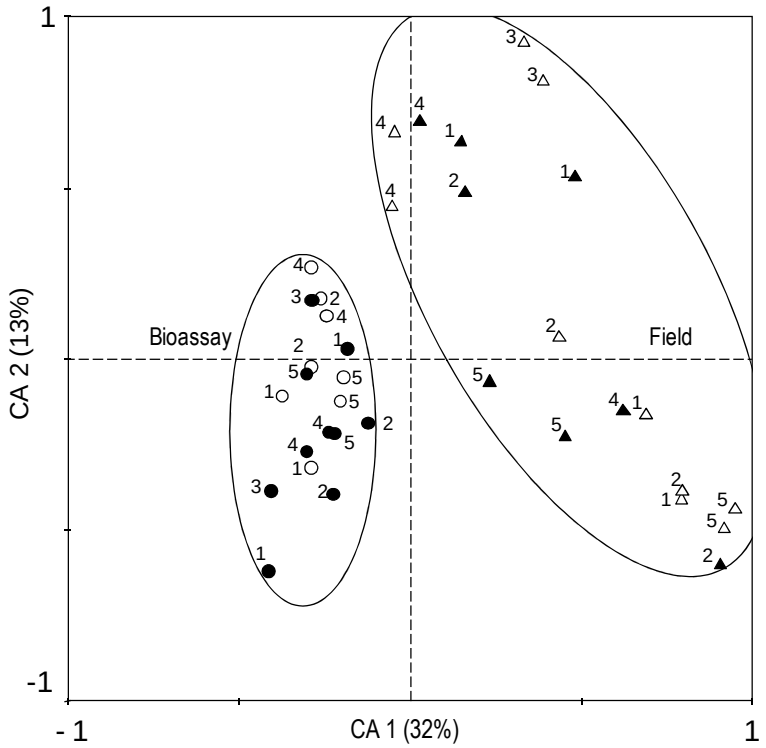


Fig. 6.3 Unconstrained unimodal canonical analysis (CA) of the arbuscular mycorrhizal fungal (AMF) community composition in *Jacobaea vulgaris* plants from the field (triangles) and from the glasshouse bioassay (circles). Open symbols, AMF communities from plants from the sown field plots; closed symbols, unsown plots. Symbols with the same number originate from the same field plot or were grown in soil from the same field plot. AMF communities in plants from the field differ significantly from those of bioassay plants (canonical correspondence analysis (CCA), F = 7.17, *P* = 0.001). Percentages of total explained variation by CA axes are given in parentheses.

AMF colonization did not differ between the two sowing treatments, while plants in sterilized soil remained almost completely devoid of AMF (Table S6.4). The average number of TRFs for the different enzyme–dye combinations also did not differ significantly between the two treatments (Table S6.1). Root samples of plants from sterilized soil did not yield any TRFs (data not shown). As was also observed in the plant roots collected directly from the field, the composition of TRFs in the bioassay plants was not significantly influenced by sowing treatment (CCA, F = 1.16, *P* = 0.12; Table S6.2). Of the TRFs found, 98% were found in both plants originating from the sown and unsown plots, and 2% were found only in plants from the unsown plots. However, in contrast to the field situation, the AMF assemblages of the two replicate plants per plot were not more dissimilar in soil from

the unsown plots than in soil from the sown field plots ($F_{1,4}$ = 3.22, P = 0.16; Fig. 6.2B). The AMF community composition of field and bioassay plants differed significantly (CCA, F = 7.17, P = 0.001; Fig. 6.3, Table S6.5). Moreover, there was considerably more variation in AMF composition between individual plants from the field than between plants from the bioassay, which can be seen by a more scattered distribution of the field samples than of the bioassay samples in the CA ordination (Fig. 6.3). Interestingly, 70% of all TRFs that were detected were present in plants from both experiments. When the similarity analyses were limited to those TRFs that were present in both field and bioassay plants, the pattern remained the same (field AMF dissimilarity, sown 0.86 ± 0.28, unsown 2.47 ± 0.50, $F_{1,4}$ = 10.11, P = 0.03; bioassay AMF dissimilarity, sown 1.77 ± 0.52, unsown 1.70 ± 0.36, $F_{1,4}$ = 0.014, P = 0.93).

Discussion

We studied the effects of plant community assembly history on AMF assemblages of individual *J. vulgaris* plants using experimental field plots. We examined AMF in plant roots that were directly collected from the field, as well as in roots of plants grown in homogenized field soil under glasshouse conditions. Despite the differences in plant community composition and assembly history of sown and unsown plant communities, we did not detect a difference in AMF community composition and richness in *J. vulgaris* plants of the sown and unsown plots, either in roots directly collected from the field or in roots of plants grown in the glasshouse. It is important to note that we used a qualitative method to determine AMF community composition. Hence, although we found no difference in the presence of TRFs, we cannot exclude the possibility that there were differences in the relative abundance of the TRFs between the treatments. Interestingly, in plant roots collected directly from the field, the average dissimilarity in AMF community was higher in the unsown than in the sown plant communities. The unsown plots had the most spatially heterogeneous plant community. Moreover, the plant communities in these unsown plots had the lowest temporal stability, because over time they had the highest rates of extinction and colonization of species and the strongest fluctuation in productivity (Bezemer and van der Putten 2007).

A controlled glasshouse bioassay with homogenized soil collected from the sown and unsown plots enabled us to study the composition of AMF communities in *J. vulgaris* roots under conditions without spatial variation. AMF diversity (number of TRFs) was higher in the bioassay plants than in plants collected from the field. This could be because bioassay plants were grown in homogenized soil, in which soil properties (*e.g.* mycorrhizal inocula) were homogeneously present throughout the soil. Moreover, the bioassay plants were still in the rosette stage, whereas the field plants were flowering. Studies on other plant species have shown that the development stage of the host plant can alter the AMF community (Šmilauer 2001; Husband *et al.* 2002). AMF communities in the roots of

bioassay plants differed significantly from AMF communities in roots of field plants, as has been reported in other studies (Sýkorová *et al.* 2007). This could be a result of the shorter growing period of the bioassay plants, or of the soil homogenization itself. Soil disturbance can favour colonization by fast-growing AMF (r-strategists) as discussed by Sýkorová *et al.* (2007). However, in our study, 70% of all TRFs that were detected were present in plants from both experiments. Since the field plants were growing in relatively old, and not recently disturbed plots, this suggests that the TRFs that make up this 70% do not resemble fast colonizing AMF taxa. Analyses based exclusively on the TRFs shared by the field and the glasshouse bioassay plants showed patterns that were similar to what we found for analyses with all TRFs present in the field or glasshouse. This indicates that the difference in dissimilarity found between field and glasshouse plants is not caused solely by the presence of different TRFs resulting from changes in environmental conditions. Our results therefore suggest that the sowing treatment caused differences in the spatial heterogeneity of the plant community and that this, in turn, has led to increased dissimilarity of AMF communities of individual plants growing in those communities. The positive relationship between spatial heterogeneity of the plant community and the dissimilarity among the AMF communities of two plant individuals supports this view.

Alternatively, it is possible that other external factors, for example, differences in resource availability or AMF control by fungal grazers, or plant pathogen pressure may have enhanced spatial variation of AMF community composition between individual plants in the field. As pot experiments with a number of other plant species have pointed at direct plant neighbour effects on the AMF community composition (Hausmann and Hawkes 2009), it is likely that our results also apply to plant species other than *J. vulgaris*.

The sowing treatment resulted in plant communities with largely the same plant species, but differing in plant community characteristics such as diversity, heterogeneity and stability (Bezemer and van der Putten 2007). The sown plant communities were more homogeneous and stable than the unsown plant communities (Bezemer and van der Putten 2007). The cause of these differences is unknown. However, what we have now shown is that individual plants from unsown communities, which are more heterogeneous and unstable, harbour much more dissimilar mycorrhizal assemblages than plants from sown communities. The observed relationship between instability and dissimilarity may be either causal or consequential, something that should be determined in further studies. Designing experiments that can separate cause from consequence will be challenging. The observed positive relationship between mycorrhizal community diversity and plant community diversity (van der Heijden *et al.* 1998b) suggests that increased spatial heterogeneity in plant–AMF interactions, as we observed, can ultimately influence diversity and functioning of ecosystems.

Symbiotic interactions with AMF can buffer plants against abiotic changes or disturbances (Smith and Read 2008). Moreover, plant–mycorrhizal interactions have been proposed to increase the potential for redundancy of plant species and to weaken the relationship

between plant diversity and ecosystem functioning (Johnson *et al.* 1996; Loreau *et al.* 2001). Our study does not provide direct evidence for such a feedback loop, but strongly suggests that in the further unraveling of the relationships between plant diversity and root symbionts, the effects of plant community characteristics, such as heterogeneity and stability, can play a profound role.

We detected up to 26 TRFs per enzyme–dye combination in T-RFLP analyses. TRFs that were present in less than three samples were excluded from further analyses and we were interested in heterogeneity rather than AMF identity; however, this number could be influenced slightly as a result of sequence heterogeneity within a single individual (Sanders *et al.* 1995; Rosendahl and Stukenbrock 2004). The AMF community of individual plants varied greatly between and within different plant communities. The diversity of TRFs and sequences found within one individual plant suggests that plant–AMF interaction studies carried out with only a limited set of AMF strains should be interpreted with caution, particularly since the effects of AMF on plant growth can differ greatly between AMF species and strains (Johnson *et al.* 1997; Klironomos 2003; Koch *et al.* 2006). Earlier microcosm and macrocosm studies have pointed out the importance of AMF community composition for plant performance, plant community composition and ecosystem functioning (van der Heijden *et al.* 1998b). Most likely, this is not a one-way interaction. In nature, AMF community composition and functioning are also influenced by the plant community dynamics, so that plant and soil community composition are tightly intertwined, as was proposed in the 'driver ⁄ passenger' hypothesis (Hart *et al.* 2001).

In conclusion, our results show that, in a long-term field experiment, AMF communities in plant roots were more dissimilar when collected from sown *versus* non-sown plant communities. As a major difference is that the non-sown plant communities were the most heterogeneous and the least stable over time, our results suggest that these factors may contribute to AMF dissimilarity among root systems of individual field plants. Reduced AMF dissimilarity among plants grown in homogenized field soil further supports this suggestion. The awareness that experimental manipulation of plant communities in the field, and hence plant community assembly history, can influence the AMF communities of individual plants growing in those plant communities is important when interpreting results from field surveys and experimental ecological studies in relation to plant–symbiont interactions.

Acknowledgements

We thank Tanja Bakx-Schotman, Agata Pijl and Wiecher Smant for technical assistance.

Supporting Information

Methods S6.1

Molecular characterization of AMF communities

DNA extraction: Frozen root samples of approximately 100 mg fresh weight were ground in liquid nitrogen, using micro pestles. Total DNA was extracted with the DNeasy® Plant Mini Kit (Qiagen, Venlo, the Netherlands) according to the manufacturer's protocol. DNA quantity and quality were assessed using a NanoDrop® ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Delaware, USA) and agarose gel electrophoresis.

PCR and enzymatic restriction: The 5'-end of the LSU rRNA genes of AMF was amplified using a nested PCR approach with the universal fungal primer pair LR1/FLR2 (Trouvelot *et al.* 1999; Van Tuinen *et al.* 1998) and the AMF-specific labeled primer pair FAM-FLR3/NED-FLR4 (Gollotte *et al.* 2004). The first PCR reaction contained 13.75 µl milli-Q water, 2.5 µl PCR buffer with $MgCl_2$ (10 µM), 2.5 µl of 10 µM primer LR1 and FLR2 each, 2.5 µl dNTP's (10 µM), 0.125 µl BLOTTO (10% w/v fat-free milk powder), 0.125 µl Taq polymerase (5U μl^{-1}, HotStart Taq Plus, Qiagen) and 1 µl total DNA extract. For PCR amplification PTC-200 DNA Engine Thermal Cycler PCR machines (MJ Research, MA, USA) were used with the following settings: 5 min at 95 ^{o}C, 35 cycles of 30 s at 94 ^{o}C, 40 s at 58 ^{o}C and 70 s at 72 ^{o}C, followed by 7 min at 72 ^{o}C before cooling. The second PCR reaction contained 16.875 µl milli-Q water, 2.5 µl PCR buffer with $MgCl_2$ (10 µM), 0.18 µl primer FAM-FLR3 (10 µM), 0.5 µl primer NED-FLR4 (10 µM), 0.32 µl primer FLR3 (10 µM), 2.5 µl dNTP's (10µM), 0.125 µl BLOTTO, 0.125 µl Taq polymerase and of 1 µl 1:100 times diluted PCR product from the first reaction. Thermal cycling conditions were: 5 min at 95 ^{o}C, 25 cycles of 30 s at 94 ^{o}C, 40 s at 54 ^{o}C and 60 s at 72 ^{o}C, followed by 15 min at 72 ^{o}C before cooling. PCR product presence and quality were verified on 1.5% agarose gels prior to restriction digestion.

T-RFLP analyses: Three restriction enzymes, AluI, MboI, and TaqI (New England Biolabs, Ipswich, MA, USA), were used to digest dual end-labeled DNA amplicons. A mixture containing 3.6 µl ddH_2O, 1 µl buffer, 0.1 µl Bovine Serume Albumin, 5 µl PCR product and 0.3 µl restriction enzyme was incubated at 37 ^{o}C (AluI and MboI) or at 65 ^{o}C (TaqI) for 3 hrs, after which enzymes were inactivated by heating to 94 ^{o}C for 3 min.

Restriction products were sodium acetate/ethanol-purified, using glycogen as co-precipitant, according to Beckman Coulter's protocol (A-2035A, 2005). After purification the pellets were dried at 37 ^{o}C for 30 min and resuspended in 10 µl Milli-Q water by vortexing shortly and moderate shaking for 1 hr. 7 µl HiDi Formamide (Applied Biosystems, Foster City, Ca, USA) with 1:600 diluted internal size standard (GeneScan™-500 LIZ, Applied Biosystems) were mixed with 2 µl of the 1:12 diluted purified digestion product. DNA fragments were denaturated for 3 min at 95 ^{o}C and shock cooled in icy water.

Fragment length polymorphism analysis was performed on an automated 3130 Genetic Analyzer sequencer (Applied Biosystems) with POP-7™ polymer (Applied Biosystems), injection time of 16 s, the dye set G5, injection at run temperature of 60°C, and voltage of 1.2 kV. TRF sizing was carried out in GeneMapper Software 4.0 (Applied Biosystems) from where the raw data with minimal relative fluorescence intensity (rfu) of one in the size range of 40-480 bp was exported. Samples which were over- (highest peak > 10,000 rfu) or under loaded (highest peak < 400 rfu) were re-run with a different concentration.

T-RFLP data analyses: The program T-REX (Culman *et al.* 2009) was used to process the raw T -RFLP data. Background noise was statistically subtracted from the TRF signals by applying a standard deviation multiplier of 4 to the peak heights. Peaks were aligned to TRFs among the samples by applying a clustering threshold of 0.5 bp and allowing multiple peaks in one TRFs. TRFs present in less than three samples were excluded from all further analyses.

Clone libraries: Twelve clone libraries from the AMF-specific PCR amplicons were prepared for the root samples from the two plants from the sown and unsown plots of the experimental field blocks 2, 4 and 5, using the pGEM-T vector (Promega, Leiden, the Netherlands) and *Escherichia coli* JM109 High Efficiency Competent cells (Promega). Twelve to 30 clones per library (i.e. plant root system) were randomly selected for sequencing with the SP6 and T7 vector primers. Electropherograms of 143 successfully sequenced clones were checked in Chromas (vers. 1.45; McCarthy, 1996-1998), before the sequences were compared against those in the public databases by BLASTN searches (http://www.ncbi.nlm.nih.gov; Altschul *et al.* 1997). All non-redundant sequences were deposited in GenBank under the accession numbers FJ820857 - FJ820960.

Phylogenetic analyses: The sequences were aligned with reference sequences of AMF isolates in Clustal X (version 1.83, Thompson *et al.* 1997). MacClade (version 4.08, Sinauer Associates, Sunderland, MA, USA) was used for manual adjustments and exclusion of ambiguously aligned regions. The most appropriate sequence evolutionary model was determined in ModelTest (version 3.7, Posada and Crandall 1998) and used for constructing a distance neighbor joining tree (10,000 bootstraps) in Paup*4b10 (Swofford 2003) and to make Bayesian phylogenetic inferences (3,000,000 generations) in MrBayes (version 3.1.2, Ronquist and Huelsenbeck 2003). A maximum likelihood phylogenetic analysis was performed with PhyML 2.4.4 (5,000 bootstrap replicates) (Guindon and Gascuel 2003). Tree topologies were compared for consistency.

Table S6.1 Mean (± SE) (a) number of AMF specific TRFs found in individual plants growing in sown and unsown plots, and (b) Euclidean distance between the AMF community compositions of the two plants originating from one plot. Shown are results per enzyme (AluI, MboI and TaqI) and dye (FAM and NED) in *Jacobaea vulgaris* roots. Results of linear mixed models (REML) ($F_{1,10}$ and *P*) for field and bioassay plants are shown, with treatment (sown or unsown) as fixed and block as random factor.

(a) number	Assay	Sown	Unsown	F	***P***
Field	AluI – FAM	9.2 ± 0.8	9.8 ± 0.9	0.93	0.50
	AluI – NED	5.4 ± 0.6	6.5 ± 0.6	2.18	0.14
	MboI – FAM	16.0 ± 1.2	14.2 ± 1.8	1.08	0.43
	MboI – NED	22.9 ± 1.3	17.9 ± 1.9	1.20	0.38
	TaqI – FAM	11.2 ± 1.6	13.2 ± 1.2	3.35	0.05
	TaqI – NED	9.6 ± 1.7	12.2 ± 1.5	1.69	0.22
Bioassay	AluI – FAM	15.0 ± 1.1	12.4 ± 1.4	1.19	0.38
	AluI – NED	7.9 ± 0.5	7.3 ± 0.8	0.64	0.68
	MboI – FAM	23.1 ± 1.1	24.9 ± 1.4	1.01	0.95
	MboI – NED	23.4 ± 1.4	26.5 ± 1.5	0.73	0.62
	TaqI – FAM	19.2 ± 1.5	20.5 ± 1.3	3.18	0.06
	TaqI – NED	20.5 ± 1.4	23.4 ± 1.1	6.77	0.01

(b) distance	Assay	Sown	Unsown	F	***P***
Field	AluI – FAM	1.14 ± 0.45	1.98 ± 0.25	2.18	0.16
	AluI – NED	0.90 ± 0.30	2.67 ± 0.43	9.37	0.04
	MboI – FAM	0.83 ± 0.21	2.92 ± 0.58	7.01	0.08
	MboI – NED	1.41 ± 0.35	2.95 ± 0.41	10.43	0.05
	TaqI – FAM	1.16 ± 0.21	2.07 ± 0.53	2.23	0.21
	TaqI – NED	1.90 ± 0.39	2.25 ± 0.52	1.65	0.30
Bioassay	AluI – FAM	1.53 ± 0.47	2.47 ± 0.21	5.07	0.09
	AluI – NED	1.65 ± 0.43	2.84 ± 0.07	9.01	0.04
	MboI – FAM	2.03 ± 0.48	2.81 ± 0.12	1.89	0.26
	MboI – NED	1.62 ± 0.37	2.45 ± 0.42	0.96	0.40
	TaqI – FAM	1.85 ± 0.32	2.55 ± 0.65	1.82	0.24
	TaqI – NED	2.12 ± 0.68	2.21 ± 0.40	0.01	0.93

Table S6.2 Results of Monte Carlo permutation tests (999 permutations) on canonical correspondence analyses (CCA) analyses comparing AMF community composition in *Jacobaea vulgaris* plants growing in sown and unsown plots in the field, or in soil collected from sown and unsown plots in a greenhouse bioassay. The two plants per plot were analysed as split-plots within each whole plot (field plot).

	Field		Bioassay	
	F	P	F	***P***
AluI – FAM	0.37	0.99	1.93	0.08
AluI – NED	0.52	0.85	1.88	0.12
MboI – FAM	0.52	0.91	1.23	0.28
MboI – NED	0.57	0.93	1.40	0.85
TaqI – FAM	1.08	0.66	1.09	0.49
TaqI – NED	1.41	0.28	1.95	0.14

Table S6.3 Overview of (a) the clades (Fig. S6.1) from which sequences were present in the investigated plants (A or B) from the three (2, 4 or 5) sown and unsown plots, and (b) the number of TRFs found for the different enzyme/dye combinations for these plants. Presence of a sequence of the specified clade in the plant is indicated with 'x'.

(a)		Clade 1	Clade 2	Clade 3	Clade 4	Clade 5	Clade 6	Clade 7	Clade 8
Sown	2A		x		x	x		x	
	2B		x		x	x			
	4A			x				x	
	4B	x	x		x		x		
	5A		x		x				
	5B		x		x				
Unsown	2A	x	x		x		x	x	x
	2B		x		x		x	x	
	4A	x	x		x		x		x
	4B	x			x	x			
	5B		x		x			x	x

(b)		AluI – FAM	AluI – NED	MboI – FAM	MboI – NED	TaqI – FAM	TaqI – NED
Sown	2A	5	3	18	21	12	8
	2B	9	6	18	21	9	6
	4A	10	5	20	24	17	18
	4B	14	9	19	20	19	18
	5A	9	7	11	20	7	4
	5B	10	6	20	28	9	7
Unsown	2A	8	7	15	18	15	14
	2B	11	7	18	19	12	6
	4A	12	5	17	24	15	18
	4B	3	4	2	4	4	4
	5B	10	9	9	18	13	12

Table S6.4 The effect of soil treatment (inoculation), in the bioassay, on above- and belowground biomass (g pot^{-1}), and AMF colonization (%) of *Jacobaea vulgaris*. Shown are means (± SE) and results of a linear mixed model (REML) with treatment (sown or unsown) as fixed, and block as random factor. Soil was collected from sown and unsown field plots, and control soil consisted of a sterilized (gamma irradiated) mixture of sown and unsown soil. Within rows, means followed by the same letter are not significantly different.

Measurement	Sown	Unsown	Control	F	***P***	df
Aboveground DW	2.03 ± 0.06a	1.96 ± 0.08a	2.24 ± 0.13a	1.27	0.36	10
Belowground DW	5.80 ± 0.22a	4.57 ± 0.38a	5.64 ± 0.26a	1.15	0.42	10
AMF colonisation	42.0 ± 9.9a	39.3 ± 2.4a	7.7 ± 0.5b	8.81	0.01	8

Table S6.5 Results of Monte Carlo permutation tests (999 permutations) on canonical correspondence analyses (CCA) analyses comparing AMF community composition in *Jacobaea vulgaris* plants from the field and bioassay. The two plants per plot were analysed as split-plots within each whole plot (field plot).

	F	***P***
AluI – FAM	5.403	0.001
AluI – NED	7.435	0.001
MboI – FAM	5.668	0.001
MboI – NED	10.728	0.001
TaqI – FAM	5.860	0.001
TaqI – NED	10.114	0.001

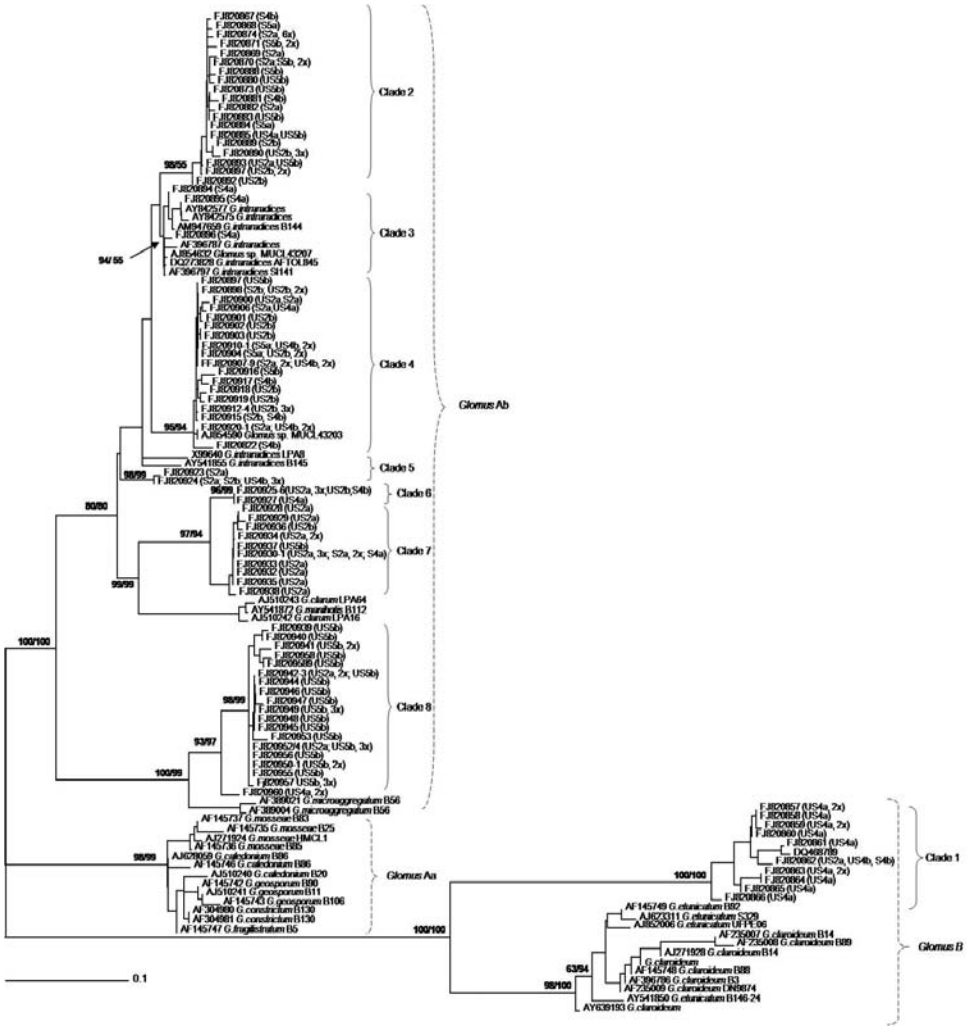


Fig. S6.1 Phylogram derived of partial 28S rDNA sequences (259 aligned sites, Model test-AIC: GTR+G, 10.000 bootstrap pseudoreplications). Sequences were generated from *Jacobaea vulgaris* root DNA root extracts, using a nested PCR approach with the primer pairs LR1/FLR2 and FLR3-FLR4. Sequences are given with accession number (GenBank), and plant origin (number indicates field block), sowing origin (US is unsown and S is sown plots) and the number of times a particular sequence was found in the same clone library (2x – 6x). Reference sequences were obtained from public sequence databases and are given with their accession numbers, species name and, when present, clone identity (B = BEG). Branch topology wee derived from neighbor joining (NJ), NJ and PHYML-analyses support values of the resolved phylogroups are given at nodes (NJ/PHYML). Taxonomic clades were numbered and their affiliation to the Glomus groups as defined by Schüßler *et al.* (2001) is indicated within dashed brackets. User-defined clades, based on support values are indicated within solid brackets.

Chapter 7

Discussion and synthesis

Tess F. J. van de Voorde

The main aim of this thesis was to investigate the importance of individual plant-soil interactions in a plant community. I used *Jacobaea vulgaris* ssp. *vulgaris* (synonym *Senecio jacobaea*), which is an early successional plant species native to the Netherlands. I studied the importance of plant-soil interactions for the performance and population dynamics of *J. vulgaris.* I studied both the interactions between *J. vulgaris* individuals and between *J. vulgaris* and co-occurring plant species. I also studied other processes that can influence the performance and dynamics of *J. vulgaris* during different stages of succession in old-fields in the Netherlands. An overview of these processes and their relative importance during old-field succession is given in Fig. 7.1. Based on the results of Chapter 2 - 6, the importance of the processes in Fig. 7.1 is shown for two stages of old-field succession: before *J. vulgaris* has reached peak abundance ('before peak abundance') and from the moment *J. vulgaris* reaches peak abundance and onwards ('after peak abundance').

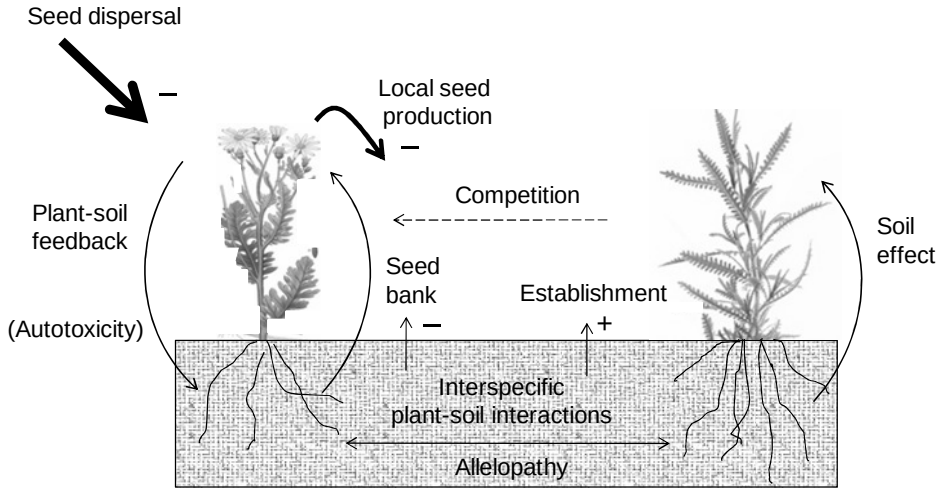
In addition, I examined the effect of the surrounding plant community on plant-symbiont interactions of individual *J. vulgaris* plants. In this chapter I will discuss and synthesize the main findings of my thesis research and propose some directions for future research and implications for management.

Jacobaea vulgaris in old-fields

In ten old-fields I studied the population dynamics of *J. vulgaris*. These ten fields form a chronosequence, ranging from 2 to 25 years, of agricultural land abandonment (Chapter 2). *J. vulgaris* cover peaked about 5 years after land abandonment, hereafter cover declined again. Such a boom-burst pattern is common for early successional plant species (Olff and Bakker 1991; Meiners *et al.* 2009). The fields of the chronosequence show some field-specific differences, which can be due to differences in agricultural practices, such as crop growing and fertilization schemes, differences in soil physical conditions or differences in the degree of isolation of the fields and the composition of the local species pool (Kardol 2007).

Overall, *J. vulgaris* cover in the chronosequence fields corresponded largely with cover measured over time at the long-term experimental field site (Chapter 2). The similarity between the chronosequence and the experimental field site justifies the use of soil samples collected from the chronosequence fields to disentangle the processes underlying the temporal pattern in *J. vulgaris* cover (*e.g.* Johnson and Miyanishi 2008).

Before *J. vulgaris* peak cover



After *J. vulgaris* peak cover

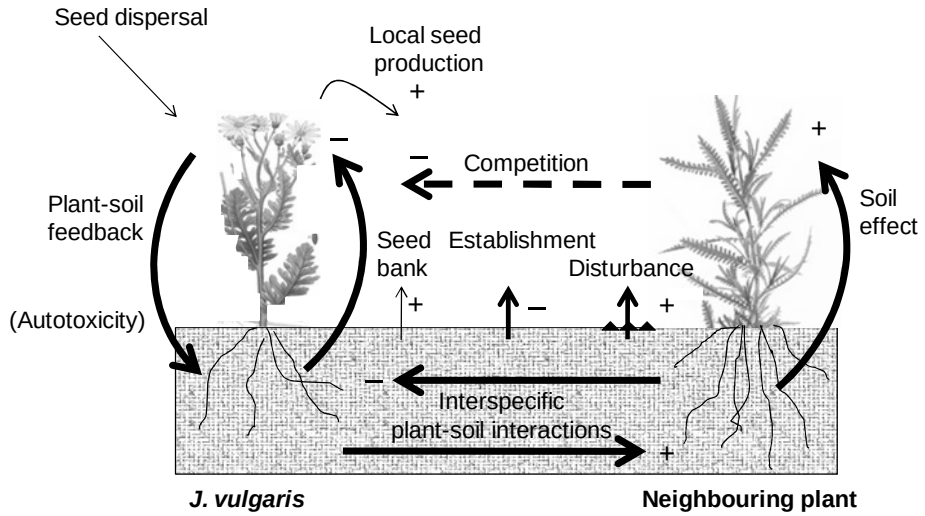


Fig. 7.1 Overview of the studied interactions that determine the performance and population dynamics of *Jacobaea vulgaris.* The importance of the processes is shown 'before peak cover' and 'after peak cover'. Arrows indicate the processes. The thickness of the arrow indicates the importance of the process. Dashed arrows indicate indirect effects. A positive effect on *J. vulgaris* is indicated by (+) and a negative effect on *J. vulgaris* by (-).

Seedling recruitment and J. vulgaris *dynamics*

The population size and development of a plant species is largely determined by its seed production, the availability and dispersal of seeds, and by subsequent germination and establishment rates (Chapin *et al.* 1994; Anderson 2007). These characteristics can be affected by soil conditions and by the plant community (van der Heijden *et al.* 2004; Wardle *et al.* 2004). The results of Chapter 2 showed that these processes are not equally important during old-field succession (Fig. 7.1). The percentage germination was generally high for seeds from all fields and did not differ with time since abandonment, suggesting that germination does not restrict *J. vulgaris* populations. However, seed availability in the soil seed bank differed considerably between fields. In the youngest fields, the number of seeds was low, probably due to the low number of *J. vulgaris* plants in the field. At this stage population development may be limited by seed dispersal. Earlier work at old fields in the same area has shown that plant community development during the initial stages following land abandonment is mainly dispersal limited, and that colonization greatly depends on what is available in the local species pool (Kardol *et al.* 2008; 2009).

Seed availability in older fields was not limiting, as the number of seedlings that emerged from the seed bank during regular disturbance of the soil was much higher than the actual number of *J. vulgaris* plants in the field. *J. vulgaris* seeds only germinate when they are close to the surface. Therefore, disturbances are needed to bring seeds closer to the surface and improve their germination chance (van der Meijden and van der Waals-Kooi 1979). This suggests that in older fields not the availability of viable propagules (*e.g.* Tilman 1997, Ozinga *et al.* 2005), but the absence of disturbances was limiting plant abundance (van der Meijden and van der Waals-Kooi 1979). In addition, based on a turf sowing experiment, seedling establishment was strongly limited in the fields where *J. vulgaris* had already reached its peak abundance (Chapter 2). This was presumably due to the lower availability of bare ground and subsequent competition by neighbouring plants (Fayolle *et al.* 2009; Chapter 2). These observations correspond with the work in Dutch dunes by Prins and Nell (1990). They showed that herbivory by rabbits indirectly stimulated *J. vulgaris* germination and growth. The gaps create opportunities for *J. vulgaris* to establish and in these gaps development is not limited by surrounding vegetation.

The finding that in older fields successful establishment is limited by the absence of bare ground and by subsequent competition with the surrounding plant community implies that interspecific competition between neighbouring plants and *J. vulgaris* becomes stronger and more important when time since abandonment proceeds. Subsequently, the creation of disturbed, open gaps that can be colonized becomes more important with time since abandonment (Fig. 7.1).

Plant-soil interactions and J. vulgaris *plant performance*

Plants can affect the performance of other individuals of the same species via their effect on the soil by plant-soil feedback (Bever *et al.* 1997). In Chapter 2 I tested the hypothesis that the decline in *J. vulgaris* cover with time since abandonment would coincide with the development of a negative plant-soil feedback. Indeed, *J. vulgaris* performance was strongly reduced in soil that was conditioned by *J. vulgaris*, but the growth reduction was neither related to time since abandonment nor to *J. vulgaris* cover in the field. Opposite to what we had expected, in soils from all stages of the chronosequence a strong negative soil feedback to *J. vulgaris* developed and the buildup of the net soil effect occurred already in a period shorter than a growth season. Hence, the establishment of a strong negative soil effect is typical for *J. vulgaris* and less depending on successional stage. We had expected that increased *J. vulgaris* abundance would have caused a legacy effect in the soil leading to greater soil feedback, which we supposed to strongly reduce the performance of subsequently establishing *J. vulgaris* plants. Indeed, the level of potential control by the soil community was positively related to *J. vulgaris* density in the field (Chapter 2). This suggests that in fields with a higher density of *J. vulgaris* plants, newly establishing *J. vulgaris* plants are more likely to encounter soil that is already conditioned by *J. vulgaris*. The development of such a negative feedback by an early successional species can accelerate the replacement of this species by later successional species (Kardol *et al.* 2006). In addition, a negative plant-soil feedback can enhance the performance of neighbouring plant species, by reducing the competitive ability of *J. vulgaris* (Bever *et al.* 1997; Bever 2003; Fig. 7.1).

In old-fields interspecific plant-soil interactions with neighbouring plants may also play a significant role (Fig. 7.1). Indeed, the results of Chapter 3 show that plants, via their legacy effect on the abiotic and, especially, the biotic component of the soil can create priority effects due to facilitation or inhibition of the growth of other plant species, including later successional ones. These interspecific plant-soil interactions between *J. vulgaris* and neighbouring species were not equal in strength. Overall, the effect of neighbouring species on *J. vulgaris* was more growth limiting than the reciprocal effect of *J. vulgaris* on the neighbouring species. As a consequence of this asymmetric relationship the successional replacement of *J. vulgaris* could be enhanced. Thus, these results on interspecific plant-soil interactions show that it is important to study plant-soil interactions in a community context and to include neighbouring species as they can also change the performance of the studied species. Similar to what we found for successional replacement, interspecific plant-soil interactions and priority effects may also play an important role in other contexts, such as exotic invasions.

In Chapter 3, I calculated a weighted feedback effect of the plant community directly surrounding a *J. vulgaris* plant. These data showed that with time since abandonment species that can cope well with soil conditioned by *J. vulgaris* increased. As far as I am aware, no other study has calculated a weighted community feedback effect. There are

some unanswered issues concerning calculating such a community feedback: Is aboveground relative cover a proper measure for belowground presence, and is this belowground relative presence a measure for a species' effect on the community feedback effect? Further, on which spatial scale should such a community effect be calculated and how long does it take for the plant community to respond? The spatial scale at which soil organisms operate is not known but most likely rather small (Ettema and Wardle 2002). Moreover, is the soil effect created by a plant growing with conspecifics similar to the feedback effect of that species when it is growing with heterospecifics plants? Bezemer *et al.* (2010) reported that the plant community in which a plant is growing can determine the composition of its soil community. Validating a weighted community feedback with experiments at different spatial scales and with multiple species would help to answer these questions and will improve our knowledge of community feedback effects.

The main growth reducing agent(s) in the soil could be sieved out by a mesh size of 20 µm, as *J. vulgaris* performance in pots inoculated with the microbial fraction did not differ from the biomass produced in pots inoculated with the sterilized microbial suspension (Chapter 4). The microbial suspension did not contain micro-arthropods, nematodes, or mycorrhizal fungi, and other organisms larger than 20 µm, whereas it should contain soil bacteria and fungi (Ames *et al.* 1987; Klironomos *et al.* 1993; Bardgett 2005). In the bioassay studies (Chapter 2) plant-feeding nematodes were almost absent in *J. vulgaris* roots, which could be due to the negative effects of pyrrolizidine alkaloids on plant parasitic nematodes (Thoden *et al.* 2009ab). Hence, a strong negative effect of plant-feeding nematodes on *J. vulgaris* in the field is not to be expected.

Autotoxicity and *J. vulgaris* plant performance

Chemicals that plants release into the soil can affect the performance of individuals of other species, called allelopathy, or individuals of the same species, called autotoxicity (Bardgett *et al.* 1999, Kulmatiski *et al.* 2008; Berg and Smalla 2009; Fig. 7.1). The results of Chapter 5 showed that the substrate in which *J. vulgaris* plants were growing, the type of plant extract, and the extract concentration are important determinants of the autotoxic effect. Unlike seedlings growing in soil that received aqueous tissue extract, the growth of seedlings growing in soil mixed with root fragments was reduced. This process can be important in the field, for example, when seeds germinate in spots left by *J. vulgaris* plants that have died (McEvoy 1984). In addition, root morphology of the seedlings that grew in water with *J. vulgaris* root fragments had changed. These alterations can eventually limit seedling performance and fitness (van der Putten *et al.* 1989) and may reduce plant performance on the longer term. Although Ahmed and Wardle (1994) showed that allelochemicals excreted by *J. vulgaris* can have allelopathic effects against pasture species, in a later study Wardle *et al.* (1995) found no allelopathic inhibition on these species anymore. They propose that negative effects were cancelled out by the beneficial

effect of *J. vulgaris*, which is in accordance with the positive soil effect of *J. vulgaris* on co-occurring species in Chapter 3. Overall, only few autotoxic effects were found when seedlings were grown in soil, which suggests that autotoxicity does not play an important role in the decline of *J. vulgaris* abundance in old-fields and growth reduction in greenhouse experiments.

Symbiont interactions in a plant community

The composition of AMF that colonize plant roots is strongly determined by environmental conditions (Vandenkoornhuyse *et al.* 2003; Hawkes *et al*. 2006; Hausmann and Hawkes 2009; Dumbrell *et al.* 2010). I showed that plant community assembly history influences the AMF assemblage dissimilarity between individual plants in those communities (Chapter 6). The question how the surrounding plant community and its history can determine AMF communities colonizing plants has been raised recently by several other studies. These studies point at multiple mechanisms that may underlie the importance of assembly history for AMF communities (Chapter 6). In line with the results of Chapter 3, Hausmann and Hawkes, (2010) recently showed that priority effects can shape AMF communities. They showed that the order of establishment of the host plant and neighbouring plants can alter the composition of the AMF that colonize plant roots. Depending on the duration of these effects, this can have consequences for plant performance and dynamics (Hausmann and Hawkes 2010).

In Chapter 6 we used plant communities that established after initial sowing or not sowing. Sown plots were all sown with a mixture of 15 mid-successional plant species at the start of the field experiment and then subjected to natural plant community development. In contrast, the unsown plots were left bare to be colonized from the seed bank and the surrounding area (van der Putten *et al.* 2000). These sowing treatments led to long-term differences in diversity, stability and richness of the plant communities (Bezemer and van der Putten 2007). Fukami *et al.* (2005) showed that the treatments at the experimental field site diverged from each other after sowing, which was suggested to be due to assembly rules and priority effects. Differences in arrival time have been part of this process, which can change the AMF community composition colonizing plant roots (Hausmann and Hawkes 2010). Species arrival in the unsown plots was less controlled and could explain the higher AMF heterogeneity in these plots.

The identity of neighbouring plant species can affect AMF community composition colonizing the host plant (Mummey *et al.* 2005; Hausmann and Hawkes 2009). At the experimental field, 91% of the plant species was present in both sowing treatments suggesting that plant community composition per se is not driving the effect observed in Chapter 6. Community assembly can also change plant community diversity. Recently, Kivlin and Hawkes, (2010) showed that plant diversity can be an important driver of fungal communities as well. König *et al.* (2010) showed that AMF diversity was also related to

plant species richness, more so than to soil parameters. In Chapter 6, plant species richness was higher in unsown plots, however AMF diversity was not higher in these plots. This could be because *J. vulgaris* is difficult to be colonized by soil organisms, such as nematodes (Thoden *et al.* 2009ab). This may also be true for AMF, such that only a selected group of AMF may colonize *J. vulgaris*. However, *J. vulgaris* plants were highly colonized with AMF (Chapter 6), and AMF diversity as measured by T-RFLP was in the same range as reported for other species (*e.g.* Mummey *et al.* 2005). Although the exact mechanism causing the effect of community assembly history on AMF communities colonizing individual plants is not clear, our results show that it is important to include the plant community and its assembly history when studying plant-symbiont interactions.

Community assembly history can influence the AMF community colonizing individual plants, but the same process could also happen in the case of other soil organisms colonizing plant roots, such as plant-feeding nematodes or fungal pathogens. Combining the results of Chapter 6 with the results of Chapters 2-5 suggests that a feedback loop can occur if the plant community alters individual plant-soil organism interactions (Fig. 7.2, arrows a), which could in turn affect the performance and competitive strength of that plant and the

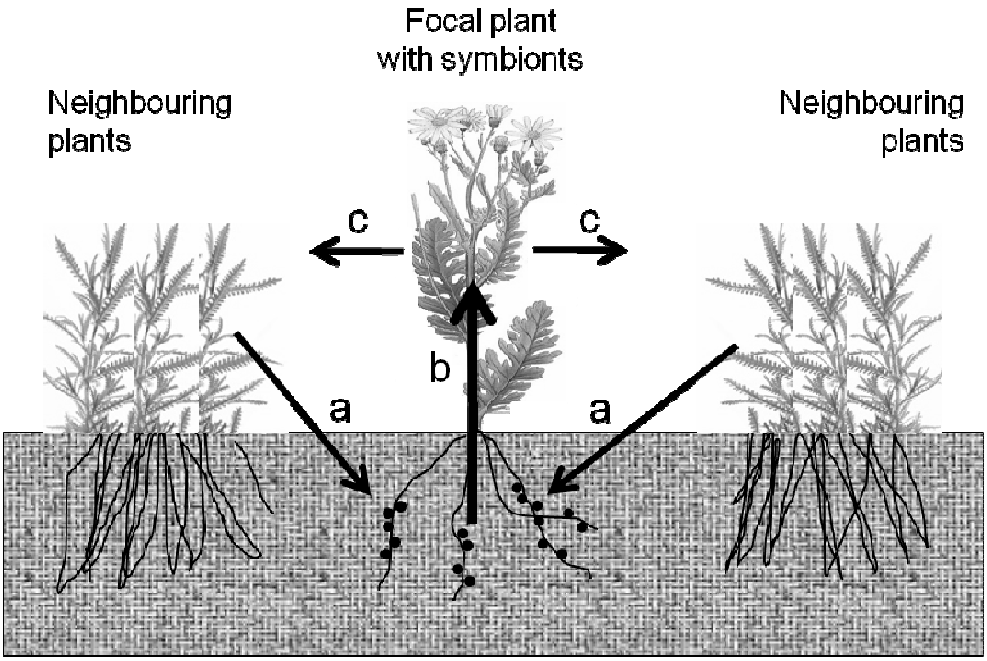


Fig. 7.2 Individual plant-soil interactions within a plant community. Plants in the community influence plant-symbiont interactions of the focal plant (a). The altered plant-symbiont interaction can change the performance of the focal plant (b). This can affect the competitive strength of that plant or abundance of that species (c), which in turn can alter plant community composition.

abundance of that plant species (Fig. 7.2, arrow b). This, in turn, can alter plant community composition and dynamics again (Fig. 7.2, arrows c; *e.g.* van der Heijden *et al.* 1998a). This effect on plant community composition could involve both aboveground and belowground processes, such as competition or plant-soil interactions respectively. These changes in diversity (Kivlin and Hawkes 2010; König *et al.* 2010), composition (Mummey *et al.* 2005), assembly history, and heterogeneity (Chapter 6) of the soil community may then subsequently affect the performance and plant-symbiont interactions of individual plants. Thus, my results provide further evidence that plant-soil biotic interactions need to be considered from a spatial perspective, also including the surrounding plant community.

Implications for management

The results of this thesis can also be used to improve management practices to control *J. vulgaris*. The management tools that should be used differ between different stages of *J. vulgaris* abundance (Table 7.1).

Immediately after ending agricultural practices or after the establishment of the pasture, there is ample bare soil available for *J. vulgaris* to colonize. In these early stages, the number of *J. vulgaris* plants in the field is still low as establishment is mainly limited by seed availability (Table 7.1: Seedling recruitment). Management in this phase should focus on preventing *J. vulgaris* seeds to reach the field, for example, from nearby *J. vulgaris* populations. This can be achieved by mowing *J. vulgaris* plants in the surrounding areas before they set seed. In addition, as long as local seed production is low, the soil seed bank will develop slowly. Removing, *J. vulgaris* plants in the field, by grazing or manual removal, will prevent the formation of a soil seed bank from which *J. vulgaris* plants can establish later on.

In the mean time, other plant species can establish and form a dense vegetation cover. The formation of a closed vegetation cover will make it difficult for *J. vulgaris* seeds to enter and become established. Creating a closed vegetation cover by sowing grassland species will also make it more difficult for *J. vulgaris* to establish and become dominant (Bezemer *et al.* 2006a). From the moment a closed vegetation cover has formed, it is important to avoid disturbances because they create new colonization opportunities for *J. vulgaris*. These disturbances can be, for example, due to overgrazing, herbivory, burrowing animals, or agricultural machines.

Once *J. vulgaris* plants have colonized the field they start producing seeds. Due to the local seed production seed availability in the field is not limiting anymore (Fig. 7.1 & Table 7.1: Plant-soil interactions). Because the soil is not yet conditioned by *J. vulgaris* and seed availability is not limiting anymore *J. vulgaris* performance and abundance can increase rapidly. However, this will only happen when other plants have not yet created a dense

vegetation cover, and when there is still space left to be colonized by *J. vulgaris*, *e.g.* bare ground. A consequence of the increase in *J. vulgaris* abundance is that more soil is conditioned by *J. vulgaris* and a negative plant-soil effect can build up (Table 7.1: Plant-soil interactions).

From the moment *J. vulgaris* plants have colonized the field management should make use of the negative soil effect, which can be formed in less than a growth season. This means that *J. vulgaris* plants (including roots and soil) should not be removed anymore, as this prevents the buildup of the negative soil effect. Instead, management should focus on the formation of the negative soil effect and on preventing seed set and dispersal towards nearby bare fields. This can be done by mowing or sheep grazing. Mowing and grazing do not disturb the creation of a negative soil effect in the field, as rosette plants can also create a negative soil feedback, while at the same moment allowing the creation of a negative soil effect. Again, herbivores should not trample the soil as this will create new colonization opportunities for *J. vulgaris*.

Table 7.1 Overview of the mechanisms that are important for the performance of *Jacobaea vulgaris* and their importance at the different stages of population dynamics: before peak abundance and after peak abundance. A negative effect on *J. vulgaris* performance or population size is represented by (-), a positive effect by (+), no effect by (o), and an unknown or unclear effect by (+/-). Management in each stage should focus on the processes that reduce *J. vulgaris* performance and abundance.

		Before peak abundance	After peak abundance
Seedling recruitment	Seed quality	+	+
	Seed availability	-	+
	Germination	+	+
	Establishment	+	-
	Dispersal	-	o
	Absence of disturbance	o	-
	Competition effect on establishment	o	-
Soil nutrients	Nutrient availability	+	+
Allelochemicals	Autotoxicity	o	o
	Allelopathy	o	+/-
Plant-soil interactions	Plant-soil feedback (***J. vulgaris***- ***J. vulgaris***)	o/-	-
	Plant-soil interactions (neighbours – ***J. vulgaris***)	o/-	-
	Plant-soil interactions (***J. vulgaris*** – neighbours)	o/-	-
	Competition effect on performance	o	-

Directions for future research

Here, I would like to point out a number of topics that I consider of great importance and that will assist in gaining a better understanding of individual plant-soil interactions in plant communities.

1. Study plant-soil-plant interactions

During my thesis research I studied different plant-soil interactions that *J. vulgaris* is exposed to and that are caused by individuals from the same and other species, *e.g.* (in) direct intra- and interspecific plant-soil interactions. In the field however, these plant-soil interactions co-occur simultaneously and interact with other plant-plant interactions, such as competition, facilitation and priority effects. In order to understand the relevance of the different plant-soil interactions and their combined importance for population dynamics in plant communities, these plant-soil and plant-plant interactions should be studied simultaneously.

2. Incorporate plant-soil interactions in population dynamics models

Plant populations can be modeled to enhance our understanding of their dynamics (Hoffmann 1999; Caswell 2001; Jongejans *et al.* 2006). Often these population models do not include plant-soil interactions, even though they can be important drivers of population dynamics. Including plant-soil interactions in plant population models could help to study the importance of plant-soil interactions for the population dynamics of *J. vulgaris* and other species.

3. Incorporate spatial dynamics in the field

Soil conditioning by *J. vulgaris* creates a negative soil effect (Chapter 2). One way for plants to escape from a negative soil effect is by occurring in shifting mosaics (Olff *et al.* 2000; Blomqvist *et al.* 2000), which is a feature that can be observed for *J. vulgaris* in the field (van der Meijden 1971; TFJ van de Voorde, personal observation). The occurrence in shifting mosaics could explain why *J. vulgaris* can become so abundant even though it experiences a strong negative plant-soil feedback. However, by using mixed field soil, I could not test the importance of these spatial dynamics. Including within-field variation will give more information about the dynamics of *J. vulgaris* and could help explain the observed field-specific differences (Chapter 2).

4. Autotoxicity and allelopathy under ecological conditions

Allelopathy and autotoxicity have been studied frequently, but often with an artificial experimental set up, such as artificial extraction techniques, substrates or an irrelevant species choice for ecology or agriculture. This artificiality makes it hard to translate the results of these experiments to the field. Investigating autotoxicity and allelopathy under field conditions can help us to learn more about the consequences of allelochemicals for plant performance and population dynamics in old-fields.

5. Incorporate disturbances

In the Veluwe region, wild boars, *Sus scrofa*, are abundant. They grub the soil to find food. While grubbing, they disturb the soil and plant community and create gaps in the vegetation. These gaps are perfect locations for *J. vulgaris* to colonize and establish (Chapter 2; van der Meijden and van der Waals-Kooi 1979), even in dense later-successional fields. The consequences and implications of wild boars on *J. vulgaris* abundance and dynamics in the Veluwe region requires more attention.

References

Aerts, R. & Chapin III, F. S. (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research,* 30, 1-67.

Ahmed, M. & Wardle, D. A. (1994) Allelopathic potential of vegetative and flowering ragwort (*Senecio jacobaea* L) plants against associated pasture species. *Plant and Soil,* 164, 61-68.

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J. H., Zhang, Z., Miller, W. & Lipman, D. J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research,* 25, 3389-3402.

Ames, R. N., Mihara, K. L. & Bethlenfalvay, G. J. (1987) The establishment of micro-organisms in vesicular arbuscular mycorrhizal and control treatments. *Biology and Fertillity of Soils,* 3, 217-223.

Anderson, K. J. (2007) Temporal patterns in rates of community change during succession. *American Naturalist,* 169, 780-793.

Armesto, J. J. & Pickett, S. T. A. (1986) Removal experiments to test mechanisms of plant succession in oldfields. *Vegetatio,* 66, 85-93.

Augspurger, C. K. (1983) Seed dispersal of the tropical tree, *Platypodium elegans*, and the escape of its seedlings from fungal pathogens. *Journal of Ecology,* 71, 759-771.

Ayres, E., Steltzer, H., Berg, S. & Wall, D. H. (2009) Soil biota accelerate decomposition in high-elevation forests by specializing in the breakdown of litter produced by the plant species above them. *Journal of Ecology,* 97, 901-912.

Baer, S. G., Kitchen, D. J., Blair, J. M. & Rice, C. W. (2002) Changes in ecosystem structure and function along a chronosequence of restored grasslands. *Ecological Applications,* 12, 1688-1701.

Bain, J. F. (1991) The biology of Canadian weeds 96: *Senecio jacobaea* L. *Canadian Journal of Plant Science,* 71, 127-140.

Bais, H. P., Vepachedu, R., Gilroy, S., Callaway, R. M. & Vivanco, J. M. (2003) Allelopathy and exotic plant invasion: From molecules and genes to species interactions. *Science,* 301, 1377-1380.

Bakker, J. P. & Berendse, F. (1999) Constraints in the restoration of ecological diversity in grassland and heathland communities. *Trends in Ecology & Evolution,* 14, 63-68.

Bakker, J. P., Grootjans, A. P., Hermy, M. & Poschlod, P. (2000) How to define targets for ecological restoration? - Introduction. *Applied Vegetation Science,* 3, 3-6.

Barbosa, P., Hines, J., Kaplan, I., Martinson, H., Szczepaniec, A. & Szendrei, Z. (2009) Associational resistance and associational susceptibility: Having right or wrong neighbors. *Annual Review of Ecology Evolution and Systematics,* 40, 1-20.

Bardgett, R. D. (2005) *The biology of soil: A community and ecosystem approach.* Oxford University Press, Oxford.

Bardgett, R. D., Bowman, W. D., Kaufmann, R. & Schmidt, S. K. (2005) A temporal approach to linking aboveground and belowground ecology. *Trends in Ecology & Evolution,* 20, 634-641.

Bardgett, R. D., Mawdsley, J. L., Edwards, S., Hobbs, P. J., Rodwell, J. S. & Davies, W. J. (1999) Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. *Functional Ecology,* 13, 650-660.

Bardgett, R. D. & Wardle, D. A. (2010) *Aboveground-belowground linkages: Biotic Interactions, ecosystem processes, and global change.* Oxford University Press.

Berendse, F. (1998) Effects of dominant plant species on soils during duccession in nutrient-poor ecosystems. *Biogeochemistry,* 42, 73-88.

Berg, G. & Smalla, K. (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *Fems Microbiology Ecology,* 68, 1-13.

Bever, J. D. (1994) Feedback between plants and their soil communities in an old field community. *Ecology,* 75, 1965-1977.

Bever, J. D. (2003) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytologist,* 157, 465-473.

Bever, J. D., Morton, J. B., Antonovics, J. & Schultz, P. A. (1996) Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. *Journal of Ecology,* 84, 71-82.

Bever, J. D., Westover, K. M. & Antonovics, J. (1997) Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology,* 85, 561-573.

Bezemer, T. M., Fountain, M. T., Barea, J. M., Christensen, S., Dekker, S. C., Duyts, H., van Hal, R., Harvey, J. A., Hedlund, K., Maraun, M., Mikola, J., Mladenov, A. G., Robin, C., de Ruiter, P. C., Scheu, S., Setälä, H., Šmilauer, P. & van der Putten, W. H. (2010) Divergent composition but similar function of soil food webs of individual plants: plant species and community effects. *Ecology,* 91, 3027-3036.

Bezemer, T. M., Harvey, J. A., Kowalchuk, G. A., Korpershoek, H. & van der Putten, W. H. (2006a) Interplay between *Senecio jacobaea* and plant, soil, and aboveground insect community composition. *Ecology,* 87, 2002-2013.

Bezemer, T. M., Lawson, C. S., Hedlund, K., Edwards, A. R., Brook, A. J., Igual, J. M., Mortimer, S. R. & van der Putten, W. H. (2006b) Plant species and functional group effects on abiotic and microbial soil properties and plant-soil feedback responses in two grasslands. *Journal of Ecology,* 94, 893-904.

Bezemer, T. M. & van der Putten, W. H. (2007) Ecology - Diversity and stability in plant communities. *Nature,* 446, E6-E7.

Blomqvist, M. M., Olff, H., Blaauw, M. B., Bongers, T. & van der Putten, W. H. (2000) Interactions between above- and belowground biota: importance for small-scale vegetation mosaics in a grassland ecosystem. *Oikos,* 90, 582-598.

Blum, U. & Shafer, S. R. (1988) Microbial population and phenolic acids in soil. *Soil Biology & Biochemistry,* 20, 793-800.

Blum, U., Shafer, S. R. & Lehman, M. E. (1999) Evidence for inhibitory allelopathic interactions involving phenolic acids in field soils: concepts vs. experimental model. *Critical Reviews in Plant Sciences* 18, 673-693.

Boekhoff, M. (2005) Jacobskruiskruid: Informatie over verspreiding, giftigheid en management.

Bonanomi, G., Rietkerk, M., Dekker, S. C. & Mazzoleni, S. (2005) Negative plant-soil feedback and positive species interaction in a herbaceous plant community. *Plant Ecology,* 181, 269-278.

Bonanomi, G., Rietkerk, M., Dekker, S. C. & Mazzoleni, S. (2008) Islands of fertility induce co-occurring negative and positive plant-soil feedbacks promoting coexistence. *Plant Ecology,* 197, 207-218.

Bongers, T. (1988) *De nematoden van Nederland.* Pirola, Schoorl.

Bonkowski, M., Villenave, C. & Griffiths, B. (2009) Rhizosphere fauna: the functional and structural diversity of intimate interactions of soil fauna with plant roots. *Plant and Soil,* 321, 213-233.

Borowicz, V. A. (2001) Do arbuscular mycorrhizal fungi alter plant-pathogen relations?. *Ecology,* 82, 3057-3068.

Borstler, B., Renker, C., Kahmen, A. & Buscot, F. (2006) Species composition of arbuscular mycorrhizal fungi in two mountain meadows with differing management types and levels of plant biodiversity. *Biology and Fertility of Soils,* 42, 286-298.

Bradford, M. A., Jones, T. H., Bardgett, R. D., Black, H. I. J., Boag, B., Bonkowski, M., Cook, R., Eggers, T., Gange, A. C., Grayston, S. J., Kandeler, E., McCaig, A. E., Newington, J. E., Prosser, J. I., Setälä, H., Staddon, P. L., Tordoff, G. M., Tscherko, D. & Lawton, J. H. (2002) Impacts of soil faunal community composition on model grassland ecosystems. *Science,* 298, 615-618.

Brinkman, E. P., Van der Putten, W. H., Bakker, E. J. & Verhoeven, K. J. F. (2010) Plant-soil feedback: experimental approaches, statistical analyses and ecological interpretations. *Journal of Ecology,* 98, 1063-1073.

Brown, V. K. & Gange, A. C. (1992) Secondary plant succession - How is it modified by insect herbivory? *Vegetatio,* 101, 3-13.

Burrows, R. L. & Pfleger, F. L. (2002) Host responses to AMF from plots differing in plant diversity. *Plant and Soil,* 240, 169-179.

Callaway, R. M. & Aschehoug, E. T. (2000) Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. *Science,* 290, 521-523.

Callaway, R. M. & Ridenour, W. M. (2004) Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment,* 2, 436-443.

Cameron, E. (1935) A study of the natural control of ragwort (*Senecio jacobaea* L.). *Journal of Ecology,* 23, 265-322.

Canals, R. M., Emeterio, L. S. & Peralta, J. (2005) Autotoxicity in *Lolium rigidum*: analyzing the role of chemically mediated interactions in annual plant populations. *Journal of Theoretical Biology,* 235, 402-407.

Casper, B. B., Bentivenga, S. P., Ji, B. M., Doherty, J. H., Edenborn, H. M. & Gustafson, D. J. (2008) Plant-soil feedback: Testing the generality with the same grasses in serpentine and prairie soils. *Ecology,* 89, 2154-2164.

Casper, B. B. & Castelli, J. P. (2007) Evaluating plant-soil feedback together with competition in a serpentine grassland. *Ecology Letters,* 10, 394-400.

Caswell, H. (2001) *Matrix population models construction, analysis, and interpretation. 2nd edn.* Sinauer Associates Inc., Sunderland, MA.

Chapin III, F. S., Walker, L. R., Fastie, C. L. & Sharman, L. C. (1994) Mechanisms of primary succession following deglaciation at Glacier bay, Alaska. *Ecological Monographs,* 64, 149-175.

Chon, S.-U. & Kim, J.-D. (2002) Biological activity and quantification of suspected allelochemicals from Alfalfa plant parts. *Journal of Agronomy & Crop Science,* 188, 281-285.

Chon, S. U., Jang, H. G., Kim, D. K., Kim, Y. M., Boo, H. O. & Kim, Y. J. (2005) Allelopathic potential in lettuce (*Lactuca sativa* L.) plants. *Scientia Horticulturae,* 106, 309-317.

Clapp, J. P., Young, J. P. W., Merryweather, J. W. & Fitter, A. H. (1995) Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. *New Phytologist,* 130, 259-265.

Classen, A. T., Chapman, S. K., Whitham, T. G., Hart, S. C. & Koch, G. W. (2007) Genetic-based plant resistance and susceptibility traits to herbivory influence needle and root litter nutrient dynamics. *Journal of Ecology,* 95, 1181-1194.

Connell, J. H. & Slatyer, R. O. (1977) Mechanisms of succession in natural communities and their role in community stability and organization *American Naturalist,* 111, 1119-1144.

Cramer, V. A., Hobbs, R. J. & Standish, R. J. (2008) What's new about old fileds? Land abandonment and ecosystem assembly. *Trends in Ecology & Evolution,* 23, 104-112.

Crawley, M. J. & Nachapong, M. (1985) The establishment of seedlings from primary and regrowth seeds of ragwort (*Senecio jacobaea*). *Journal of Ecology,* 73, 255-261.

Crutsinger, G. M., Reynolds, W. N., Classen, A. T. & Sanders, N. J. (2008) Disparate effects of plant genotypic diversity on foliage and litter arthropod communities. *Oecologia,* 158.

Culman, S. W., Bukowski, R., Gauch, H. G., Cadillo-Quiroz, H. & Buckley, D. H. (2009) T-REX: Software for the processing and analysis of T-RFLP data. *BMC Bioinformatics,* 10, 171-.

Davies, F. T., Potter, J. R. & Linuerman, R. G. (1993) Drought resistance of mycorrhizal pepper plants independent of leaf P concentration - response in gas exchange and water relations. *Physiologia Plantarum,* 87, 45-53.

Davis, M. A., Grime, J. P. & K., T. (2000) Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology* 528-534.

De Deyn, G. B., Raaijmakers, C. E. & van der Putten, W. H. (2004) Plant community development is affected by nutrients and soil biota. *Journal of Ecology,* 92, 824-834.

De Deyn, G. B., Raaijmakers, C. E., Zoomer, H. R., Berg, M. P., de Ruiter, P. C., Verhoef, H. A., Bezemer, T. M. & van der Putten, W. H. (2003) Soil invertebrate fauna enhances grassland succession and diversity. *Nature,* 422, 711-713.

de Rooij-van der Goes, P., van Dijk, C., van der Putten, W. & Jungerius, P. (1997) Effects of sand movement by wind on nematodes and soil-borne fungi in coastal foredunes. *Journal of Coastal Conservation,* 3, 133-142.

de Rooij-van der Goes, P. C. E. M., van der Putten, W. H. & van Dijk, C. (1995) Analysis of nematodes and soil-borne fungi from *Ammophila arenaria* (Marram grass) in Dutch coastal foredunes by multivariate techniques. *European Journal of Plant Pathology,* 101, 149-162.

Dorning, M. & Cipollini, D. (2006) Leaf and root extracts of the invasive shrub, *Lonicera maackii*, inhibit seed germination of three herbs with no autotoxic effects. *Plant Ecology,* 184, 287-296.

Dumbrell, A. J., Nelson, M., Helgason, T., Dytham, C. & Fitter, A. H. (2010) Relative roles of niche and neutral processes in structuring a soil microbial community. *ISME Journal,* 4, 337-345.

Egerton-Warburton, L. M., Johnson, N. C. & Allen, E. B. (2007) Mycorrhizal community dynamics following nitrogen fertilization: A cross-site test in five grasslands. *Ecological Monographs,* 77, 527 -544.

Ehrenfeld, J. G., Ravit, B. & Elgersma, K. (2005) Feedback in the plant-soil system. *Annual Review of Environment and Resources,* 30, 75-115.

Ejrnæs, R., Bruun, H. H. & Graae, B. J. (2006) Community assembly in experimental grasslands: Suitable environment or timely arrival? *Ecology,* 87, 1225-1233.

Ejrnæs, R., Hansen, D. N. & Aude, E. (2003) Changing course of secondary succession in abandoned sandy fields. *Biological Conservation,* 109, 343-350.

Eschen, R., Müller-Schärer, H. & Schaffner, U. (2009) Aboveground environment type, soil nutrient content and arbuscular mycorrhizal fungi explain establishment success of *Centaurea jacea* on ex-arable land and in late-successional grasslands. *Plant and Soil,* 322, 115-123.

Ettema, C. H. & Wardle, D. A. (2002) Spatial soil ecology. *Trends in Ecology & Evolution,* 17, 177-183.

Facelli, J. M. & Pickett, S. T. A. (1991) Plant litters: Its dynamics and effects on plant community structure. *Botanical Review,* 57, 1-32.

Fayolle, A., Violle, C. & Navas, M.-L. (2009) Differential impacts of plant interactions on herbaceous species recruitment: disentangling factors controlling emergence, survival and growth of seedlings. *Oecologia,* 159, 817-825.

Fitter, A. H., Gilligan, C. A., Hollingworth, K., Kleczkowski, A., Twyman, R. M. & Pitchford, J. W. (2005) Biodiversity and ecosystem function in soil. *Functional Ecology,* 19, 369-377.

Fitzsimons, M. S., Miller, R. M. & Jastrow, J. D. (2008) Scale-dependent niche axes of arbuscular mycorrhizal fungi. *Oecologia,* 158, 117-127.

Forbes, J. C. (1977) Population flux and mortality in a ragwort (*Senecio jacobaea* L) infestation. *Weed Research* 17, 387-391.

Fujieda, K., Shoyama, Y., Matsunaka, H. & Nishioka, I. (1988) Plant growth inhibiting properties of phalaenopsine T from *Pharaenopsis* SPP. *Phytochemistry,* 27, 1564-1566.

Fukami, T., Bezemer, T. M., Mortimer, S. R. & van der Putten, W. H. (2005) Species divergence and trait convergence in experimental plant community assembly. *Ecology Letters,* 8, 1283-1290.

Gatti, A. B., Ferreira, A. G., Arduin, M. & Perez, S. (2010) Allelopathic effects of aqueous extracts of *Aristoluchia esperanzae* O. *Kuntze* on development of *Sesamum indicum* L. seedlings. *Acta Botanica Brasilica,* 24, 454-461.

Gollotte, A., van Tuinen, D. & Atkinson, D. (2004) Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species *Agrostis capillaris* and *Lolium perenne* in a field experiment. *Mycorrhiza,* 14, 111-117.

Grime, J. P. (1998) Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *Journal of Ecology,* 86, 902-910.

Grman, E. & Suding, K. N. (2010) Within-year soil legacies contribute to strong priority effects of exotics on native California grassland communities. *Restoration Ecology,* 18, 664-670.

Halpern, C. B., Antos, J. A., Geyer, M. A. & Olson, A. M. (1997) Species replacement during early secondary succession: the abrupt decline of a winter annual. *Ecology,* 78, 621-631.

Harper, J. L. (1958) The ecology of ragwort (*Senecio jacobaea* L) with especial reference to control. *Herbage Abstracts,* 28, 151-157.

Harper, J. L. & Wood, W. A. (1957) Biological flora of the British Isles: *Senecio jacobaea* L. *Journal of Ecology,* 45, 617-637.

Harrison, K. A. & Bardgett, R. D. (2010) Influence of plant species and soil conditions on plant-soil feedback in mixed grassland communities. *Journal of Ecology,* 98, 384-395.

Hart, M. M., Reader, R. J. & Klironomos, J. N. (2001) Life-history strategies of arbuscular mycorrhizal fungi in relation to their successional dynamics. *Mycologia,* 93, 1186-1194.

Hartmann, T. & Dierich, B. (1998) Chemical diversity and variation of pyrrolizidine alkaloids of the senecionine type: biological need or coincidence? *Planta,* 206, 443-451.

Hartnett, D. C. & Wilson, G. W. T. (1999) Mycorrhizae influence plant community structure and diversity in tallgras prairie *Ecology,* 80, 1187-1195.

Hausmann, N. T. & Hawkes, C. V. (2009) Plant neighborhood control of arbuscular mycorrhizal community composition. *New Phytologist,* 183, 1188-1200.

Hausmann, N. T. & Hawkes, C. V. (2010) Order of plant host establishment alters the composition of arbuscular mycorrhizal communities. *Ecology,* 91, 2333-2343.

Hawkes, C. V., Belnap, J., D'Antonio, C. & Firestone, M. K. (2006) Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. *Plant and Soil,* 281, 369-380.

Helgason, T., Daniell, T. J., Husband, R., Fitter, A. H. & Young, J. P. W. (1998) Ploughing up the wood -wide web? *Nature,* 394, 431-431.

Hierro, J. L. & Callaway, R. M. (2003) Allelopathy and exotic plant invasion. *Plant and Soil,* 256, 29-39.

Hoagland, D. R. & Arnon, D. I. (1950) The water-culture method for growing plants without soil. *California Agricultural Experiment Station Circular,* 347.

Hoffmann, J. H. & Moran, V. C. (1998) The population dynamics of an introduced tree, *Sesbania punicea*, in South Africa, in response to long-term damage caused by different combinations of three species of biological control agents. *Oecologia,* 114, 343-348.

Hol, W. H. G., de Boer, W., Termorshuizen, A. J., Meyer, K. M., Schneider, J. H. M., van Dam, N. M., van Veen, J. A. & van der Putten, W. H. (2010) Reduction of rare soil microbes modifies plant-herbivore interactions. *Ecology Letters,* 13, 292-301.

Hol, W. H. G., Macel, M., van Veen, J. A. & van der Meijden, E. (2004) Root damage and aboveground herbivory change concentration and composition of pyrrolizidine alkaloids of *Senecio jacobaea*. *Basic and Applied Ecology,* 5, 253-260.

Hol, W. H. G. & van Veen, J. A. (2002) Pyrrolizidine alkaloids from *Senecio jacobaea* affect fungal growth. *Journal of Chemical Ecology,* 28, 1763-1772.

Hol, W. H. G., Vrieling, K. & van Veen, J. A. (2003) Nutrients decrease pyrrolizidine alkaloid concentrations in *Senecio jacobaea*. *New Phytologist,* 158, 175-181.

Holtkamp, R. (2010) *Changing soils: Belowground food webs during secondary succession*. PhD thesis, Netherlands Institute of Ecology / Utrecht University.

Husband, R., Herre, E. A. & Young, J. P. W. (2002) Temporal variation in the arbuscular mycorrhizal communities colonising seedlings in a tropical forest. *Fems Microbiology Ecology,* 42, 131-136.

Huston, M. & Smith, T. (1987) Plant succession - Life history and competition. *American Naturalist,* 130, 168-198.

Huston, M. A. & Deangelis, D. L. (1994) Competition and coexistence - the effects of resource transport and supply rates. *American Naturalist,* 144, 954-977.

Inderjit (1996) Plant phenolics in allelopathy. *Botanical Review,* 62, 186-202.

Inderjit & Nilsen, E. T. (2003) Bioassays and field studies for allelopathy in terrestrial plants: Progress and problems. *Critical Reviews in Plant Sciences,* 22, 221-238.

Inderjit & Weiner, J. (2001) Plant allelochemical interference or soil chemical ecology? *Perspectives in Plant Ecology Evolution and Systematics,* 4, 3-12.

Inouye, R. S., Huntly, N. J., Tilman, D., Tester, J. R., Stillwell, M. & Zinnel, K. C. (1987) Old-Field Succession on a Minnesota Sand Plain. *Ecology,* 68, 12-26.

Johnson, D., Anderson, I. C., Williams, A., Whitlock, R. & Grime, J. P. (2010) Plant genotypic diversity does not beget root-fungal species diversity. *Plant and Soil,* 336, 107-111.

Johnson, D., Vandenkoornhuyse, P. J., Leake, J. R., Gilbert, L., Booth, R. E., Grime, J. P., Young, J. P. W. & Read, D. J. (2004) Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. *New Phytologist,* 161, 503-515.

Johnson, E. A. & Miyanishi, K. (2008) Testing the assumptions of chronosequences in succession. *Ecology Letters,* 11, 419-431.

Johnson, K. H., Vogt, K. A., Clark, H. J., Schmitz, O. J. & Vogt, D. J. (1996) Biodiversity and the productivity and stability of ecosystems. *Trends in Ecology & Evolution,* 11, 372-377.

Johnson, N. C., Graham, J. H. & Smith, F. A. (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist,* 135, 575-586.

Johnson, N. C., Tilman, D. & Wedin, D. (1992) Plant and soil controls on mycorrhizal fungal communities *Ecology,* 73, 2034-2042.

Johnson, N. C., Zak, D. R., Tilman, D. & Pfleger, F. L. (1991) Dynamics of vesicular-arbuscular mycorrhizae during old field succession. *Oecologia,* 86, 349-358.

Jongejans, E., Sheppard, A. W. & Shea, K. (2006) What controls the population dynamics of the invasive thistle Carduus nutans in its native range? *Journal of Applied Ecology,* 43, 877-886.

Joosten, L., Mulder, P. P. J., Klinkhamer, P. G. L. & van Veen, J. A. (2009) Soil-borne microorganisms and soil-type affect pyrrolizidine alkaloids in *Jacobaea vulgaris*. *Plant and Soil,* 325, 133-143.

Kabouw, P., van der Putten, W., van Dam, N. & Biere, A. (2010) Effects of intraspecific variation in white cabbage (*Brassica oleracea* var. *capitata*) on soil organisms. *Plant and Soil*, 1-10.

Kardol, P. (2007) *Plant and soil community assembly in secondary succession on ex-arable land - Fundamental and applied approaches*. PhD thesis, Netherlands Institute of Ecology / Wageningen University.

Kardol, P., Bezemer, T. M. & van der Putten, W. H. (2006) Temporal variation in plant-soil feedback controls succession. *Ecology Letters,* 9, 1080-1088.

Kardol, P., Bezemer, T. M. & Van Der Putten, W. H. (2009) Soil organism and plant introductions in restoration of species-rich grassland communities. *Restoration Ecology,* 17, 258-269.

Kardol, P., Bezemer, T. M., van der Wal, A. & van der Putten, W. H. (2005) Successional trajectories of soil nematode and plant communities in a chronosequence of ex-arable lands. *Biological Conservation,* 126, 317-327.

Kardol, P., Cornips, N. J., van Kempen, M. M. L., Bakx-Schotman, J. M. T. & van der Putten, W. H. (2007) Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. *Ecological Monographs,* 77, 147-162.

Kardol, P., Van der Wal, A., Bezemer, T. M., de Boer, W., Duyts, H., Holtkamp, R. & Van der Putten, W. H. (2008) Restoration of species-rich grasslands on ex-arable land: Seed addition outweighs soil fertility reduction. *Biological Conservation,* 141, 2208-2217.

Kivlin, S. N. & Hawkes, C. V. (2010) Differentiating between effects of invasion and diversity: impacts of aboveground plant communities on belowground fungal communities. *New Phytologist*, 526-535.

Klironomos, J. N. (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature,* 417, 67-70.

Klironomos, J. N. (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology,* 84, 2292-2301.

Klironomos, J. N. & Hart, M. M. (2002) Colonization of roots by arbuscular mycorrhizal fungi using different sources of inoculum. *Mycorrhiza,* 12, 181-184.

Klironomos, J. N., McCune, J., Hart, M. & Neville, J. (2000) The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. *Ecology Letters,* 3, 137-141.

Klironomos, J. N., Moutoglis, P., Kendrick, B. & Widden, P. (1993) A comparison of spatial heterogeneity of vesicular-arbuscular mycorrhizal fungi in 2 maple forest soils *Canadian Journal of Botany-Revue Canadienne De Botanique,* 71, 1472-1480.

Knops, J. M. H. & Tilman, D. (2000) Dynamics of soil nitrogen and carbon accumulation for 61 years after agricultural abandonment *Ecology,* 81, 88-98.

Kobayashi, K. (2004) Factors affecting phytotoxic activity of allelochemicals in soil. *Weed Biology and Management,* 4, 1-7.

Koch, A. M., Croll, D. & Sanders, I. R. (2006) Genetic variability in a population of arbuscular mycorrhizal fungi causes variation in plant growth. *Ecology Letters,* 9, 103-110.

Koide, R. T. & Dickie, I. A. (2002) Effects of mycorrhizal fungi on plant populations. *Plant and Soil,* 244, 307-317.

König, S., Wubet, T., Dormann, C. F., Hempel, S., Renker, C. & Buscot, F. (2010) TaqMan Real-Time PCR assays to assess arbuscular mycorrhizal responses to field manipulation of grassland biodiversity: Effects of soil characteristics, plant species richness, and functional traits. *Applied and Environmental Microbiology,* 76, 3765-3775.

Körner, C., Stöcklin, J., Reuther-Thiébaud, L. & Pelaez-Riedl, S. (2008) Small differences in arrival time influence composition and productivity of plant communities. *New Phytologist,* 177, 698-705.

Korthals, G. W., Šmilauer, P., van Dijk, C. & van der Putten, W. H. (2001) Linking above- and below-ground biodiversity: abundance and trophic complexity in soil as a response to experimental plant communities on abandoned arable land. *Functional Ecology,* 15, 506-514.

Kowalchuk, G. A., Hol, W. H. G. & van Veen, J. A. (2006) Rhizosphere fungal communities are influenced by *Senecio jacobaea* pyrrolizidine alkaloid content and composition. *Soil Biology & Biochemistry,* 38, 2852-2859.

Krogmeier, M. J. & Bremner, J. M. (1989) Effects of phenolic acids on seed germination and seedling growth in soil. *Biology and Fertility of Soils,* 8, 116-122.

Kulmatiski, A., Beard, K. H., Stevens, J. R. & Cobbold, S. M. (2008) Plant-soil feedbacks: a meta-analytical review. *Ecology Letters,* 11, 980-992.

Kumari, A. & Kohli, R. K. (1987) Autotoxicity of ragweed parthenium (*Parthenium hysterophorus*). *Weed Science,* 35, 629-632.

Ladygina, N. & Hedlund, K. (2010) Plant species influence microbial diversity and carbon allocation in the rhizosphere. *Soil Biology & Biochemistry,* 42, 162-168.

Lambers, H., Chapin III, F. S. & Pons, T. L. (2008) *Plant physiological ecology.* Springer, New York.

Lepš, J., Dolezal, J., Bezemer, T. M., Brown, V. K., Hedlund, K., Igual, A. M., Jorgensen, H. B., Lawson, C. S., Mortimer, S. R., Peix Geldart, A., Rodriguez Barrueco, C., Santa Regina, I., Šmilauer, P. & van der Putten, W. H. (2007) Long-term effectiveness of sowing high and low diversity seed mixtures to enhance plant community development on ex-arable fields. *Applied Vegetation Science,* 10, 97-110.

Lepš, J. & Šmilauer, P. (2003) *Multivariate analysis of ecological data using CANOCO.* Cambridge University Press

Lipinska, H. & Harkot, W. (2007) Allelopathic activity of grassland species. *Allelopathy Journal,* 19, 3-36.

Liu, W. T., Marsh, T. L., Cheng, H. & Forney, L. J. (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Applied and Environmental Microbiology,* 63, 4516-4522.

Liu, Y. H., Zeng, R. S., An, M., Mallik, A. U. & Luo, S. M. (2008) Autotoxicity in agriculture and forestry. *Allelopathy in Sustainable Agriculture and Forestry* (eds R. S. Zeng, A. U. Mallik & S. M. Luo), pp. 283-301. Springer New York.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J. P., Hector, A., Hooper, D. U., Huston, M. A., Raffaelli, D., Schmid, B., Tilman, D. & Wardle, D. A. (2001) Ecology - Biodiversity and ecosystem functioning: Current knowledge and future challenges. *Science,* 294, 804-808.

Luzuriaga, A. L. & Escudero, A. (2008) What determines emergence and net recruitment in an early succession plant community? Disentangling biotic and abiotic effects. *Journal of Vegetation Science,* 19, 445-U5.

Macel, M., Bruinsma, M., Dijkstra, S. M., Ooijendijk, T., Niemeyer, H. M. & Klinkhamer, P. G. L. (2005) Differences in effects of pyrrolizidine alkaloids on five generalist insect herbivore species. *Journal of Chemical Ecology,* 31, 1493-1508.

Macel, M., Klinkhamer, P. G. L., Vrieling, K. & Meijden, E. v. d. (2002) Diversity of pyrrolizidine alkaloids in Senecio species does not affect the specialist herbivore *Tyria jacobaeae*. *Oecologia,* 133, 541-550.

Mangan, S. A., Schnitzer, S. A., Herre, E. A., Mack, K. M. L., Valencia, M. C., Sanchez, E. I. & Bever, J. D. (2010) Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature,* 466, 752-755.

Manning, P., Morrison, S. A., Bonkowski, M. & Bardgett, R. D. (2008) Nitrogen enrichment modifies plant community structure via changes to plant-soil feedback. *Oecologia,* 157, 661-673.

Markham, J. H., Grime, J. P. & Buckland, S. (2009) Reciprocal interactions between plants and soil in an upland grassland. *Ecological Research,* 24, 93-98.

Marrs, R. H. (1993) Soil fertility and nature conservation in Europe: Theoretical considerations and practical management solutions. *Advances in Ecological Research* (eds M. Begon & A. H. Fitter), pp. 241-300. Academic Press.

Mattocks, A. R. (1968) Toxicity of pyrrolizidine alkaloids. *Nature,* 217, 723-728.

Mattocks, A. R. (1986) *Chemistry and toxicology of pyrrolizidine alkaloids.* Academic Press, London, UK.

McCarthy-Neumann, S. & Kobe, R. K. (2010) Conspecific and heterospecific plant–soil feedbacks influence survivorship and growth of temperate tree seedlings. *Journal of Ecology,* **98,** 408-418.

McEvoy, P., Cox, C. & Coombs, E. (1991) Successful biological control of ragwort, *Senecio jacobaea*, by introduced insects in Oregon. *Ecological Applications,* 1, 430-442.

McEvoy, P. B. (1984) Dormancy and dispersal in domorphic achenes of tansy ragwort, *Senecio jacobaea* L (compositae) *Oecologia,* 61, 160-168.

McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L. & Swan, J. A. (1990) A new method which gives an objective-measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. pp. 495-501. Cambridge University Press.

Meiners, S. J., Rye, T. A. & Klass, J. R. (2009) On a level field: the utility of studying native and non-native species in successional systems. *Applied Vegetation Science,* 12, 45-53.

Merryweather, J. & Fitter, A. (1998) The arbuscular mycorrhizal fungi of *Hyacinthoides non-scripta* - I. Diversity of fungal taxa. *New Phytologist,* 138, 117-129.

Miller, D. A. (1996) Allelopathy in forage crop systems. *Agronomy Journal,* 88, 854-859.

Mummey, D. L. & Rillig, M. C. (2007) Evaluation of LSU rRNA-gene PCR primers for analysis of arbuscular mycorrhizal fungal communities via terminal restriction fragment length polymorphism analysis. *Journal of Microbiological Methods,* 70, 200-204.

Mummey, D. L., Rillig, M. C. & Holben, W. E. (2005) Neighboring plant influences on arbuscular mycorrhizal fungal community composition as assessed by T-RFLP analysis. *Plant and Soil,* 271, 83 -90.

Muyzer, G., Dewaal, E. C. & Uitterlinden, A. G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S ribosomal rRNA. *Applied and Environmental Microbiology,* 59, 695-700.

Myster, R. W. & Pickett, S. T. A. (1994) A comparison of rate of succession over 18 yr in 10 contrasting old fields. *Ecology,* 75, 387-392.

Nelson, D. E. & Sommers, L. E. (1982) Total carbon, organic carbon, and organic matter. *Methods of Soil Analysis* (eds A. L. Page, R. H. Miller & D. R. Keeney), pp. 539-580. American Society of Agronomy.

Newsham, K. K., Fitter, A. H. & Watkinson, A. R. (1995) Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *Journal of Ecology,* 83, 991-1000.

Olff, H. & Bakker, J. P. (1991) Long-term dynamics of standing crop and species composition after the cessation of fertilizer application to mown grassland. *Journal of Applied Ecology,* 28, 1040-1052.

Olff, H., Berendse, F. & de Visser, W. (1994) Changes in nitrogen mineralization, tissue nutrient concentrations and biomass compartmentation after cessation of fertilizer application to mown grassland. *Journal of Ecology,* 82, 611-620.

Olff, H., Hoorens, B., de Goede, R. G. M., van der Putten, W. H. & Gleichman, J. M. (2000) Small-scale shifting mosaics of two dominant grassland species: the possible role of soil-borne pathogens. *Oecologia,* 125, 45-54.

Olff, H., Pegtel, D. M., van Groenendael, J. M. & Bakker, J. P. (1994) Germination strategies during grassland succession. *Journal of Ecology,* 82, 69-77.

Olsen, S. R., Cole, C. V., Watanabe, F. S. & Dean, L. A. (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *USDA circular,* 139, 1-19.

Öpik, M., Moora, M., Liira, J. & Zobel, M. (2006) Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. *Journal of Ecology,* 94, 778-790.

Orr, S. P., Rudgers, J. A. & Clay, K. (2005) Invasive plants can inhibit native tree seedlings: testing potential allelopathic mechanisms. *Plant Ecology,* 181, 153-165.

Ozinga, W. A., Schaminée, J. H. J., Bekker, R. M., Bonn, S., Poschlod, P., Tackenberg, O., Bakker, J. & van Groenendael, J. M. (2005) Predictability of plant species composition from environmental conditions is constrained by dispersal limitation. *Oikos,* 108, 555-561.

Packer, A. & Clay, K. (2000) Soil pathogens and spatial patterns of seedling mortality in a temperate tree. *Nature,* 404, 278-281.

Packer, A. & Clay, K. (2003) Soil pathogens and *Prunus serotina* seedling and sapling growth near conspecific trees *Ecology,* 84, 108-119.

Payne, R. W., Harding, S. A., Murray, D. A., Soutar, D. M., Baird, D. B., Welham, S. J., Kane, A. F., Gilmour, A. R., Thompson, R., Webster, R. & Tunnicliffe Wilson, G. (2008) *The guide to GenStat release 11, Part 2: Statistics*. VSN International, Hemel Hempstead.

Pelser, P. B., Veldkamp, J. F. & van der Meijden, R. (2006) New combinationsin *Jacobaea* Mill. (Asteraceae - Senecioneae). *Compositae Newsletter,* 44, 1-11.

Petermann, J. S., Fergus, A. J. F., Turnbull, L. A. & Schmid, B. (2008) Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. *Ecology,* 89, 2399-2406.

Pickett, S. T. A., Collins, S. L. & Armesto, J. J. (1987) Models, mechanisms and pathways of successions. *Botanical Review,* 53, 335-371.

Poole, A. & Cairns, D. (1940) Botanical aspects of ragwort (*Senecio jacobaea* L.) control. *Bulletin of the New Zealand Department of Science and Industrial Research* 82, 1-66.

Pregitzer, C., Bailey, J., Hart, S. & Schweitzer, J. (2010) Soils as agents of selection: feedbacks between plants and soils alter seedling survival and performance. *Evolutionary Ecology,* 24, 1045-1059.

Prins, A. H. & Nell, H. W. (1990) Positive and negative effects of herbivory on the population-dynamics of *Senecio jacobaea* and *Cynoglossum officinale* L. *Oecologia,* 83, 325-332.

Prins, A. H., Vrieling, K., Klinkhamer, P. G. L. & de Jong, T. J. (1990) Flowering behavior of *Senecio jacobaea*: Effects of nutrient availability and size-dependent vernalization. *Oikos,* 59, 248-252.

Quinn, J. A. (1974) *Convolvulus sepium* in old field succession on the New Jersey Piedmont. *Bulletin of the Torrey Botanical Club,* 101, 89-95.

Ramos-Zapata, J. A., Campos-Navarrete, M. J., Parra-Tabla, V., Abdala-Roberts, L. & Navarro-Alberto, J. (2010) Genetic variation in the response of the weed *Ruellia nudiflora* (Acanthaceae) to arbuscular mycorrhizal fungi. *Mycorrhiza,* 20, 275-280.

Reinhart, K. O. & Callaway, R. M. (2006) Soil biota and invasive plants. *New Phytologist,* 170, 445-457.

Reinhart, K. O., Royo, A. A., Van der Putten, W. H. & Clay, K. (2005) Soil feedback and pathogen activity in Prunus serotina throughout its native range. *Journal of Ecology,* 93, 890-898.

Reynolds, H. L., Packer, A., Bever, J. D. & Clay, K. (2003) Grassroots ecology: Plant-microbe-soil interactions as drivers of plant community structure and dynamics. *Ecology,* 84, 2281-2291.

Rhoades, C. C., Eckert, G. E. & Coleman, D. C. (1998) Effect of pasture trees on soil nitrogen and organic matter: Implications for tropical montane forest restoration. *Restoration Ecology,* 6, 262-270.

Rice, E. L. (1984) *Allelopathy.* Academic Press, New York.

Ridenour, W. M. & Callaway, R. M. (2001) The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. *Oecologia* 126, 444-450.

Rillig, M. C. (2004) Arbuscular mycorrhizae, glomalin, and soil aggregation. *Canadian Journal of Soil Science,* 84, 355-363.

Rinaudo, V., Barberi, P., Giovannetti, M. & van der Heijden, M. G. A. (2010) Mycorrhizal fungi suppress aggressive agricultural weeds. *Plant and Soil,* 333, 7-20.

Rodriguez-Echeverria, S., Hol, W. H. G., Freitas, H., Eason, W. R. & Cook, R. (2008) Arbuscular mycorrhizal fungi of *Ammophila arenaria* (L.) Link: Spore abundance and root colonisation in six locations of the European coast. *European Journal of Soil Biology,* 44, 30-36.

Ronquist, F. & Huelsenbeck, J. P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics,* 19, 1572-1574.

Ronsheim, M. L. & Anderson, S. E. (2001) Population-level specificity in the plant-mycorrhizae association alters intraspecific interactions among neighboring plants. *Oecologia,* 128, 77-84.

Rosendahl, S. & Stukenbrock, E. H. (2004) Community structure of arbuscular mycorrhizal fungi in undisturbed vegetation revealed by analyses of LSU rDNA sequences. *Molecular Ecology,* 13, 3179 -3186.

Sanders, I. R., Alt, M., Groppe, K., Boller, T. & Wiemken, A. (1995) Identification of ribosomal DNA polymorphisms among and within spores of the Glomales: application to studies on the genetic diversity of arbuscular mycorrhizal fungal communities. *New Phytologist,* 130, 419-427.

Schädler, M., Jung, G., Auge, H. & Brandl, R. (2003) Palatability, decomposition and insect herbivory: patterns in a successional old-field plant community. *Oikos,* **103,** 121-132.

Scheublin, T. R., Ridgway, K. P., Young, J. P. W. & van der Heijden, M. G. A. (2004) Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. *Applied and Environmental Microbiology,* 70, 6240-6246.

Scheublin, T. R., Van Logtestijn, R. S. P. & Van der Heijden, M. G. A. (2007) Presence and identity of arbuscular mycorrhizal fungi influence competitive interactions between plant species. *Journal of Ecology,* 95, 631-638.

Schüßler, A., Schwarzott, D. & Walker, C. (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research,* 105, 1413-1421.

Schweitzer, J. A., Bailey, J. K., Fischer, D. G., Leroy, C. J., Lonsdorf, E. V., Whitham, T. G. & Hart, S. C. (2008) Plant-soil-microorganism interactions: Heritable relationship between plant genotype and associated soil microorganisms. *Ecology,* 89, 773-781.

Schweitzer, J. A., Bailey, J. K., Rehill, B. J., Martinsen, G. D., Hart, S. C., Lindroth, R. L., Keim, P. & Whitham, T. G. (2004) Genetically based trait in a dominant tree affects ecosystem processes. *Ecology Letters,* 7, 127-134.

Scowcroft, P. G. & Jeffrey, J. (1999) Potential significance of frost, topographic relief, and *Acacia koa* stands to restoration of mesic Hawaiian forests on abandoned rangeland. *Forest Ecology and Management,* 114, 447-458.

Singh, H. P., Batish, D. R. & Kohli, R. K. (1999) Autotoxicity: Concept, organisms, and ecological significance. *Critical Reviews in Plant Sciences,* 18, 757-772.

Six, J., Bossuyt, H., Degryze, S. & Denef, K. (2004) A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics. *Soil and Tillage Research,* 79, 7-31.

Šmilauer, P. (2001) Communities of arbuscular mycorrhizal fungi in grassland: Seasonal variability and effects of environment and host plants. *Folia Geobotanica,* 36, 243-263.

Smith, S. E. & Read, D. J. (2008) *Mycorrhizal symbiosis (third edn).* Academic Press, London, UK.

Smittenberg, J. C. (2005) Jakobskruiskruid: Waardevolle plant of sluipmoordenaar? *Natura*, 48-51.

Stampe, E. D. & Daehler, C. C. (2003) Mycorrhizal species identity affects plant community structure and invasion: a microcosm study. *Oikos,* 100, 362-372.

Stevens, J. M. & Fehmi, J. S. (2009) Early establishment of a native grass reduces the competitive effect of a non-native grass. *Restoration Ecology*, in press.

Swift, M. J., Heal, O. W. & Anderson, J. M. (1979) *Decomposition in terrestrial ecosystems.* Blackwell Scientific Publications, Oxford.

Sýkorová, Z., Ineichen, K., Wiemken, A. & Redecker, D. (2007) The cultivation bias: different communities of arbuscular mycorrhizal fungi detected in roots from the field, from bait plants transplanted to the field, and from a greenhouse trap experiment. *Mycorrhiza,* 18, 1-14.

Tawaha, A. M. & Turk, M. A. (2003) Allelopathic Effects of Black Mustard (*Brassica nigra*) on Germination and Growth of Wild Barley (*Hordeum spontaneum*). *Journal of Agronomy and Crop Science*, 298-303.

Ter Braak, C. J. F. & Šmilauer, P. (2002) CANOCO Reference manual and CanoDraw for Windows user's guide: Software for canonical community ordination (version 4.5). Microcomputer Power, Ithaca, New York.

Thoden, T., Hallmann, J. & Boppré, M. (2009) Effects of plants containing pyrrolizidine alkaloids on the northern root-knot nematode *Meloidogyne hapla*. *European Journal of Plant Pathology,* 123, 27-36.

Thoden, T. C., Boppre, M. & Hallmann, J. (2009) Effects of pyrrolizidine alkaloids on the performance of plant-parasitic and free-living nematodes. *Pest Management Science,* 65, 823-830.

Thomas, J. A., Telfer, M. G., Roy, D. B., Preston, C. D., Greenwood, J. J. D., Asher, J., Fox, R., Clarke, R. T. & Lawton, J. H. (2004) Comparative losses of British butterflies, birds, and plants and the global extinction crisis. *Science,* 303, 1879-1881.

Thompson, A. (1980) Ragwort populations and control studies. *Proceedings of the 33rd New Zealand Weed and Pest Control Conference*, pp. 59-62.

Thompson, A. & Makepeace, W. (1983) Longevity of buried ragwort (*Senecio jacobaea* L.) seed. *New Zealand Journal of Experimental Agriculture,* 11, 89-90.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997) The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research,* 25, 4876-4882.

Tilman, D. (1982) *Resource competition and community structure.* Princeton University Press, Princeton.

Tilman, D. (1988) *Plant strategies and the dynamics and structure of plant communities.* Princeton University

Press, Princeton.

Tilman, D. (1997) Community invasibility, recruitment limitation, and grassland biodiversity. *Ecology,* 78, 81-92.

Tilman, D. (2009) Mechanisms of plant competition. *Plant Ecology* (ed M. J. Crawley). Blackwell Publishing Ltd., Oxford, UK.

Tilman, D. & Wedin, D. (1991) Plant traits and resource reduction for five grasses growing on a nitrogen gradient. *Ecology,* 72, 685-700.

Trappe, J. M. (1987) Phylogenetic and ecological aspects of mycotrophy in the angiosperms from an evolutionary standpoint. *Ecophysiology of VA mycorrhizal plants.* (ed G. R. Safir), pp. 5-25. CRC Press, Boca Raton, Florida.

Troelstra, S. R., Wagenaar, R., Smant, W. & Peters, B. A. M. (2001) Interpretation of bioassays in the study of interactions between soil organisms and plants: involvement of nutrient factors. *New Phytologist,* 150, 697-706.

Trouvelot, S., van Tuinen, D., Hijri, M. & Gianinazzi-Pearson, V. (1999) Visualization of ribosomal DNA loci in spore interphasic nuclei of glomalean fungi by fluorescence in situ hyhridization. *Mycorrhiza,* 8, 203-206.

van Dam, N. M. (2009) Belowground Herbivory and Plant Defenses. *Annual Review of Ecology Evolution and Systematics,* 40, 373-391.

van Dam, N. M., Vuister, L. W. M., Bergshoeff, C., Devos, H. & van der Meijden, E. (1995) The raison d'etre of pyrrolizidine alkaloids in *Cynoglossum officinale*: Deterrent effect against generalist herbivores *Journal of Chemical Ecology,* 21, 507-523.

van de Voorde, T. F. J., van der Putten, W. H., Gamper, H. A., Hol, W. H. G. & Bezemer, T. M. (2010) Comparing arbuscular mycorrhizal communities of individual plants in a grassland biodiversity experiment. *New Phytologist,* 186, 746-754.

van der Heijden, M. G. A. (2004) Arbuscular mycorrhizal fungi as support systems for seedling establishment in grassland. *Ecology Letters,* 7, 293-303.

van der Heijden, M. G. A., Bardgett, R. D. & van Straalen, N. M. (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters,* 11, 296-310.

van der Heijden, M. G. A., Boller, T., Wiemken, A. & Sanders, I. R. (1998a) Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology,* 79, 2082-2091.

van der Heijden, M. G. A. & Horton, T. R. (2009) Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. *Journal of Ecology,* 97, 1139-1150.

van der Heijden, M. G. A., Klironomos, J. N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A. & Sanders, I. R. (1998b) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature,* 396, 69-72.

van der Meijden, E. (1971) *Senecio* and *Tyria* (Callimorpha) in a Dutch dune area: a study on an interaction between a monophagous consumer and its host plant. *Dynamics of populations* (eds P. J. den Boer & G. R. Gradwell), pp. 390-404. Centre for Agricultural Publishing and Documentation, Wageningen.

van der Meijden, E., de Boer, N. J. & van der Veen-van Wijk, C. A. (2000) Pattern of storage and regrowth in ragwort. *Evolutionary Ecology,* 14, 439-455.

van der Meijden, E. & van der Waals-Kooi, R. E. (1979) The population ecology of *Senecio jacobaea* in the sand dune system. *Journal of Ecology,* 67, 131-153.

van der Meijden, R. (2005) *Heukels' flora van Nederland. 23e druk.* Wolters-Noordhoff Groningen/ Houten

van der Putten, W. H. (2003) Plant defense belowground and spatiotemporal processes in natural vegetation. *Ecology,* 84, 2269-2280.

van der Putten, W. H. (2009) A multitrophic perspective on functioning and evolution of facilitation in plant communities. *Journal of Ecology,* 97, 1131-1138.

van der Putten, W. H., Breteler, J. T. V. & van Dijk, C. (1989) Colonization of the root zone of *Ammophila arenaria* by harmful soil organisms. *Plant and Soil,* 120, 213-223.

van der Putten, W. H., Mortimer, S. R., Hedlund, K., van Dijk, C., Brown, V. K., Lepš, J., Rodriguez-Barrueco, C., Roy, J., Len, T. A. D., Gormsen, D., Korthals, G. W., Lavorel, S., Regina, I. S. & Šmilauer, P. (2000) Plant species diversity as a driver of early succession in abandoned fields: a multi-site approach. *Oecologia,* 124, 91-99.

van der Putten, W. H., van Dijk, C. & Peters, B. A. M. (1993) Plant-specific soil-borne diseases contribute to succession in foredune vegetation. *Nature,* 362, 53-56.

van der Wal, A. (2007) *Soils in transition: dynamics and functioning of fungi*. PhD thesis, Netherlands Institute of Ecology / Leiden University.

van der Wal, A., de Boer, W., Klein Gunnewiek, P. J. A. & van Veen, J. A. (2009) Possible mechanism for spontaneous establishment of *Calluna vulgaris* in a recently abandoned agricultural field. *Restoration Ecology,* 17, 308-313.

van der Wal, A., van Veen, J. A., Smant, W., Boschker, H. T. S., Bloem, J., Kardol, P., van der Putten, W. H. & de Boer, W. (2006) Fungal biomass development in a chronosequence of land abandonment. *Soil Biology & Biochemistry,* 38, 51-60.

Van Tuinen, D., Jacquot, E., Zhao, B., Gollotte, A. & Gianinazzi-Pearson, V. (1998) Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR. *Molecular Ecology,* 7, 879-887.

Vandegehuchte, M. L., de la Peña, E. & Bonte, D. (2010a) Contrasting covariation of above- and belowground invertebrate species across plant genotypes. *Journal of Animal Ecology*, in press.

Vandegehuchte, M. L., de la Peña, E. & Bonte, D. (2010b) Relative importance of biotic and abiotic soil components to plant growth and insect herbivore population dynamics. *PLoS ONE,* 5, e12937.

Vandenkoornhuyse, P., Ridgway, K. P., Watson, I. J., Fitter, A. H. & Young, J. P. W. (2003) Co-existing grass species have distinctive arbuscular mycorrhizal communities. *Molecular Ecology,* 12, 3085-3095.

Veblen, K. E. (2008) Season- and herbivore dependent competition and facilitation in a semiarid savanna *Ecology,* 89, 1532-1540.

Viketoft, M., Bengtsson, J., Sohlenius, B., Berg, M. P., Petchey, O., Palmborg, C. & Huss-Danell, K. (2009) Long-term effects of plant diversity and composition on soil nematode communities in model grasslands. *Ecology,* 90, 90-99.

Vitousek, P. M., Mooney, H. A., Lubchenco, J. & Melillo, J. M. (1997) Human domination of earth's ecosystems. *Science,* 277, 494-499.

Vivanco, J. M., Bais, H. P., Stermitz, F. R., Thelen, G. C. & Callaway, R. M. (2004) Biogeographical variation in community response to root allelochemistry: novel weapons and exotic invasion. *Ecology Letters,* 7, 285-292.

Vrieling, K., Smit, W. & van der Meijden, E. (1991) Tritrophic interactions between aphids (*Aphis jacobaeae* Schrank), ant species, *Tyria jacobaeae* L., and *Senecio jacobaea* L. lead to maintenance of genetic variation in pyrrolizidine alkaloid concentration. *Oecologia,* 86, 177-182.

Vrieling, K., Soldaat, L. L. & Smit, W. (1991) The influence of pyrrolizidine alkaloids of *Senecio jacobaea* on *Tyria jacobaeae*, *Brachycaudus cardii* and *Haplothrips senecionis Netherlands Journal of Zoology,* 41, 228-239.

Walker, K. J., Stevens, P. A., Stevens, D. P., Mountford, J. O., Manchester, S. J. & Pywell, R. F. (2004) The restoration and re-creation of species-rich lowland grassland on land formerly managed for intensive agriculture in the UK. *Biological Conservation,* 119, 1-18.

Walker, L. R. & Chapin, F. S., III (1987) Interactions among processes controlling successional change. *Oikos,* 50, 131-135.

Walker, L. R. & del Moral, R. (2003) *Primary succession and ecosystem rehabilitation.* Cambridge University Press, Cambridge, UK.

Wardle, D. A. (1987) The ecology of ragwort (*Senecio jacobaea* L.) - a review. *New Zealand Journal of Ecology,* 10, 67-76.

Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., van der Putten, W. H. & Wall, D. H. (2004) Ecological linkages between aboveground and belowground biota. *Science,* 304, 1629-1633.

Wardle, D. A., Nicholson, K. S. & Rahman, A. (1995) Ecological effects of the invasive weed species *Senecio jacobaea* L. (ragwort) in a New Zealand pasture. *Agriculture Ecosystems & Environment,* 56, 19-28.

Weiner, J. (1990) Asymmetric competition in plant populations. *Trends in Ecology & Evolution,* 5, 360-364.

White, T. J., Bruns, T. D., Lee, S. B. & Taylor, J. W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols. A guide to methods and applications.* pp. 315-322. Academic Press, San Diego, California.

Wilby, A. & Brown, V. K. (2001) Herbivory, litter and soil disturbance as determinants of vegetation dynamics during early old-field succession under set-aside. *Oecologia,* 127, 259-265.

Wilson, R. E. & Rice, E. L. (1968) Allelopathy as expressed by *Helianthus annuus* and its role in old-field succession. *Bulletin of the Torrey Botanical Club,* 95, 432-448.

Wink, M., Latz-Brüning, B. & Schmeller, T. (1999) Biochemical effects of allelopathic alkaloids. *Principles and Practices in Plant Ecology: Allelochemical Interactions* (eds Inderjit, K. M. M. Dakshini & C. L. Foy), pp. 411-422. CRC Press, Boca Raton, FL.

Witte, L., Ernst, L., Adam, H. & Hartmannt, T. (1992) Chemotypes of two pyrrolizidine alkaloid-containing *Senecio* species. *Phytochemistry,* 31, 559-565.

Young, T. P., Chase, J. M. & Huddleston, R. T. (2001) Community succession and assembly: comparing, contrasting and combining paradigms in the context of ecological restoration. *Ecological restoration,* 19, 5-18.

Zeller, V., Bardgett, R. D. & Tappeiner, U. (2001) Site and management effects on soil microbial properties of subalpine meadows: a study of land abandonment along a north-south gradient in the European Alps. *Soil Biology & Biochemistry,* 33, 639-649.

Zeng, R. S., Luo, S. M., Shi, Y. H., Shi, M. B. & Tu, C. Y. (2001) Physiological and biochemical mechanism of allelopathy of secalonic acid F on higher plants. *Agronomy Journal,* 93, 72-79.

Nederlandse samenvatting

In Europa zijn de laatste decennia veel landbouwgronden uit productie genomen om ze om te vormen tot semi-natuurlijke graslandecosystemen. Deze transitie wordt secundaire successie genoemd en deze gebieden oude landbouwgronden. Gedurende secundaire successie komen en gaan individuele plantensoorten. Veel van deze soorten komen op, hebben een piek in bedekking en nemen dan weer af waarna hun plek wordt ingenomen door een andere soort. De prestatie en bedekking van de individuele soorten wordt beïnvloed door terugkoppelingsmechanismen tussen de abiotische en biotische kenmerken van de bodem en de plant. Dit betekent dat planten en de plantengemeenschap als geheel de bodem kunnen veranderen en dat deze veranderingen in de bodem op hun beurt weer grote gevolgen kunnen hebben voor de planten. Het doel van dit onderzoek was 1. het begrijpen van het belang van plant-bodeminteracties voor de populatiedynamiek van een vroege successie-soort gedurende secundaire successie in oude landbouwgronden; 2. Daarnaast bestudeerde ik hoe de samenstelling van de plantengemeenschap de relatie tussen individuele planten en bodemsymbionten in de gemeenschap kan beïnvloeden. Ik gebruikte voor dit onderzoek de plantensoort Jakobskruiskruid, *Jacobaea vulgaris* ssp. *vulgaris*, omdat deze plant een groot effect kan hebben op vroege-successie plantengemeenschappen in Nederland.

Allereerst bestudeerde ik de populatiedynamiek van Jakobskruiskruid in tien oude landbouwgronden. Deze velden verschillen in het moment dat ze uit productie genomen zijn (2 tot 25 jaar geleden), maar hebben verder vergelijkbare geschiedenis en karakteristieken. Daardoor vormen ze een chronosequentie van uit-productiename. De bedekking met Jakobskruiskruid in de velden piekte ongeveer 5 jaar na uit-productiename, waarna de bedekking snel weer afnam. Ik testte de hypothese dat de achteruitgang van Jakobskruiskruid komt door de opbouw van een negatief plant-bodem effect (plant-bodem feedback). Ik testte het effect van de bodemgemeenschap op de prestatie van Jakobskruiskruid in een kasexperiment met grond die was verzameld in de velden. Er was een positieve relatie tussen het aantal Jakobskruiskruidplanten in het veld en de mate waarin de bodemgemeenschap de groei van Jakobskruiskruid remde. Als de grond eerst was geconditioneerd door er gedurende één groeifase Jakobskruiskruid in te groeien, dan remde grond uit alle velden de groei van Jakobskruiskruid sterk.

In een tuinexperiment zaaide ik Jakobskruiskruidzaden in plaggen met intacte vegetatie die waren verzameld in de velden. De succesvolle opkomst van zaden was significant lager in plagen afkomstig van oude velden dan in plaggen uit de jonge velden. In een zaadbankstudie nam het aantal opgekomen zaailingen af met tijd sinds uit-productiename. Echter, het aantal zaailingen was in alle gevallen hoger dan het aantal Jakobskruiskruidplanten in het veld. Dit laat zien dat het aantal zaden in de zaadbank niet limiterend is, maar dat de condities ongunstiger worden met tijd sinds uit-productiename. Andere plantensoorten die samen met Jakobskruiskruid voorkomen in de velden kunnen ook de kenmerken van de bodem veranderen, welke op hun beurt weer de prestatie van

Jakobskruiskruid kunnen beïnvloeden. In een kasexperiment testte ik hoe veranderingen in de bodem die zijn veroorzaakt door Jakobskruiskruid de prestatie van Jakobskruiskruid en die van 30 andere plantensoorten die samen met Jakobskruiskruid voorkomen veranderen. Daarnaast testte ik het omgekeerde, dus het effect van iedere soort afzonderlijk via de bodem op Jakobskruiskruid. Ik vergelijk dus interspecificieke plant-bodem interacties tussen Jakobskruiskruid en omringende planten met het intraspecifieke plant-bodem feedbackeffect van Jakobskruiskruid op zichzelf. Deze studie bevestigde dat Jakobskruiskruid een sterk negatief feedbackeffect op zichzelf heeft. Dit experiment liet zien dat er grote verschillen zijn tussen plantensoorten en hun bodemeffect op Jakobskruiskruid (ongeveer de helft van de soorten verminderde de prestatie van Jakobskruiskruid, terwijl de rest geen effect had). Bodem die geconditioneerd was door Jakobskruiskruid had daarentegen een positief tot neutraal effect op de groei van de omringende soorten. Deze resultaten laten drie mechanismen zien waarmee de nalatenschap van plant-bodem interacties, alleen of in combinatie, de snelheid van secundaire successie kunnen beïnvloeden door prioriteitseffecten. 1. Soorten die typisch zijn voor vroege successie hebben een negatief bodemeffect op soortgenoten; 2. Omringende plantensoorten hebben een negatief bodemeffect op vroege successiesoorten; 3. Vroege successiesoorten hebben over het algemeen een positief bodemeffect op de omringende plantensoorten.

Om een beter idee te krijgen welke bodemorganismen belangrijk zijn voor plant-bodem interacties bestudeerde ik het bodemeffect van verschillende groepen van bodemorganismen in een kasexperiment. In dit experiment vergeleek ik de groei van Jakobskruiskruid in gesteriliseerde veldgrond die was geïnoculeerd met levende veldgrond gezeefd door een 1 mm zeef, geïnoculeerd met een waterige oplossing van veldgrond gezeefd door een 1 mm zeef (grondoplossing), of geïnoculeerd met een waterige oplossing van veldgrond gezeefd door een 20 µm zeef (microbiële oplossing). De biomassa van Jakobskruiskruidplanten was het laagst in grond geïnoculeerd met de gezeefde grond en het hoogst in grond geïnoculeerd met de microbiële oplossing. Deze studie laat ook zien dat resultaten die verkregen worden met gezeefde grond of met een grondoplossing niet vergelijkbaar zijn en dus dat de methode waarmee de inocula zijn gemaakt effect kan hebben op de gemeten feedbacksterkte. Bij het plannen van plant-bodem experimenten zou men hier rekening mee moeten houden.

Ik bestudeerde ook of de verminderde groei van Jakobskruiskruid in grond die is geconditioneerd door soortgenoten kan komen door chemische stoffen die Jakobskruiskruid bevat. Ik testte dit door extracten van verschillende sterkte en gemaakt van verschillende delen van Jakobskruiskruid of wortelfragmenten te geven aan zaailingen en ontkiemende zaden. De prestatie van zaailingen die in water groeiden en die extracten toegediend kregen was in sommige gevallen significant minder, maar de groei van zaailingen in grond was niet verminderd. Het mengen van wortelfragmenten verminderde significant de maximum wortellengte van deze zaailingen, ook als deze in grond groeiden.

Deze resultaten laten zien dat Jakobskruiskruid een autotoxische effect kan hebben onder laboratoriumcondities, maar dat deze effecten zwak zijn wanneer de zaailingen in grond groeien. Dit suggereert dat autotoxiciteit waarschijnlijk geen sterk effect heeft in de afname van Jakobskruiskruidbedekking in het veld.

De samenstelling van de omringende plantengemeenschap kan potentieel ook de bodemorganismen die de plant koloniseren beïnvloeden. Om dit te bestuderen bepaalde ik de samenstelling van arbusculaire mycorrhiza (AM) schimmels in de wortels van afzonderlijke Jakobskruiskruidplanten. Deze planten groeiden in experimentele plantengemeenschappen. Vijf gemeenschappen waren ingezaaid met midden-successiesoorten in een voormalige landbouwgrond. De andere vijf waren niet ingezaaid maar op natuurlijke wijze gekoloniseerd. De plantengemeenschappen waren tien jaar oud toen de samenstelling van de AM-schimmels in de wortels van de Jakobskruiskruidplanten geanalyseerd werd. De samenstelling van de AM-schimmelgemeenschap werd in twee planten per gemeenschap geanalyseerd met 'terminal restriction fragment length polymorphism' (T-RFLP). Op dat moment waren de niet ingezaaide plantengemeenschappen meer divers en vertoonden meer spatiële heterogeniteit dan de ingezaaide gemeenschappen, maar beide gemeenschappen deelden grotendeels dezelfde plantensoorten. De diversiteit van de AM-schimmels was niet verschillend tussen beide plantengemeenschappen, maar de dissimilariteit tussen AM-schimmelgemeenschap die afkomstig waren van planten uit een niet ingezaaide gemeenschap was groter dan tussen planten uit ingezaaide gemeenschappen. Als de planten in de kasproef groeiden in gehomogeniseerde grond, dus zonder spatiële variatie, dan was er geen verschil meer in AM-schimmel dissimilarieit tussen de wel en niet ingezaaide gemeenschappen. Dus, de wijze en geschiedenis waarop een plantengemeenschap zich heeft gevormd heeft effect op de AM-schimmel gemeenschap die individuele planten in deze gemeenschap koloniseert.

Dit onderzoek laat zien dat interacties tussen planten en bodemorganismen een belangrijke rol spelen bij het verklaren van de populatieontwikkeling van Jakobskruiskruid, maar het laat ook zien dat deze interacties moeten worden bestudeerd in combinatie met de opkomst en succes van zaailingen en competitie met andere plantensoorten.

BEHEER

De resultaten van dit promotieonderzoek kunnen ook gebruikt worden om management ten aanzien het beheersen van Jakobskruiskruid te verbeteren. Bij het beheer zou men gebruik kunnen maken van de opbouw van het negatieve bodemeffect tegen Jakobskruiskruid. Men zou ook de fase waarin Jakobskruiskruid zich bevindt, voor piek- of na piekbedekking, moeten meenemen in de te kiezen beheersmaatregelen (Tabel 1).

Direct nadat landbouwgronden uit-productie worden genomen of dat een weiland wordt ontwikkeld is er vaak nog veel open grond die Jakobskruiskruid kan koloniseren. In dit

vroege stadium is het aantal Jakobskruiskruidplanten in het veld echter nog laag, omdat er nog maar weinig zaden aanwezig zijn (Tabel 7.1). In dit stadium zou het management zich kunnen richten op het voorkomen dat zaden het nog makkelijk te koloniseren veld bereiken. Dit zou kunnen door Jakobskruiskruidplanten in de omgeving te maaien voordat ze hun zaden verspreiden, zodat deze zich niet naar het nieuwe veld verspreiden. Zolang er nog weinig zaden zijn bouwt de zaadbank zich ook slechts langzaam op. Om te voorkomen dat er zich een zaadbank vormt zouden planten die zich vestigen verwijderd moeten worden. Dit kan door ze handmatig te verwijderen of door middel van begrazing.

Ondertussen kunnen andere planten het veld wel koloniseren en een dichte begroeiing vormen. Deze dichte begroeiing is moeilijk voor Jakobskruiskruidplanten om te koloniseren en zich te vestigen. Het stimuleren van een dichte begroeiing door soorten in te zaaien maakt het ook moeilijker voor Jakobskruiskruid om zich te vestigen en hoge dichtheden te halen (Bezemer *et al*. 2006a). Vanaf het moment dat zich een dichte mat heeft gevormd is het belangrijk om verstoringen hiervan te verkomen, zodat er geen nieuwe vestigingskansen voor Jakobskruiskruid ontstaan. Deze verstoringen kunnen bijvoorbeeld ontstaan door herbivoren, overbegrazing, betreding of landbouwmachines.

Vanaf het moment dat Jakobskruiskruidplanten zich vestigen op het veld gaan ze ook zaden produceren. Door deze lokale productie is de beschikbaarheid van zaden in het veld niet meer limiterend (Figuur 7.1 & Tabel 1). Omdat de bodem nog niet is geconditioneerd door Jakobskruiskruid en zaadbeschikbaarheid niet meer limiterend is kunnen Jakobskruiskruidplanten er snel groeien en zich vermeerderen. Echter, dit kan alleen wanneer andere planten nog geen dichte mat hebben gevormd en wanneer er nog ruimte, bijvoorbeeld kale grond, beschikbaar is voor Jakobskruiskruid om te koloniseren. Als er meer Jakobskruiskruidplanten komen zal ook meer grond geconditioneerd worden door Jakobskruiskruid en een negatief bodemeffect zal nu worden opgebouwd (Tabel 1).

Als Jakobskruiskruid eenmaal het veld heeft gekoloniseerd zou het beheer meer gebruik moeten gaan maken van het negatieve bodemeffect dat zich op bouwt. Dit negatieve effect kan zich al opbouwen in minder dan één groeiseizoen, echter Jakobskruiskruidplanten, inclusief wortels en grond, moeten dan niet verwijderd worden, omdat dit de opbouw van een negatief bodemeffect verhindert. Daarentegen zou het beheer zich moeten richten op het voorkomen dat zaden zich verspreiden naar naastgelegen jonge velden. Dit kan door de bloeiende planten te maaien of te begrazen. Dit verhindert niet de opbouw van een negatief bodemeffect, aangezien deze zich ook kan opbouwen bij rozetten. Ook hier geldt dat verstoring van de bodem voorkomen moet worden omdat dit juist nieuwe vestigingskansen voor Jakobskruiskruid creëert.

Tabel 1: Overzicht van de mechanismen die belangrijk zijn voor de prestatie van Jakobskruiskruid en hun belang gedurende twee verschillende fases: voordat de piek in bedekking is bereikt en daarna. Een negatief effect op het presteren of de populatieomvang van Jakobskruiskruid is aangegeven met (-), een positief effect met (+), geen effect met (o) en een onbekend of onduidelijk effect met (+/-). Beheer in beide fases zou zich moeten richten op processen die Jakobskruiskruid limiteren.

		Voor piek	Na piek
Succes van zaailingen	Zaadkwaliteit	+	+
	Zaadbeschikbaarheid	-	+
	Kieming	+	+
	Vestiging	+	-
	Verspreiding/ dispersie	-	o
	Verstoringen	o	-
	Effect van competitie op vestiging	o	-
Bodemnutriënten	Nutriëntenbeschikbaarheid	+	+
Allelochemicaliën	Autotoxiciteit	o	o
	Allelopatie	o	+/-
Plant-bodem interacties	Plant-bodem feedback (***J. vulgaris*** – ***J. vulgaris***)	o/-	-
	Plant-bodem interacties (***J. vulgaris***—omringende planten)	o/-	-
	Plant-bodem interacties (omringende planten—***J. vulgaris***)	o/-	-
	Effect van competitie op Jakobskruiskruid	o	-

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Curriculum vitae

Tess Frieda Jozef van de Voorde was born on May 14, 1982 in Terneuzen, the Netherlands. She attended the Reynaertcollege in Hulst for her secondary education (Atheneum). In 2000, she started her propaedeutics Landscape Planning at Wageningen University and Research centre (WUR). After one year, she switched to the Bachelor program of Soil, Water, Atmosphere. After this bachelor, she started the Master Soil science with a major in Earth system science and a minor in Geohydrology at WUR. During her master she carried out a master project where she performed fieldwork and modeling to link hydrology to gravity in a small river catchment area in Germany, supervised by dr. Roel Dijksma and dr. Henny van Lanen of the Hydrology and Quantitative Water Management group of WUR and in cooperation with the Friedrich-Schiller University Jena, Germany. For a second master thesis, she went to the University of California - Davis, where she studied rhizodeposition under elevated atmospheric CO_2 conditions, supervised by Dr. Marie-Anne de Graaff, Prof. dr. Johan Six and Prof. dr. Chris van Kessel. She finished her master in 2006 with an internship at the department of Terrestrial Ecology of the Netherlands Institute of Ecology (NIOO-KNAW) where she studied the effect of arbuscular mycorrhizal fungi on ragwort, supervised by her later PhD supervisors. In 2006 she started her PhD at the laboratory of Nematology of Wageningen University in cooperation with the Terrestrial Ecology group of NIOO-KNAW. She was supervised by dr. ir. Martijn Bezemer and Prof. dr. ir. Wim van der Putten. Her PhD study focused on the importance of plant-soil feedback for the population dynamics of ragwort in ex-agricultural fields at the Veluwe, the Netherlands. The research involved laboratory, greenhouse and field experiments. After her PhD she worked as a post-doctoral researcher at NIOO-KNAW examining problems with *Prunus serotina* and *Jacobaea vulgaris* in the Netherlands. In April 2011 she started as a post-doctoral researcher within the project "Ecology of the biobased economy: soil amelioration with biochar in a natural ecosystem", being a joint project of WUR and NIOO-KNAW.

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PE&RC PhD Education certificate

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (4.2 ECTS)

Belowground multitrophic interactions and *Jacobaea vulgaris*

Post-graduate courses (10.4 ECTS)

Multivariate analysis of ecological data; University of South Bohemia, Czech Republic (2009)

PhD Course "Soil ecology: crossing the frontier between below and above ground; PE&RC (2007)

Nematode identification course; Laboratory of Nematology, Wageningen UR (2007)

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Career Perspectives; Wageningen Graduate schools, Wageningen UR (2010)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.7 ECTS)

PE&RC Day (2009)

PE&RC Symposium: the biobased economy: back to earth? (2010)

Current themes in ecology (2 x 2007, 2008)

Discussion groups / local seminars / other scientific meetings (7.6 ECTS)

Terrestrial Ecology (NIOO-KNAW); PhD discussion group (2006-2010)
NIOO-days (2009, 2010)
Netherlands Annual Ecology Meeting (2009, 2010)

International symposia, workshops and conferences (11.9 ECTS)

Rhizophere 2; International conference, Montpellier, France (2007)
British Ecological Society (BES) annual meeting; Imperial College, London, UK (2008)
Ecological Society of Germany; Austria and Switzerland (GFOE), Leipzig, Germany (2008)
Ecological Society of America (ESA) annual meeting; Albuquerque, USA (2009)
Ecological Society of Germany; Austria and Switzerland (GFOE), Giessen, Germany (2010)

Supervision of MSc student: 1 hour/week; 4 months

Investigating the autotoxic potential of ragwort (*Jacobaea vulgaris*)