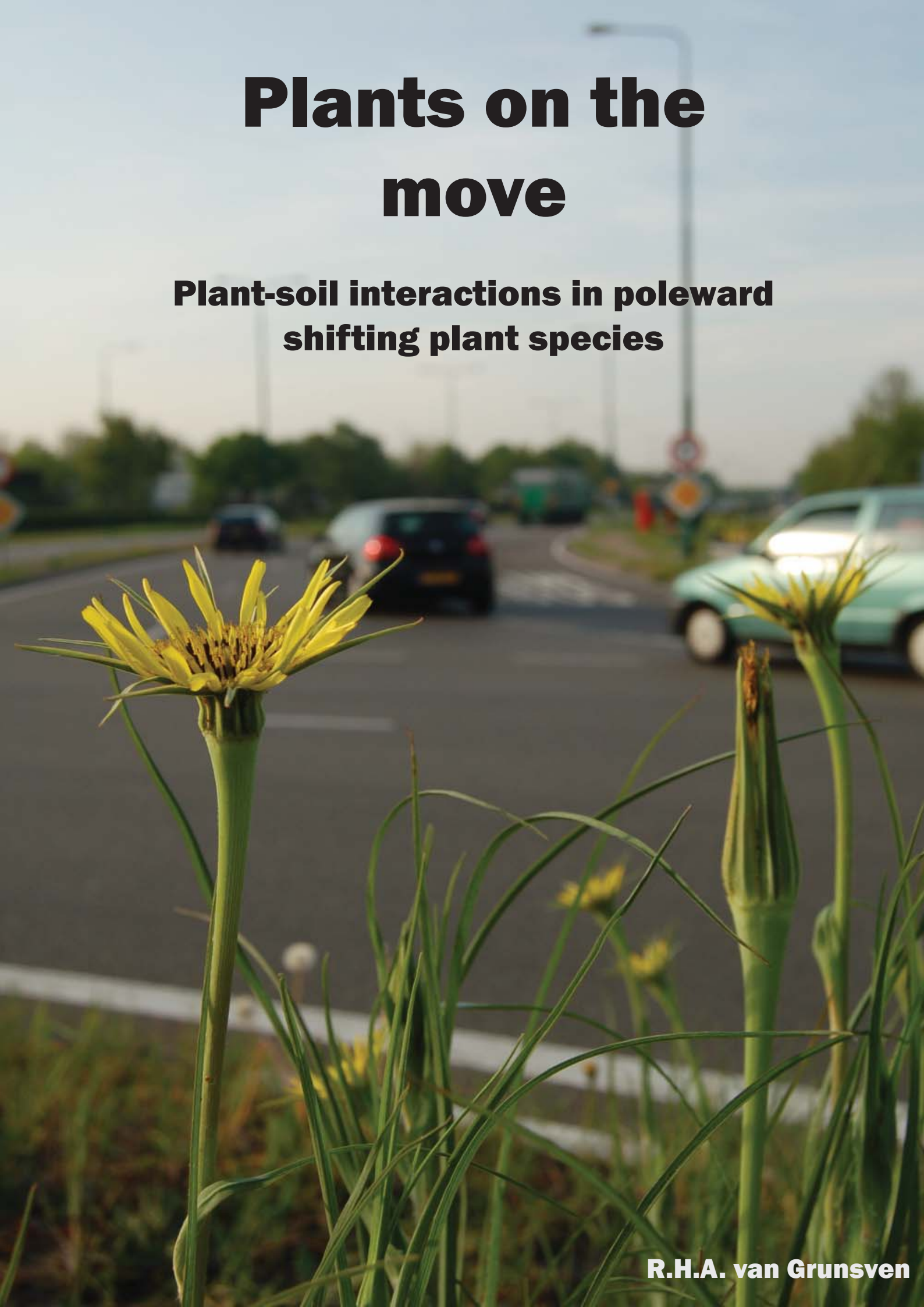# Plants on the move

Plant-soil interactions in poleward shifting plant species

R.H.A. van Grunsven

Plants on the move:

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Roy H. A. van Grunsven

Abstract

As a result of recent global climate change, areas that have previously been climatically unsuitable for species have now become suitable new habitats. Many plant-species are expanding their range polewards, colonizing these newly available areas. If these species are able to expand their range faster than their natural enemies they can become released from these limiting factors. A similar mechanism has been reported for invasive plant species, introduced into foreign continent, which are often found to be released from natural enemies.

An example of an invasive plant species that is introduced into a foreing contintinent is *Carpobrotus edulis.* This species was found to be negatively affected by the soil community collected in the native range, while the soil communities from the invaded range did not have an effect on plant performance compared to a sterilized control. I hypothesized that a similar reduction of the negative effects of the soil community can occur when plant species shift their range. This hypothesis was tested in a greenhouse experiment. I compared plant-soil feedbacks of three plant species that have recently expanded their range into The Netherlands, with three related native species. The non-native species experienced a significantly less negative effect of plant-soil feedback than the native plant species.

Concurrently with these range shifts local climate is changing and this might affect plant-soil feedback as well. In order to test this plant-soil feedbacks of six range expanding and six related native species were compared at two temperatures, 20°C and 25°C daytime temperature. While again native species showed a more negative plant-soil feedback than the non-native species, temperature did not affect the strength or direction of plant-soil feedback.

Besides pair wise comparisons between native and non-native species in the invaded range, comparisons between the native and non-native range of a range expanding plant can be used to test for effects of range shifts on plant-soil interactions. Rhizosphere soil was collected from populations of *Tragopogon dubius*

in both the native and the recently colonized range. The soil communities from the native range had a more negative effect on plant performance than the soil communities from the invaded range as compared to sterilized controls. *T. pratensis*, which is native to the entire studied range, did not show this pattern.

As plant-soil interactions are the net effect of many positive and negative factors the less negative effect of plant-soil feedback can be either a result of more positive or less negative effects of the soil community. One of the mutualistic groups of organisms, the arbuscular mycorrhizal fungi (AMF) are known to be a major factor contributing to ecosystem functioning and to the maintenance of plant biodiversity and the most important soil-borne mutualists for many plants. I therefore focus on this group of soil organisms. I compared the association of *T.dubius* with AMF in the new part of its range with *T. pratensis* native to this area. Three measures for plant-fungal affinity were compared between these two plant species; the density of AMF propagules able to colonize the plant, the percentage of root length colonized by arbuscular mycorrhiza, and the composition of the resulting AMF community in the roots. This was done for four replicate soil inocula from different sites in The Netherlands. The two plant species did not differ in any of the tested factors. As there are no differences in the association with the most important mutualist the observed differences in plant-soil interaction are likely an effect of release from negative components in the soil community, e.g. soil pathogens, but further studies are needed to test this.

Alterations in biotic interactions, through climate change and range shifts, such as a release of soil-borne natural enemies, can have significant effects on the performance of plants. Predictions of future ranges and impact of range expanding plant species on invaded ecosystems can therefore not be accurately made without a thorough understanding of its biotic interactions and the way these interactions are changed by the range shifts.

Contents

General introduction

"May you live in interesting times" is a well-known Chinese curse or, more likely, a western saying claimed to be Chinese. Ecologists currently do live in interesting times, this being a curse and a blessing at the same time. Biodiversity is threatened worldwide by habitat destruction, pollution, overharvesting, invasive species and climate change. Currently, the number one threat to biodiversity is habitat destruction and second the spread of invasive species (Wilcove *et al.* 1998). The effect of climate change is only starting to become apparent, but is thought by some to be a major threat to biodiversity (Thomas *et al.* 2004). Although the current increase in environmental awareness is slowly resulting in action, it is clear that the present threats on biodiversity cannot be easily removed.

These changes, however, also provide us with valuable insights. The way systems change when affected can give us insights into the functioning of the natural system and raises new questions. When observing an invasive species outcompeting native plant species, changing vegetation composition and structure, one cannot help but wonder how it can be so successful in an area it has not evolved in (Elton 1958). Also, the arrival of species in The Netherlands that used to be restricted to more southern areas, raises the question how they perform in this new area (Parmesan *et al.* 1999; Tamis *et al.* 2005) and how they interact with the species already present.

In this thesis I focus on plants that are introduced into foreign regions and plants that are shifting their range and more specifically how their interactions with the soil community are affected by these range shifts.

Introductions and range shifts can affect interactions between organisms. Species that shift their range come into contact with new species and lose contact with species from their original environment. The ecological characteristics of a plant

species are co-determined by these biotic interactions. Many trees could not grow in places where they now do grow, if they did not have fungi on their roots that supply them with nutrients. On the other hand, many plants do well in gardens but do not spread into natural areas, because they cannot compete with native species or are heavily attacked by herbivores when not nurtured by a gardener. We can only understand the distribution and abundance of species when both physiological characteristics and biotic interactions are considered. When these interactions change, the "behavior" of the species considered change. Most people associate these biotic interactions with caterpillars feeding on leaves, cows grazing or plants being overshaded by other plants. But an important battleground is actually on and around the roots of plants (Bever 1994; Wardle *et al.* 2004).

Plants do not move. Although this may seem obvious there are many less obvious consequences on plant ecology (Silvertown 2001). Most animals can move when a site becomes less suitable. Plants do not have this option, at least not in the way most animals do. If a plant changes its environment, for better or worse, this will also affect the plant itself. This is especially true in the soil. Soils are very heterogeneous and dispersal through the soil is limited for both organisms and many chemical compounds. Therefore, plants can affect their own performance through their effect on the soil (fig. 1.1). This can be in the form of nutrient depletion, litter formation (Berendse 1994), accumulation of pathogens or mutualists (Bever *et al.* 1997). Many plant species accumulate pathogens, resulting in a negative feedback on their own performance (van der Putten *et al.* 1993; Bever 1994; Reynolds *et al.* 2003; Kardol *et al.* 2007). This has long been recognized in agriculture and is the main reason why crop-rotations increase yield (Ghorbani *et al.* 2008). If plants can disperse, or be dispersed, to new sites that are unaffected by themselves or a conspecific, they can escape these negative effects. The way plants change the soil, and how they affect their own performance through these changes, differs between species. Some species have a negative effect on themselves through the soil, resulting in species that occur in low densities. Other species can improve conditions for themselves on a local scale by increasing mineralization or accumulating mutualistic organisms. This positive feedback results in high local densities and these plants can become dominant (Klironomos 2002).
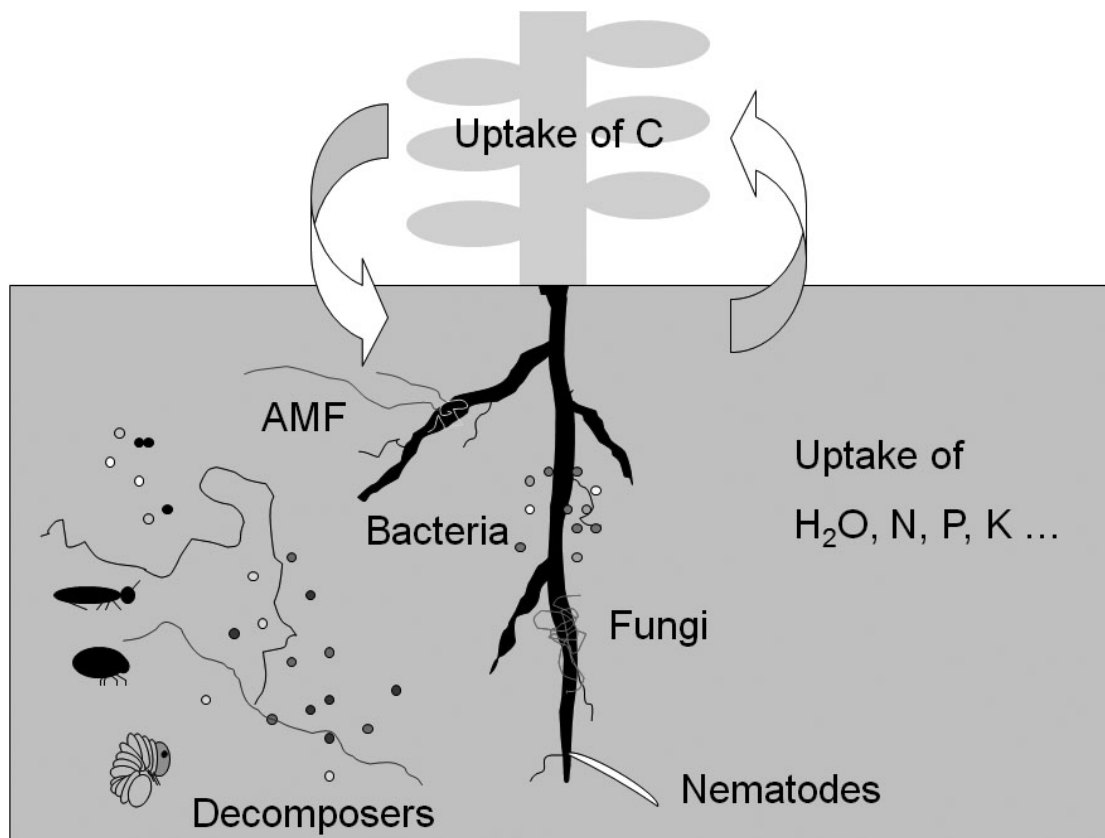
Figure1.1: Plants interact with many belowground organisms. Some of these organisms directly interact with the plant, such as plant-feeding nematodes, arbuscular mycorrhizal fungi and pathogenic bacteria and fungi. Other organisms only interact with the plant indirectly, for instance through feeding on plant litter and thereby increasing the nutrient availability to the plant. These soil organisms are all affected by the plant and in their turn affect the performance of the plant; this is called plant-soil feedback (Wardle *et al.* 2004).

Although I stated that plants stand still, anyone who has blown dandelion seeds in his youth knows that plants can move very well in some stages of their life (Soons *et al.* 2004). This gives them the ability to colonize new sites over relatively short distances. Plants with a negative plant-soil feedback can colonize a new site, unaffected by plant-soil feedback, when the site where it is growing becomes unsuitable. This results in plant species moving through the landscape (Olff *et al.* 2000; Packer & Clay 2000) followed by their pathogens, a kind of natural "crop-rotation". In plant species with a neutral or positive plant-soil feedback this does not occur. Under certain circumstances, the effect of this plant-soil feedback can even work directional. For example, fast growing plants with poor defense can thrive in a

pioneer situation, i.e. a soil with very few pathogens (Herms & Mattson 1992). These species are called early-succesional species. After their arrival, pathogens start to accumulate which negatively affects these plants. This opens up the opportunity for another group of plants that are insensitive to these pathogens to invade, i.e. the mid-successional species. These in their turn also accumulate pathogens that have a negative effect on both the early-succesional species and on themselves. Therefore a third group, the late-succesional species can invade. And this group accumulates pathogens that have a stronger negative effect on the early- and mid-succesional species than on themselves. This results in a soil-borne pathogen-driven succession (Kardol *et al.* 2007).

Invasive species

While dispersal within the natural range can result in a temporal release of pathogens, plants can also be more or less permanently released from their natural enemies. Man-mediated dispersal can cross barriers that are impossible to cross through natural dispersal. This can be intentional, for instance as an ornamental or crop, or accidental through hitchhiking on the massive transport of all types of material around the world. This results in the introduction of species into areas that were previously inaccessible to them (Mack *et al.* 2000; Colautti *et al.* 2006). Some of these species can establish in these new areas, thrive or even become invasive (Mack *et al.* 2000). The small subset of introduced species that does become invasive can become very dominant and cause economic and ecological damage. One of the hypotheses explaining this dominance is the enemy release hypothesis. This hypothesis states that an introduced species can find itself released from the natural enemies of its native range, and lacks specialist enemies in the invaded range, and therefore has an advantage over native species that do have to cope with their natural enemies (Elton 1958). In contrast to the temporal release in natural systems, this is a permanent release until the natural enemies are introduced, intentionally or unintentionally.

Numerous studies support the enemy release hypothesis (Colautti *et al.* 2004; Carpenter & Cappuccino 2005; Vila *et al.* 2005; Liu & Stiling 2006). However, most of these studies focus on the role of insect herbivores or aboveground pathogens while soil-borne pathogens are underrepresented (Reinhart & Callaway 2006). This is not because soil-borne pathogens are thought to be less important, but because

these are harder to study (Reinhart & Callaway 2006). Soil organisms are not obviously present on a plant, like for instance caterpillars or aphids. The damage done by soil-borne organisms is hard to measure and they operate in a complex community with many interactions (Callaway *et al.* 2004; Piśkiewicz *et al.* 2007). The few studies that focused on the possible release of soil-borne enemies in invasive species confirmed such a release (Reinhart *et al.* 2003; Reinhart & Callaway 2004; van der Putten *et al.* 2005). Release from soil-borne enemies appears to play an important role in at least some invasive plant species. However, the evidence is still limited to very few species in comparison to aboveground enemy-release. Therefore the question if release from soil borne enemies is common or restricted to a few species remains valid. In chapter 2, I consider a creeping succulent from South Africa that has invaded Mediterranean dune areas. I compare the impact of soil communities from the native and invaded range on the performance of this invader.

Climate change

The fact that introductions on other continents can result in a release from natural enemies has long been recognized (Elton 1958). However, the acceptance of plant-soil feedback as a driving force in plant ecology and its importance in small scale shifts, is of a much more recent date (van der Putten *et al.* 1993; Bever 1994; van der Putten 1997; Olff *et al.* 2000; Kardol *et al.* 2006). Introductions on other continents and small shifts within a habitat are two extremes in dispersal. Dispersal events of intermediate distance also occur; these are relatively long distance but do not cross any actual barriers. These events occur when previously unavailable areas become suitable for a species. An example of this is the current range-shifting of many species as a result of climate change. The climate in Europe is changing and has done so for the last decades. All years from 2003 to 2008 have been warmer than the 30 year average (1960-1990) (KNMI 2008). The last two years, 2006 and 2007 have both been 1.4 degrees warmer than average. The current weather in The Netherlands has more in common with the weather typical for France than the characteristic Dutch weather (fig. 1.2). Therefore, areas that were previously too cold and thus unsuitable for certain species have now become suitable.

Figure 1.2: The annual average temperature as measured at Dutch weather stations in 2006 en 2007 was similar to the 30 year average (1961-1990) of cities in France, 600 to 800 km south of The Netherlands. (Adapted from "De toestand van het klimaat in Nederland" 2008, KNMI)

As a result of this climatic change many plants and animals are shifting their range polewards (Bakkenes *et al.* 2002; Parmesan & Yohe 2003; Hickling *et al.* 2005). Species that used to occur in France spread north into The Netherlands. Many southern species have already colonized The Netherlands in recent years. This is true for both animals (e.g. *Meconema meridionale*, *Argiope bruennichi* and *Crocothemis erythraea,* fig. 1.3) and plants (e.g. *Tragopogon dubius*, *Eragrostis pilosa*). The number of "new" plant species is thought to outnumber the "old" species in large parts of Europe by 2050 (Bakkenes *et al.* 2002). Some species will be able to shift their range rapidly while others have a more limited dispersal and need more time to expand their range. The faster species, able to colonize the now suitable area first, will find themselves among foreign species. This situation is similar to that of introduced species but without the actual introduction. If these species are able to shift their range faster than their natural enemies they can escape some of them

during this range expansion and experience the same advantages of enemy release as introduced species in comparison to native species, at least temporarily.

Range shifts and plant-soil feedback

Plants have to cope with many different natural enemies, ranging from large grazers to soil bacteria and from caterpillars to fungi. Some of these enemies are very good dispersers or generalists and are therefore unlikely to escape from during a range shift. However, many soil-borne organisms are poor dispersers as they lack targeted dispersal (van der Putten *et al.* 2001). Therefore, plants shifting their range might be released from a negative plant-soil feedback and experience an advantage in comparison to native species. There are two main approaches to study release from negative plant-soil feedback. Firstly, the plant-soil feedback of species that have recently colonized The Netherlands can be compared with related native species (Agrawal *et al.* 2005). Secondly, the impact of natural soil-borne enemies can be compared between the native and the invaded range (Liu & Stiling 2006). In the first approach a more positive, or less negative, effect of plant-soil feedback is expected for the non-native species than for the native species. This approach is used in chapter 3 and 4. It does not directly test the enemy release hypothesis itself, but rather the effect enemy release has on the performance of the native and non-native species (Klironomos 2002).

The ecosystems that are invaded by range-shifting plant species simultaneously experience an increase in ambient temperature. This temperature increase might also affect plant-soil interactions directly. Temperature increase is known to affect many soil processes such as decomposition (Cornelissen *et al.* 2007), yet its effect on plant-soil interactions was so far unknown. As temperature increase might interact with the effect of the range-shift, I included temperature as a factor in chapter 4 by performing the plant-soil feedback experiment at two temperatures.

As a second approach to test enemy release by range expansion, a comparison between the newly colonized and the native range can be made. This approach was used in chapter 5. Enemy release will result in a less negative effect of plant-soil interaction on plant performance in the newly colonized range compared to the native range. This approach tests the effect of enemy release more directly but is not as informative on the competitive advantage in the newly invaded range.

Furthermore, this method is sensitive to trends in plant-soil feedback from south to north. Therefore I included a closely related species native to the entire range as a yardstick.

Mutualists

Plant soil interactions are the net effect of many biotic and abiotic interactions (Wardle *et al.* 2004). Some soil organisms, such as pathogens and herbivores, have negative effects on plant performance while other organisms have a positive effect on plant performance, so called mutualists. In the chapters 2 to 5 of this thesis, the different groups of soil-organisms are not separated but the effect of the soil community as a whole is studied. In chapter 6, I focus on the role of one of these groups, the arbuscular mycorrhizal fungi or AMF. AMF grow partly in the roots of a plant and partly in the soil. They receive sugars from the plant in exchange for a.o. phosphorous, increased drought resistance and defense and are considered the most important soil-borne mutualists for many plant species (Newsham *et al.* 1995; Kiers & Van Der Heijden 2006; Bennett & Bever 2007). And AMF are considered to be a major factor contributing to ecosystem functioning and to the maintenance of plant biodiversity (van der Heijden *et al.* 1998).

Despite the intimate interactions, with fungal hyphae growing inside plant cells, AMF have often been thought to be generalistic. Loss of AMF mutualism in invading or range shifting species was therefore thought to be highly unlikely (Richardson *et al.* 2000). Recently it has become clear that interactions between plants and AMF are much less generalistic than previously assumed (Klironomos 2003), and some invasive species have been shown to associate with very few mycorrhizal species in the invaded range (van der Putten *et al.* 2007). In chapter 6, I test if a species that has recently colonized The Netherlands is as effective in associating with mycorrhizas as a native related species and if they associate with the same arbuscular mycorrhizal taxa. I did this by assessing the density of AMF propagules capable of colonizing the native and/or the non-native plant species in four soils, by examining the relative root length colonized by AMF of both plant species when grown in these four soils and by comparing the community of AMF in the roots. The last comparison is done using PCR-DGGE.

This thesis is concluded by a short synthesis of the findings in the separate chapters in which I draw a general conclusion.

Figure 1.3: Poleward shift is even more obvious in some insects than in plants as the first can disperse very well (Hickling *et al.* 2005). One of the nicest additions to the Dutch fauna as a result of climate change is *Crocothemis erythraea* (Photo Antoine van der Heijden)

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Release from soil pathogens plays an important role in the success of invasive *Carpobrotus* in the Mediterranean.

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Abstract

Introduced plant species can become locally dominant and threaten native flora and fauna. This dominance is often thought to be a result of enemy release, the lack of specialist enemies in the invaded range, and the evolution of increased competitive ability. Soil borne microorganisms have often been overlooked as enemies in this context, but a less negative plant soil interaction in the invaded range could explain local dominance.

Two plant species, *Carpobrotus edulis* and the hybrid *Carpobrotus X cf. acinaciformis*, are considered major pests in the Mediterranean basin. I tested if release from soil-borne enemies and/or evolution of increased competitive ability could explain this dominance. Comparing biomass production on inoculated soil with that on uninoculated soil, I found that inoculation with rhizosphere soil from the native range reduced biomass production by 32 % while inoculation with rhizosphere soil from the invaded range did not have a significant effect on plant biomass. Genotypes from the invaded range, including a hybrid, did not perform better than plants from the native range in sterile soil. Hence evolution of increased competitive ability and hybridization do not seem to play a major role.

I conclude that the reduced negative net impact of the soil community in the invaded range is an important mechanism, which may explain the success of *Carpobrotus* species in the Mediterranean basin.

Introduction

Many plant species have been introduced into areas where they previously did not occur. Most of these fail to establish or only establish locally, however a few species become dominant and displace the natural vegetation (Williamson & Fitter 1996). Several hypotheses explaining this dominance have been proposed. Three of these are enemy release (Elton 1958; Keane & Crawley 2002), evolution of increased competitive ability (Blossey & Notzold 1995), and hybridization (Ellstrand & Schierenbeck 2000). These hypotheses are often tested in isolation and rarely in combination although they are not mutually exclusive and partly depend upon each other.

Most studies on enemy release of invasive species focus on the role of aboveground herbivores (Maron & Vila 2001; Agrawal *et al.* 2005) but attention on the soil community has increased recently as the feedback between plants and soil has proven to be an important driver in ecosystem dynamics (Klironomos 2002; Kardol *et al.* 2006). Plant-soil feedback is an important determinant of plant density. Plants that quickly accumulate pathogens are found at low densities in natural communities, while plants that accumulate pathogens slowly, or are not strongly affected by these pathogens, are often locally dominant (Klironomos 2002).

Plants can undergo genetic changes after introduction resulting in more vigorous genotypes in the invaded range (Bossdorf *et al.* 2005). If an invasive species is released from its enemies, the benefit of defence mechanisms is diminished and the balance in the trade-off between defence and growth or reproduction (Herms & Mattson 1992) may shift towards growth and reproduction. Therefore these plants have a better competitive ability than, and may outcompete, plants that invest more in defence mechanisms. This hypothesis is known as the evolution of increased competitive ability or EICA (Blossey & Notzold 1995). This will result in increased performance in the absence of pathogens, e.g. in sterilized soil, compared to plants from the native range.

A third factor that may play an important role in the success of invasive species is hybridization (Ellstrand & Schierenbeck 2000). Hybridization can result in heterosis and hybrid vigour resulting in a more competitive phenotype (Wolfe *et al.* 2007). Hybridization can also increase genetic variation and thus facilitate EICA.

We test whether these hypotheses are supported for the highly invasive species *C. edulis* (L.) N.E. Br. and the hybrid *C. X cf. acinaciformis* (*C. edulis* x *C. acinaciformis* (L.) L. Bolus) of the Aizoaceae. These are invasive in the Mediterranean basin and are threatening native species by the formation of large, monospecific mats excluding native species (D'Antonio & Mahall 1991; Draper *et al.* 2003; Vila *et al.* 2006). Related species are invasive in areas with a Mediterranean climate around the world (Suehs *et al.* 2006).

We predicted that, 1) the inoculation with soil from the native range has a stronger negative effect than inoculation with soil from the invaded range. This would support the enemy release hypothesis, 2) plants from the invaded range have a higher biomass production on sterilized soil than the plants from the native range. This would support the increased competitive ability hypothesis.

Materials and methods

Study species

Carpobrotus edulis, *C. acinaciformis* and their hybrid, *C. X cf. acinaciformis*, are succulent perennials with strong clonal growth and creeping vine-like branches that root at internodes. Branches can grow up to 1 metre per year, overgrowing and replacing surrounding vegetation (D'Antonio 1993; Acosta *et al.* 2006). Both species originate from South Africa and have been introduced in order to stabilize sand dunes and road sides (Vila & D'Antonio 1998) in the western Mediterranean basin since the early 1800's (Gouffe De La Cour 1813).

Rhizosphere soil was collected from three populations of *C. edulis*. A typical native population near East London, South Africa (SA 1) and two populations from southern Spain, one near Cadiz, 10 kilometres from the seashore (Spain 1) and another near Malaga next to the Mediterranean seashore (Spain 2). For every population 10 plants were selected. Distance between selected plants was between 15 and 800 metre. Under each plant approximately 1.5 kg of soil was collected. Soil was collected no deeper than 15 cm. below the soil surface. These samples were homogenized into an aggregate sample per population. Seeds from the same populations were collected. Additional seeds, but not soil, were collected from a second population near Cape Town, South Africa (SA 2) and two populations on

Bagaud island (Hyères archipelago, France); one consisting of pure *C. edulis* (France 1) and one of the genetically more diverse hybrid *C. X cf. acinaciformis* (France 2) (Suehs *et al.* 2004; Suehs *et al.* 2006).

Experimental setup

Soils and seeds were moved to Wageningen, The Netherlands, where the experiment was performed. Half of each soil sample was autoclaved (3 hours at 121.5°C) resulting in three sterilized and three non-sterilized inocula. The inocula were mixed with bulk soil (a sterilized mixture of sandy loam soil and river sand) in a 1 to 4 w/w ratio and homogenized. This resulted in inoculated soils and soils that received a similar but sterilized inoculum as a control for the abiotic effects of the inoculum. Pots with a diameter of 14 cm and a height of 12 cm were filled with 1000 grams of this mixture.

Seeds were germinated in sterilized river sand. Two weeks after germination, similar sized seedlings were selected and transplanted into the pots. As the seeds from one of the populations in South Africa (SA1) did not germinate, these were excluded from the experiment, but the soil from this population was used. The five remaining progenies, one South African, two Spanish and two French, were grown in soils with six different inocula, two Spanish and a South African and their respective sterilized control. These five progenies and six soils result in 30 treatments. Every treatment was replicated five times, in five randomized complete blocks, a total of 150 pots each containing one seedling. Dead seedlings were replaced with similar sized seedlings during the first two weeks after planting. Pots were brought to the same weight, 1150 grams (20% soil moisture), by watering twice a week. Plants were grown for 12 weeks in a conditioned greenhouse with a temperature of 25°C/20°C for 16/8 hours. Plants were moved within blocks twice a week and blocks shifted position every two weeks.

After twelve weeks, plants were harvested and separated into roots, shoots and dead material. Roots were cleaned by rinsing with water, and all parts were dried at 70°C for at least 48 hours before weighing. Total nitrogen and phosphorous content of soil samples were measured (Novozamsky *et al.* 1984) and available nitrogen, phosphorous and potassium content of soil samples were measured using a 0.01 M $CaCl_2$ extraction (Houba *et al.* 1986).

Data analysis

To calculate the effect of the soil inoculum the relative difference in biomass between plants grown in inoculated soil and plants grown in soil with the same but sterilized inoculum is used. These calculations were made using plants grown in the same block.

Data were analysed using a general linear model (GLM), with origin of the inoculum and progeny as independent factors, including their interaction. One-sample t-tests were used to test for significant effects of inoculation. To test if seedling death was randomly distributed over the treatments probability of the found and extremer combinations were calculated. The biomass of the different progenies at the end of the experiment was compared using ANOVA and a Tukey B *post hoc* test. Potential differences in nutrient concentration between treatments were analysed using GLM (including Tukey B *post hoc* tests) with sterilization and inoculum origin as independent factors. All analyses were carried out using SPSS 12.0.1 (SPSS inc., Chicago, USA)

Figure 2.1: Average reduction (± s.e.) in biomass production of *Carpobrotus edulis* and *C. X cf. acinaciformis* between inoculated and sterilized soil for soils of South African and Spanish origin. Values are averaged over all genotypes, genotype itself did not have an effect and did not interact with soil origin (P > 0.05). (*** indicates difference from 0, P < 0.001).
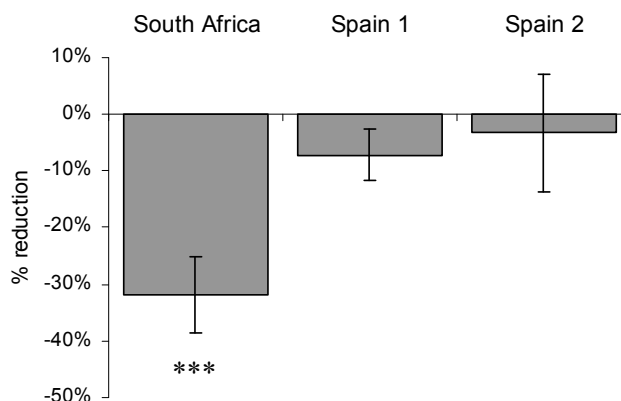
Results

Soil from the native range had a stronger negative influence on biomass than soil from the invaded range ($F_{2,55}$ = 4.105, *P* = 0.0054) irrespective of the location where the progeny was collected ($F_{4,55}$ = 0.561, *P* = 0.692). The effect of inoculation did not differ between progenies ($F_{8,55}$ = 0.973, *P*= 0.467).

Inoculating with South African soil reduced plant biomass production by 31.9 ± 6.7 %, (t_{24} = 4.737, *P* < 0.001) in comparison with the sterilized South African control. Inoculation with the two Spanish soils did not significantly reduce biomass production in comparison with their respective sterilized controls (7.1 ± 4.4 %; t_{23} =1.620, *P* = 0.119 for Spain 1 and 3.3 ± 10.0 %; t_{24} = 0.316, *P* = 0.754 for Spain 2) (fig. 2.1).

During the experiment five seedlings died. Of these, four were growing in inoculated South African soil and one in sterilized Spanish soil. This distribution significantly differs from random mortality (exact *P* < 0.01). The three seedlings that died in the first two weeks of the experiment were replaced; the two that died after two weeks were not replaced.

Plant biomass in sterilized soil did differ between progenies, ($F_{4,65}$ = 5.836, *P* < 0.01). Biomass production of plants of South African and Spanish origin was

significantly higher than the French hybrid *C. X cf. acinaciformis* (Tukey B; α = 0.05) (fig. 2.2). Biomass production of the French *C. edulis* was intermediate.

Soil nutrient contents did not differ between the soils with different inocula, except for total phosphorous, which was significantly higher in soils with an African inoculum (Tukey B, *P* < 0.05). Sterilization did not affect any of the measured nutrients, including available and total phosphorous (*P* > 0.05).

Figure 2.2: Average dry biomass (± s.e.) produced after 12 weeks for plants of different origin grown in sterilized soils. Letters indicate statistically similar subsets (Tukey HSD α = 0.05). Ce= *Carpobrotus edulis* (L.) N.E. Br., Caa= *Carpobrotus X cf. acinaciformis* (*C. edulis X C. acinaciformis* (L.) L. Bolus).
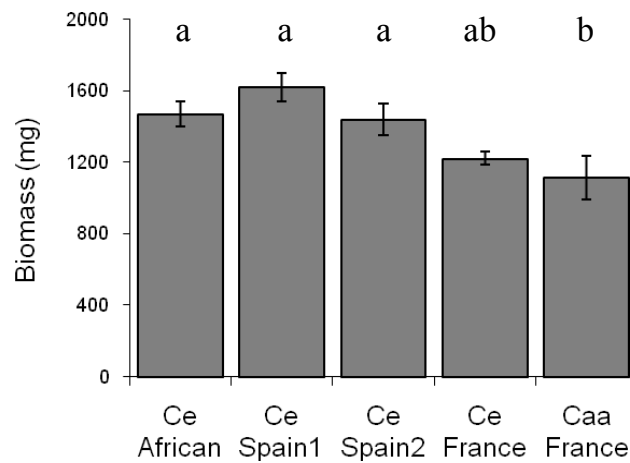
Discussion

Biomass production was strongly reduced when plants where inoculated with soil from the native range while was no significant effect of inoculation with soil from the invaded range. This supports the hypothesis that *Carpobrotus* is released from soil-borne enemies in the Mediterranean. We did not find support for the enemy release hypothesis as the invasive progenies do not show a better performance than the native progeny.

The difference between soil from the native and invasive range can be removed by sterilization, this indicates that the African inoculum contains a soil community with a strong net negative effect on plant growth. This can be explained as a stronger pathogenic activity in the African soil, as a result of greater density or stronger virulence of pathogens (or less mutualists) acting on *Carpobrotus* seedlings.

We did not find evidence for the EICA hypothesis in this species. The plants from the invaded range performed equally well as or worse than plants from the native range in sterilized soil. However, considering the small sample size this does not prove absence of EICA. We also did not find any support for hybrid vigour but this conclusion is limited as we have only sampled a single hybrid population.

The found differences in the effect of the soil community between native and invaded range can be ecologically significant. Release from soil pathogens is thought to explain the dominance of some invasive plant species (Klironomos 2002).Soil pathogens have been shown to influence succession and plant competition (van der Putten *et al.* 1993; Klironomos 2002; Kardol *et al.* 2006). Therefore a release from soil-borne pathogens can explain the dominant behavior of *C. edulis* in the invaded range

Autoclaving of soil is known to release nutrients under some circumstances and this can confound the sterility effect (Troelstra *et al.* 2001). We used inocula in a sterile bulk soil to dilute this effect and controlled for differences by measuring nutrient contents in the resulting soils. The soils did not differ in nutrients except for the concentration of total phosphorous, this being higher in soils with the African inoculum. There were no differences between inoculated and sterilized soils, therefore there confounding effects as previously mentioned can be excluded.

We acknowledge that no generalizations are possible with the limited number of sampled sites. But as typical sites have been used and soil samples are

aggregates collected throughout the populations we believe results to be of common occurrence and may represent a general phenomenon.

We conclude that release from growth reducing soil-borne factors, such as soil pathogens, plays an important role in the invasiveness of *Carpobrotus edulis s.l.* in the Mediterranean, while we did not find support for evolution of increased competitive ability.

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Reduced plant-soil feedback of plant species expanding their range as compared to natives

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Abstract

As a result of global warming, species may spread into previously cool regions. Species that disperse faster than their natural enemies may become released from top-down control. We investigated whether plants originating from southern Europe and recently established in north-western Europe experience less soil pathogen effects than native species.

We selected three plant species originating from southern Europe that have immigrated into The Netherlands and three similar native Dutch species. All six plant species were grown in sterilized soils with a soil inoculum collected from the rhizospheres of field populations. As a control we grew a series of all six plant species with a sterilized rhizosphere inoculum. We harvested the plants, added the conditioned soil to sterilized soil and grew a second generation of all six plant species in order to test for each plant pair feedback effects from the conditioned soil communities to conspecifics and heterospecifics. The effect of the soil community is dependent on plant species but on soil fertility in only one of the three pairs. Soil conditioning caused less biomass reduction to exotic plant species than to native species, suggesting that exotic immigrants are less exposed to soil pathogens than similar native plant species.

Our results suggest that plant species which expand their range as a result of climate change may become released from soil pathogenic activity. Whether the exotics are released from soil pathogens, or whether they experience enhanced benefit from mutualistic symbionts remains to be studied.

We conclude that range expansion may result in enemy release patterns that are similar to artificially introduced invasive exotic plant species. The escape from enemies through range shifts changes key biotic interactions and complicates predictions of future distribution and dominance.

Introduction

Global climate change affects the distribution and phenology of a many species (Parmesan *et al.* 1999; Tamis *et al.* 2005; Hickling *et al.* 2006). Thus far, climate change studies have mainly described and analyzed species distribution patterns (Parmesan & Yohe 2003; Hickling *et al.* 2006). Predictions of future ranges of species have been based predominantly on environmental requirements that limit a species range, so called 'climate envelopes' (Bakkenes *et al.* 2002; Thomas *et al.* 2004). The envelope approach assumes the environmental conditions of the future range will be the same as in the current range. However, environmental requirements of species are also influenced by interactions with other trophic levels, such as natural enemies, the enemies of their enemies, and mutualistic symbionts (van der Putten *et al.* 2004). While studies on phenological shifts have focussed on biotic interactions (Visser & Both 2005), these interactions have been neglected in studies that analyzed altered distribution patterns. In order to develop a more complete understanding of species distribution patterns and abundances in a warmer world, climate envelopes need to include biotic interactions within and between trophic layers. New introductions may affect biotic interactions (Liu & Stiling 2006), but whether this is also true for gradual shifts in distribution as a result of climate change is currently unknown.

As some species disperse faster than others (Hickling *et al.* 2006), range shifts due to climate change may lead to a disruption of trophic interactions in the original range, and to an assemblage of novel interactions in the new range. During trophic re-assemblage, species may become released from natural enemies and they may also be exposed to novel enemies. This process is similar to enemy release and biotic resistance, which is known from exotic invasive species that have artificially crossed geographical barriers (Elton 1958; Maron & Vila 2001; Keane & Crawley 2002). However, in contrast to exotic plants that have been released from many of their enemies by crossing geographical barriers (Wolfe 2002; Reinhart *et al.* 2003; Hinz & Schwarzlaender 2004; van der Putten *et al.* 2005), range expansion might result in partial or temporary enemy release, depending on the response of the natural enemies to the factor that drives host range expansion. To our knowledge,

enemy release has not yet been studied for plant species that immigrate into previously cold (poleward) ranges.

Soil pathogens and root herbivores generally are less capable of actively dispersing than aboveground enemies (van der Putten *et al.* 2001). Therefore, we assumed that plant range shifts could result in release from adverse soil organisms. Soil organisms are known to influence succession, plant abundance, plant competition and community composition (van der Putten *et al.* 1993; Bever *et al.* 1997; van der Putten & Peters 1997; Klironomos 2002; Kardol *et al.* 2006). They can influence plant performance negatively, through herbivorous or pathogenic activities, or they can enhance plant performance through mutualistic interactions or improved plant nutrition by decomposition of soil organic matter (Wardle *et al.* 2002). Plants, in turn, can also influence soil organisms, e.g. via input of organic matter, or rhizodeposition (Ehrenfeld *et al.* 2005). The net effects of all positive and negative interactions between plants and soil organisms is called plant-soil feedback (Bever *et al.* 1997). Separating positive and negative effects in plant-soil feedback is difficult, because individual interactions can cause chain effects, and some interacting biota can have positive or negative effects depending on genotype and environmental conditions (Borowicz 2001; Klironomos 2002; Wolfe *et al.* 2006). The effects of isolated biota can be different from their effects when occurring in a rhizosphere community. This advocates a focus on the net effect of the soil community rather than attempting to separate individual positive and negative components. Due to enemy release, plant-soil feedback effects could be more negative for local plants than for species that colonize a formerly cold range (so called thermophilic neophytes) (Tamis *et al.* 2005). When these exotic species have a less negative net feedback from the local soil community than most native species this would give them an advantage in interspecific interactions, ultimately resulting in greater dominance than in their original habitat (Klironomos 2002).

One approach to study enemy release of exotic plant species is to compare the response of a species in its native and new range (Wolfe 2002; Hinz & Schwarzlaender 2004; Hierro *et al.* 2005). However, this approach is less effective when the precise origin of the exotic plants is unknown (Reinhart *et al.* 2005). An alternative is to make a phylogenetic, or ecological, comparison between exotic species and similar related native species (Agrawal *et al.* 2005; Carpenter & Cappuccino 2005). We took the latter approach and compared plant biomass

production of three exotic and three related or ecologically similar native species. All plants were grown in sterilized soil to which an inoculum from the rhizosphere of natives and exotics in the invaded range was added. This resulted in a conditioned soil containing a soil community specific to that plant species, which was used to assess plant-soil feedback in the second part of the experiment. The confounding effects of nutrient release by sterilization (Troelstra *et al.* 2001; McNamara *et al.* 2003) were counteracted by using a relatively small inoculum in a sterilized bulk soil and addition of a nutrient treatment.

In the plant-soil feedback experiment, we tested the hypothesis that exotic plants would have less negative plant-soil feedback than the native species (Reinhart *et al.* 2003; Callaway *et al.* 2004). In the cross-inoculation experiment, we grew native species on soil inoculated with soil conditioned by the exotics, and *vice versa.* Here, we tested the hypothesis that the exotic plants may accumulate local pathogens, so that they could have indirect negative effects on the performance of related native species (Eppinga *et al.* 2006). We discuss our results in relation to climate change effects on plant release from soil-borne enemies and on possible indirect effects of the exotic plants on the performance of native plant species through soil feedback.

Materials and methods

Plant species selection
Three exotic plant species (*Heracleum mantegazzianum, Tragopogon dubius* and *Eragrostis pilosa)* were selected and compared with three native species (*Heracleum sphondylium, Tragopogon pratensis* and *Poa annua*, respectively) that naturally co-occur with the exotic species in The Netherlands. Two species pairs were of the same genus and one species pair involved species with similar ecology. Invading plant species may not necessarily be a random sample from the total species pool. In our selection of native species from the Dutch flora, we choose species that themselves have become invaders in other regions of the world (Clements *et al.* 1999; Cody *et al.* 2000; Ryan *et al.* 2003; Page *et al.* 2006).
Heracleum mantegazzianum Somm. & Lev. (Apiaceae) is a monocarpic perennial species that can become 3 m tall, and is native to the Caucasus and South-West

Asia. It was introduced as an ornamental into Western Europe in the 19th century, and it has spread northwards in the 20th century. Nowadays it is considered naturalized in The Netherlands (Weeda *et al.* 1987). *Heracleum mantegazzianum* is a relatively common plant of enriched disturbed habitats, such as road verges and public parks; it also occurs in forest edges (Weeda *et al.* 1991). *Heracleum sphondylium* L. is smaller (<1.50 m) than *H. mantegazzianum* and is found in the same habitat. Both *Heracleum* species often co-occur and sometimes produce hybrids (Grace & Nelson 1981). *Heracleum sphondylium* was introduced and became established in Canada (Page *et al.* 2006).

Tragopogon dubius Scop. (Asteraceae) originates from central and southern Europe and has extended its range northwards in the 20th century. In the 1950's *T. dubius* was first found in The Netherlands and in the 1980's it started spreading naturally along railways. Currently, *T. dubius* occurs throughout large parts of The Netherlands at sites with a warm microclimate (Weeda *et al.* 1991). The native *T. pratensis* L. is morphologically very similar to *T. dubius* and also occurs in road verges, on dikes and in semi-natural grasslands. *Tragopogon pratensis* is naturalized in Canada and Northern USA (Clements *et al.* 1999).

Eragrostis pilosa (L.) P. Beauv. (Poacaeae) is a C4 grass of warm and temperate regions, including southern and central Europe. It was first found in The Netherlands in 1958 and started to spread naturally in the 1970's (Weeda *et al.* 1994). After an apparent lag phase, it is now a very common species in The Netherlands (Tamis & van 't Zelfde 2003) occurring on sidewalks, parking lots and on highly disturbed road verges. This species is paired with *Poa annua* L. (Poacaeae), which is a C3 grass, and therefore less drought tolerant. It occurs in habitats similar to *E. pilosa*. *Poa annua* has become cosmopolitan by human dispersal (Ryan *et al.* 2003).

Soil and seed collection

Soil from the rhizosphere of all six plant species was collected from populations in The Netherlands. Soil samples from *Eragrostis*, *Poa* and *Heracleum spp.* were collected from the centre of The Netherlands (51°58N, 5°39E), soil from *Tragopogon pratensis* was collected along the river Waal (51°48N, 5°19E) and *Tragopogon dubius* in the west of The Netherlands (52°10N, 4°30E). For each species, a total of 20 litre of soil was collected from the root-zone of 20 plants. The soil of each plant

species was sieved through a 5 mm mesh size to remove roots and coarse fragments. Soils were homogenized and split into two fractions, one of which was sterilized by gamma irradiation (>45 kGray), which effectively eliminates all soil biota (McNamara *et al.* 2003). The sterilized and non-sterilized soils were used as inoculum and homogenized at a 1:6 w/w ratio with a sterilized mixture of sandy loam soil and river sand (1:5 w/w, sterilized by gamma irradiation >45 kGray). Pots (diameter 14 cm, height 13 cm) were filled with 1100 g of the resulting soil mixture. Five samples were taken for nutrient analysis per soil type (see Appendix S1 in Supplementary material).

Seeds were collected from the same plant populations as used for soil collection except for *T. pratensis*, which were bought from a small company that collects seeds from wild Dutch populations. Seeds of both *Heracleum* species were stored at 7°C for six weeks during which they were soaked in water once a week to break dormancy. All seeds were germinated on sterilized mineral sand.

Conditioning experiment

In a conditioning experiment, all three plant pairs were grown in soil with a sterilized or non-sterilized inoculum of con- or heterospecific origin. Half the pots received additional nutrients. There were five replicates of each treatment, resulting in: 2 (inoculum origin) * 2 (sterilization) * 2 (nutrients) * 5 (replicates) = 40 pots per species, and 240 pots in total. Of each forb species one similar sized seedling was planted in each pot, whereas two seedlings were planted per pot in the case of grasses.

The pots with additional nutrients received 50 ml nutrient solution pot^{-1} $week^{-1}$ (KNO_3 12.5 mmol/l; $Ca(NO_3)_2$ 6.50 mmol/l; $MgSO_4$ 3.75 mmol/l; $NH_4H_2PO_4$ 7.50 mmol/l FeEDTA 0.21 mmol/l) starting after three weeks and maintained until harvest. This resulted in 12.3 mmol N, 3.8 mmol P and 6.3 mmol K per plant, or an addition of approximately 1.5 to 2 times the available N and 2.5 to 4 times the available P and K at the start of the experiment. Nutrients were supplied during watering so that pots without nutrient addition received the same amount of water.

Pots were placed in five randomized complete blocks in a greenhouse with 70% relative humidity, 16/8 hour light/dark at 20/15°C. Philips SOL-T armatures were used for additional lighting, providing 80 µmol m^{-2} s^{-1} when sunlight was insufficient.

Twice a week, soil moisture was reset to 20% using a Theta probe and weighing. In between, water was supplied to compensate for evapotranspiration. Twice a week ripe seeds of *P. annua* were removed and stored. Total seed biomass was added to the final dry weight (determined after 12 weeks when plants were harvested, separated into shoots and roots, dried at 70 °C for at least 48 hours and weighed). Soil from each individual pot was stored separately in plastic bags.

Feedback experiment

Feedback responses were determined by growing every species in soil inoculated with soil conditioned by a conspecific to test plant-soil feedback (Reinhart *et al.* 2003; Callaway *et al.* 2004). To test for the effect of the species on each other through the soil community (Eppinga *et al.* 2006) plants were also grown in soil conditioned by the other species within the species pair. Prior to the feedback experiment, the replicates of the conditioned soils of each species were homogenized and split into two portions, one to be sterilized by autoclaving for three hours at 121.5 °C and the other to remain non-sterilized. The conditioned soils were used as inoculum and homogenized at a 1:6 w/w ratio with the same sterilized substrate as used for the conditioning phase. For each treatment there were five replicates. Pots were placed in five randomized complete blocks in a greenhouse with conditions described above, except that no additional nutrients were added. Plants were harvested after 12 weeks and root and shoot biomass determined after drying at 70 °C for at least 48 hours.

Data analysis

The difference between the biomass of plants grown in soil inoculated with sterile and non sterile soil was calculated as percentage of the biomass of plants inoculated with sterile soil. This value is the proportional reduction in biomass production by the inoculated soil community.

$$Inoculation\ Effect = \frac{Biomass\ Non\ Sterile\ Inoc - Biomass\ Sterile\ Inoc}{Biomass\ Sterile\ Inoc} \bullet 100\%$$

For the conditioning experiment, a general linear model was applied with pair, origin of the plant species (native vs. exotic), inoculum origin (native vs. exotic) and nutrients as independent factors including all their interactions, and inoculation effect as the dependent variable. Data were then analysed pair-wise with the same model,

excluding plant pair as a factor. To test if inoculation effects differed from one-sample t-tests were used. In the feedback experiment a general linear model with origin of species (native vs. exotic) and plant pair was used for both co- and heterospecific treatments. All analyses were carried out using SPSS 12.0.1.

Table 3.1: Mean effect of inoculation (and standard error), calculated as a reduction of biomass relative to sterile soil, for every combination of plant species, inoculum and nutrient level in the conditioning experiment. Significant reduction is indicated by * (T-test, $P < 0.05$)

Plant species	inoculum	nutrient poor		nutrient rich	
H. mantegazzianum	H. mantegazzianum	-0.15	(0.13)	-0.02	(0.26)
	H. spondylium	0.10	(0.26)	0.09	(0.20)
H. spondylium	H. mantegazzianum	-0.30*	(0.09)	-0.19*	(0.06)
	H. spondylium	-0.34*	(0.07)	-0.12	(0.06)
T. dubius	T. dubius	-0.11	(0.10)	0.30	(0.24)
	T. pratensis	-0.34*	(0.06)	-0.06	(0.12)
T. pratensis	T. dubius	-0.37*	(0.02)	-0.04	(0.16)
	T. pratensis	-0.46	(0.05)	0.34	(0.18)
E. pilosa	E. pilosa	-0.28*	(0.05)	-0.29	(0.14)
	P. annua	-0.20	(0.11)	0.02	(0.23)
P. annua	E. pilosa	-0.10	(0.11)	0.18	(0.33)
	P. annua	-0.27	(0.11)	-0.39*	(0.09)

Results

Conditioning experiment

In an overall ANOVA (see Appendix S2) we tested the effect of plant status (native or exotic), inoculum, plant pair and nutrient addition on the biomass production in inoculated *versus* sterilized soil (= inoculation effect). There were no significant main effects of status, inoculum origin, plant pair or nutrients ($P > 0.05$). However, there was a significant three-way interaction between status, inoculum origin, and plant pair ($F_{2,96} = 4.659$, $P = 0.012$). Other interactions were not significant ($P > 0.05$). Therefore, we decided to carry out three-way ANOVA's (see Appendix S2) for the three plant pairs in order to test the effect of status, inoculum origin, and nutrients on the inoculation effect.

For *Heracleum* the inoculation effect differed between the two species ($F_{1,30}$ = 4.238, P = 0.048, table 3.1). Soil inoculation reduced the native *H. sphondylium* more than the exotic *H. mantegazzianum*. Other main factors and their interactions did not influence the inoculation effect (inoculum origin: $F_{1,30}$ = 0.623, P = 0.436; nutrient level: $F_{1,30}$ = 0.936, P = 0.341). Therefore, although nutrient addition resulted in an 8.1-fold increase in biomass for *H. mantegazzianum* and an 11.3-fold increase for *H. sphondylium*, this did not influence the inoculation effect.

For *Tragopogon* the inoculation effect was influenced by nutrient addition ($F_{1,32}$ = 22.178, P < 0.0001), but not by species ($F_{1,32}$ = 0.738, P = 0.397) or inoculum origin ($F_{1,32}$ = 0.690, P = 0.412). There was no interaction between any of the treatments. While nutrient addition enhanced biomass 8.1- and 7.8-fold for *T. dubius* and *T. pratensis*, respectively, it tended to reduce the inoculation effect.

For *E. pilosa* and *P. annua* the inoculation effect did not differ between species ($F_{1,32}$ = 0.000, P = 0.952), inoculum origin ($F_{1,32}$ = 0.001, P = 0.927), or nutrients ($F_{1,32}$ = 0.000, P = 0.981). However, there was a significant interaction between species and inoculum origin ($F_{1,32}$ = 5.314, P = 0.028). Nutrient addition resulted in a 3.3- and 3.9-fold increase in biomass for *E. pilosa* and *P. annua*, respectively, without influencing the inoculation effect.

Feedback experiment

In a two-way ANOVA (see Appendix S2 and S3), the inoculation effect on conspecifics (= within species) differed between native and exotic species ($F_{1,30}$ = 15.95, P < 0.001), but not between species pairs ($F_{2,24}$ = 1.669, P = 0.210). There was no interaction between inoculum origin and species pair ($F_{2,24}$ = 0.795, P = 0.463). The main effect on natives *vs.* exotics was due to native species having a stronger negative inoculation effect than the exotic species. On average, inoculation reduced the biomass of the native species by 35% (SE = 4.5%), whereas the biomass of the exotic plant species were reduced by 13% (SE = 3.2%) (fig. 3.1). In all plant species in all soils the plants on sterilized soil had a significantly higher biomass than the plants in the inoculated soils (one-sample t-test p<0.05). Therefore, while the biomass of all plant species was reduced by inoculation with soil conditioned by a conspecific, this was more severe in the case of native, as opposed to exotic, species.

The feedback effect of the inoculum conditioned by the other species in the pair (the heterospecific) was not different between the native and exotic species ($F_{1,24}$ = 2.883, P = 0.102) or species pairs ($F_{2,24}$ = 1.669, P = 0.210) and there was no interaction between species and species pair ($F_{2,24}$ = 0.162, P = 0.852) (fig. 3.2). On average the biomass of the native species was reduced by 17% (SE = 6.0%) by inoculation, whereas the exotic plant species were reduced by 28 % (SE = 2.6%). Therefore, the feedback effects appeared to be species specific within pairs of native and exotic species.

In order to test our second hypothesis, we compared the feedback effect of the inoculum conditioned by the exotic species between the native and the exotic species. Interestingly, there was no difference in feedback from the soil conditioned by the exotic plant to the exotic and the native plant species ($F_{1,24}$ = 0.239, P = 0.309). This shows that, in their own soil, exotic species did not have a more positive soil feedback effect than a related native species. This result also implies that the exotic species may not exert indirect negative effects, either through the soil community or through allelopathic effects, to (related) native species.
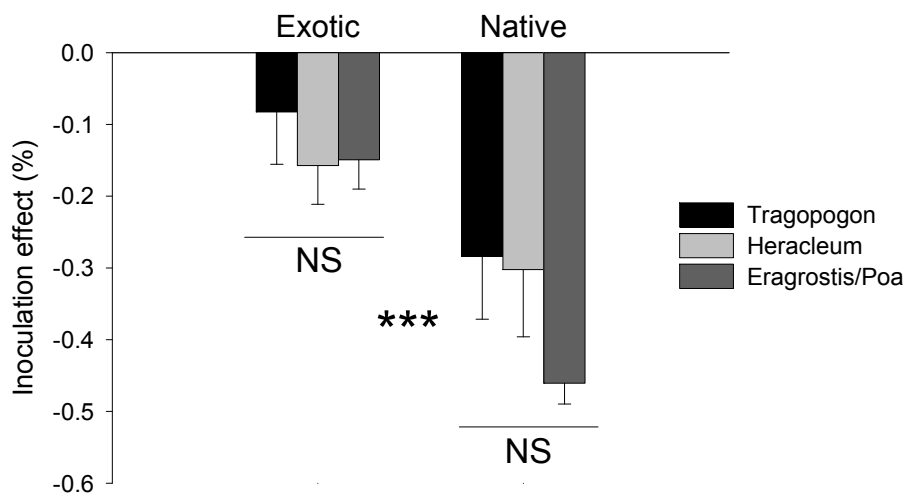
Figure 3.1: Soil inoculation effect, calculated as the reduction in biomass relative to sterile soil, for exotic and native species grown in soil inoculated with soil conditioned by the same species. Mean values (± standard error) and significance are shown between natives and exotics, and between species within those two groups. NS indicates not significant, *** P < 0.001 (ANOVA).
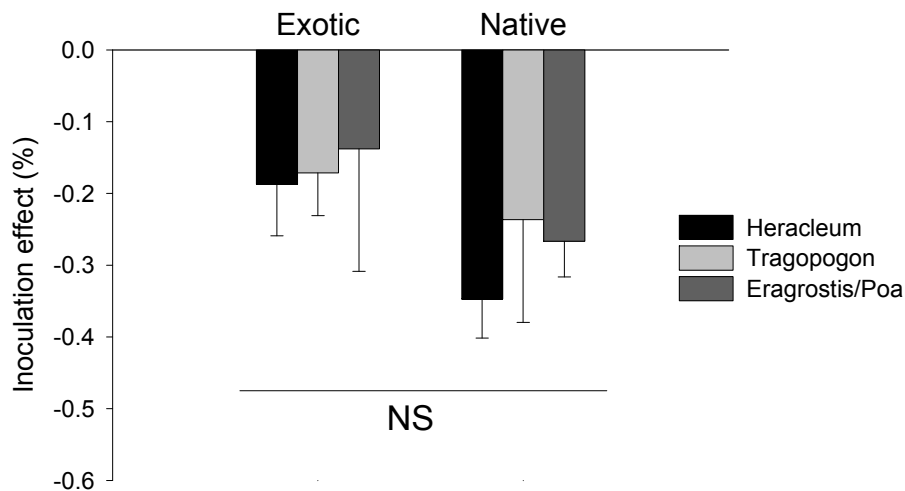
Figure 3.2: Soil inoculation effect, calculated as the reduction in biomass relative to sterile soil, for exotic and native species grown in soil inoculated with soil conditioned by the other species within the same native/exotic plant pair. Mean (± SE) are shown. NS indicates not significant (ANOVA).

Discussion

Our results show that exotic plant species that have colonized north-western Europe from southern climate regions have a less net negative plant-soil feedback than similar species which are native in the new range. Whereas plant-soil feedback reduced the average biomass production of native species by 35%, the exotic species demonstrated only a 13% reduction. It appears that the exotic species are less negatively affected by the soil community, either due to reduced exposure to pathogens or due to more effective mutualists, such as arbuscular mycorrhizal fungi or even allelopathic effects. This difference is absent in the reciprocal comparison, indicating that it is an effect of the interaction between plant species and soil community. Our results show that the net pathogenic activity must be specific at the species level, such as has been demonstrated for dune plants that succeed each other (van der Putten *et al.* 1993).

Besides specificity, another prerequisite for enemy release along a climate gradient is that the soil pathogens are less mobile than their host plants. In contrast to agricultural systems (e.g. Levenfors & Fatehi 2004), the specificity and mobility of soil pathogens in natural systems has been poorly studied. Escape from soil

pathogens has been shown at local scales (D'Hertefeldt & van der Putten 1998; Blomqvist *et al.* 2000; Olff *et al.* 2000; van der Stoel & van der Putten 2006) and at large spatial scales (van der Putten *et al.* 1993; Beckstead & Parker 2003; Reinhart *et al.* 2003; van der Stoel & van der Putten 2006). Little is known, however, about the role of dispersal of soil organisms in the population dynamics of their host plants. Soil pathogens are thought to be relatively immobile when compared to most aboveground plant enemies (van der Putten *et al.* 2001) and our results suggest that range expansion of plants, for example due to climate change, may, at least temporarily, release the plants from their natural soil-borne enemies. Range expansions are often considered as natural, gradual phenomena and invasion of exotics as abrupt ones, due to anthropogenic introduction. This would imply that different mechanisms underlie these processes. Here, we show that the effects can be comparable. Although biotic interactions are often neglected in climate change studies (Schmitz *et al.* 2003; Brooker *et al.* 2007), the need to incorporate their effects in predictions of future distributions is increasingly recognized (Davis *et al.* 1998), as is also demonstrated in our study.

The plants used in the present study represent three of the largest families of the European flora which themselves are unrelated. The different pairs of plants occur in different habitats and are different functional types. The similarity observed between the pairs, therefore, can not be attributed to a phylogenetic or ecological bias but indicates that a reduced plant-soil feedback is common amongst expanding plant species.

Experiments with sterilized soil are often confounded by nutrient release due to the sterilization procedure (Troelstra *et al.* 2001; McNamara *et al.* 2003). It is difficult to tease apart positive effects of eliminating soil pathogens, negative effects of killing symbionts and positive effects due to enhanced availability of mineral nitrogen and phosphate. In our experiment the inocula were used in a 1:6 w/w ratio in a sterilized bulk soil, thus reducing the difference in nutrient content between treatments as the bulk soil has the same nutrient input for all treatments. In the conditioning experiment we would expect that addition of nutrients would decrease the difference between biomass production with sterilized or non sterilized inocula if this was due to nutrient release. In two of the three pairs the nutrient level did not influence the effect of inoculation even though the addition of nutrients strongly increased biomass production. It is, therefore, unlikely that the observed effects were

caused by nutrient release due to sterilization of the inocula. In the third pair, *Tragopogon*, the addition of nutrients did reduce the inoculation effect. The amount of nutrients added was relatively large compared to the soil nutrient content (app 1) and had a strong effect on biomass production (3 to 11 fold increase). The differences between native and exotic species in the feedback experiment cannot be explained by differences in nutrient availability as this would result in the same pattern in the reciprocal treatment and not in a species specific result.

Abiotic plant-soil feedback responses via allelopathic effects have also been reported (Pellissier 1998; Souto *et al.* 2001) and the structure of some allelochemicals present in the soil can change due to autoclaving, thus causing an apparent pathogenic effect (McPherson & Muller 1969). In particular *Heracleum* species are known to exhibit allopathic effects on other plant species (Junttila 1975; Myras & Junttila 1981). Allelopathic substances typically have a more negative effect on heterospecifics than on conspecifics (Inderjit *et al.* 2005) and this would result in a strong feedback effect to the other species in the pair, but not to itself. We did not observe such an effect and therefore it is less likely that allelopathy has played an important role in our study. Moreover, we diluted rhizosphere soil by using a sterilized standard bulk soil and this also would have reduced the direct effects of allelopathy.

The effect of the heterospecific soil community was the same for both native and exotic species. Futhermore, exotic and native plants did not differ in the conditioning phase. This means that when establishing, native and exotic species do not show different responses and that the advantage of exotic species over native species becomes apparent only in the feedback stage. Plants have been suggested to gain an advantage of plant-soil feedback even if the direct effect is negative, for example when the soil community developed in association with a species has a more negative effect on competitors than on the species itself (Eppinga *et al.* 2006). However, in our study the effect of the soil conditioned by the exotic plants on the native and exotic plants did not differ, so at least in these species this mechanism does not play a role.

Plant species colonizing a new area often exhibit reduced pathogen and/or herbivore load, potentially increasing their competitive ability, growth and reproductive output (Blaney & Kotanen 2001; Mitchell & Power 2003; Callaway *et al.* 2004; Van der Putten *et al.* 2005). Recently, soil pathogens have been identified as a

potentially important group of organisms that can drive plant colonization and competition processes (Torchin & Mitchell 2004; Agrawal *et al.* 2005; Van der Putten *et al.* 2005). Here we show that the negative effect of soil pathogens on plant growth can be quite substantial and differs between native plant species and species that recently colonized an area, even when this is due to a range expansion rather than due to a remote introduction. To study the effects of the soil community as a whole is helpful and sufficient to understand general mechanisms (Colautti *et al.* 2004). Further studies, however, need to identify key soil organisms and their roles (Brinkman *et al.* 2005). This might provide insight to the relative importance of the different groups of organisms and underlying mechanisms for the different plant species. Separating the effects of pathogens and mutualists (Richardson *et al.* 2000) is especially important in order to distinguish the positive and negative components of the net feedback.

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Supplementary Material
The following supplementary material is available online:

Appendix S1: Soil nutrient content
Appendix S2: ANOVA tables
Appendix S3: Mean and SE for biomass measurements.

Plant-soil feedback of native and range expanding plant species is insensitive to temperature.

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Elmar M. Veenendaal

Abstract

Temperature change affects many above and below ground ecosystem processes. Although effects of temperature on decomposition of soil organic matter have been widely explored, the net effects of temperature-induced changes in the soil on plant community development have received far less attention. Here we investigate the effect of a 5°C temperature increase on plant-soil feedback. As global warming results in pole ward range shifts of plant species, we compare plant species from a temperate climate region with immigrant plants that originate from warmer regions. We tested whether the magnitude of plant-soil feedback is affected by ambient temperature and whether the effect of temperature differs between these groups of plant species. We selected six European/Eurasian plant species that recently colonized The Netherlands (non-natives), and six species (natives) from The Netherlands. During a conditioning phase, all plant species were grown for twelve weeks in sterilized soil with a live soil inoculum. In a second phase, all plant species were grown in conditioned conspecific soil and, as a control, in a mixture of all conditioned soils. Plant-soil feedback was determined by comparing performance in the con- and heterospecific soils. In order to test the effect of temperature on these plant-soil feedback interactions, the experiments were performed at two greenhouse temperatures (20/15°C and 25/20°C).

Whereas soil inoculation had the same effect on natives and non-natives, soil conditioning resulted in a negative feedback to natives, but a positive feedback to non-natives. In both cases, plant-soil interactions were not affected by temperature. Therefore, we conclude that climate warming does not affect the direction, or strength of plant-soil feedback, nor for native nor for non-native plant species. However, as the non-natives have a more positive soil feedback than natives, climate warming may introduce new plant species in temperate regions that have less soil-borne control of abundance.

Introduction

As a result of global climate change, ecological interactions between organisms are changing (e.g. Voigt *et al.* 2003; Roy *et al.* 2004; Visser & Both 2005).This is also true for the interaction between plants and the decomposer community in the soil (Walther 2004; Cornelissen *et al.* 2007). The soil community, however, does not just consist of the decomposer community, as it also contains organisms that directly interact with plants, such as mutualists and pathogens (Bever 1994; Klironomos 2002; Wardle *et al.* 2004). The sensitivity of the interaction between plants and these groups of soil organisms to temperature changes is currently unknown.

Feedback effects between plants, soil organisms and physical-chemical soil conditions play a major role in structuring the composition and dynamics of natural plant communities (van der Putten *et al.* 1993; Bever 1994; Ehrenfeld *et al.* 2005). Plant-soil feedback is the phenomenon that plants influence properties of the rhizosphere, which in return influence the performance of the same or other plants (Bever *et al.* 1997). The effect of plant-soil feedback can be positive, through enhanced nutrient availability or the accumulation of mutualists in the rhizosphere, or negative due to depletion or immobilization of nutrients, or the accumulation of root herbivores and soil pathogens (Wardle *et al.* 2004). Plant-soil feedback includes direct interactions between plant roots and mutualists, herbivores and pathogens, but also indirect interactions between plants and decomposer organisms that are responsible for nutrient turnover (Wardle *et al.* 2004). Although both direct and indirect interactions can be affected by global warming, effects of temperature on indirect interactions-related pathways have received most attention, especially litter decomposition (Cornelissen *et al.* 2007). Decomposition, soil respiration and mineralization tend to increase with increasing ambient temperature (Bardgett *et al.* 1999; Davidson & Janssens 2006; Bengtson & Bengtsson 2007), The effect of an increased temperature on direct interactions, such as those between plants and pathogens or mutualists have remained largely unclarified. In the present study the effects of atmospheric temperature on plant-soil feedback as a whole, including both direct and indirect aspects, is tested.

The direct effect of an atmospheric temperature increase on plant-soil feedback is the sum of the effects on many components and their interactions. The outcome of plant-soil feedback can be positive, negative or neutral for plant

performance (Klironomos 2002; Kardol *et al.* 2006). A temperature increase can change this outcome through changes in decomposition and mutualistic effects but also pathogenic activity. Therefore, the net effect of atmospheric temperature on plant-soil feedback cannot yet be predicted.

Apart from local effects of climate warming on community composition and dynamics, warming also causes pole-ward shifts of many plants and other species groups (Parmesan & Yohe 2003; Tamis *et al.* 2005; Hickling *et al.* 2006). As not all species shift their range at the same speed, biotic interactions between plants and other organisms can become disrupted at a local scale (van der Putten *et al.* 2004; Visser *et al.* 2006). Plants with well dispersed seeds can expand their range quite fast (Higgins & Richardson 1999), whereas soil-borne pathogens lack targeted dispersal (van der Putten *et al.* 2001). This may result in a temporary release from soil-borne enemies of range expanding plant species, so called thermophylic neophytes (Tamis *et al.* 2005; van Grunsven *et al.* 2007). While release from soil-borne enemies has been reported for non-native plant species that colonize new continents (Klironomos 2002; Reinhart et al. 2003) such effects of climate warming on local plant-soil interactions have received little attention. One study showed that three plant species from warmer climate regions had a less negative soil feedback in their new range than plants which are native in that range (van Grunsven et al. 2007). However, it is not known if plant-soil feedback effects are sensitive to ambient temperature and if plants from temperate regions differ from plants from warmer climate regions in their response to temperature.

The main question addressed in the present study is how plant-soil feedback may be affected by an elevated ambient temperature and whether warming affects soil feedback of native plants and non-native plants differently. As a null hypothesis, we expect no differences of warming on plant-soil feedback. Alternatively, warming can result in a change in plant-soil feedback, either in positive or in negative direction.

Additionally, we expected plant species from warm climate regions to benefit more from high ambient temperature than the plants that were native to the temperate zone.

Materials and Methods

Plant species with a first record in The Netherlands after 1900 and a European origin were selected from the standard list of the Dutch flora (Tamis et al. 2004). Each plant was paired with a native species (present before 1500 AD) that has both a comparable ecology, life history, morphology and that is phylogenetically related (same genus or same family). Many selected species were rejected on practical grounds (unavailable seeds, parasitic or aquatic plants, poor germination, etc.) and if a similar native species was unavailable. Thus, we ended up with six plant pairs from four different families. We selected two plant pairs from the families *Asteraceae* and *Chenopodiaceae*. These two families contain many neophytes in the Dutch flora (Tamis et al. 2004). The 12 selected species are (non-native/native): *Chenopodium botrys* L. /*Ch. polyspermum* L.; *Corispermum intermedium* Schweigg. /*Ch. album* L.; *Eragrostis pilosa* (L.) P. Beauv. /*Poa annua* L.; *Geranium lucidum* L. /*G. molle* L.; *Senecio vernalis* Waldst. & Kit. /*S. vulgaris* L. and *Tragopogon dubius* Scop. /*T. pratensis* L. (table 4.1).

Table 4.1. Origin and seed source of the used plant species. Seed origin: B&T Worldseeds, Paguignan, 34210 Aigues-Vives, France and Cruydt-hoeck, P.O. box 88, 9400 AB Assen, The Netherlands

Species	Invasive in other continents	Seed origin	First record	Native range
Poa annua	yes	Collected in NL	Indigenous	Global
Eragrostis pilosa	yes	Collected in NL	1958	Southern Europe
Senecio vulgaris	yes	B&T worldseeds	Indigenous	
Senecio vernalis	unknown	Cruydt-hoeck	1915	Eastern Europe
Tragopogon pratensis	yes	Cruydt-hoeck	Indigenous	
Tragopogon dubius	yes	Collected in Austria	1950's	Central Europe
Chenopodium album	yes	Cruydt-hoeck	Indigenous	
Corispermum intermedium	yes	Collected in NL	1900	Central Europe
Chenopodium polyspermum	yes	B&T worldseeds	Archeophyte (< 1500)	Europe
Chenopodium botrys	yes	Collected in NL	1900-1924	Mediterranean
Geranium molle	yes	B&T worldseeds	Indigenous	
Geranium lucidum	yes	B&T worldseeds	1975-1999	Central and Southern Europe

Conditioning phase

Soils were conditioned by growing plants for 12 weeks in sterilized soil inoculated with a non-sterile or sterile general soil-inoculum. During this period, specific soil communities of plant species develop (Bever *et al.* 1997; Klironomos & Hart 2002; Kardol *et al.* 2007). Seeds were collected, or purchased from commercial suppliers that collect seeds from wild plant populations (table 4.1). Soil (top layer, 25 cm deep) was collected from an ex-agricultural grassland in Wageningen, The Netherlands, where none of the species that were used in the experiment occurred. This grassland is relatively rich in plant species, so that it is expected to contain a large collection of soil biota. The soil was homogenized and split in two halves of which one was sterilized by autoclaving at 121.5 °C for 3 hours to serve as a sterilized control. The other half was used as a non-sterile inoculum source. The mineral sub-soil from the same grassland was collected and autoclaved (121.5 °C for 3 hours) to form the bulk soil to which inocula were added before establishing the plants. Half of the inocula and substrate was stored at 4°C for later use.

The sterilized bulk soil was inoculated with the non-sterilized or sterilized top soil at a 1:5 (w/w) ratio. Pots of 19 cm ø and 15 cm height were filled with 2200 g of the resulting soil mixture (15% moisture w/w). These pots were planted with seedlings that were germinated in trays with autoclaved river sand. Each pot was planted with three similar sized seedlings of the same species. The pots were equally distributed over four greenhouses, two with 20/15°C (Day-Night) and two with 25/20°C. The greenhouses had a day/night vapour pressure deficit of 0.70/0.51 kPa for both temperatures, Philips SOL-T armatures were used for additional light to a minimum of 80 µmol m^{-2} s^{-1} between 6:00 and 22:00 h. We used three randomized complete replicate blocks per greenhouse, so that the experimental design consisted of 12 species (6 pairs), grown in two soil treatments in 4 greenhouses (two with a low and two with a high temperature) with three replicates per greenhouse and three plants per pot. This experimental design resulted in 288 pots.

Plants were watered every other day and harvested after 12 weeks of growth. Shoots were dried for minimally 48 hours at 70°C and weighed. In order to prepare the soil for the feedback phase, coarse roots were removed to prevent re-sprouting, but fine roots were left in the soil to serve as inoculum, as most microbial activity is situated in the rhizosphere. This procedure, therefore, prohibited determination of

root biomass. Soil that had been inoculated with sterilized inoculum was not used for the feedback phase, because likely colonization by air-borne microorganisms during this growth period.

Feedback phase

Plant-soil feedback was examined by comparing biomass production of plants grown in conspecific conditioned soil with plants grown in a mixture of soils conditioned by all the different pecies, the latter forming a heterospecific control. The con- and heterospecific soils were prepared as follows: Conditioned soil that originated from non-sterilized inocula was collected from each individual pot, homogenized and split into two parts of 1000 gram. One part was placed in a new pot serving as conditioned conspecific soil. The other part was mixed with all other conditioned soils from the same block and then subdivided over individual pots, serving as heterospecific soil. Since there were 12 plant species the specific soil community was diluted 12-fold. Effects on plant performance of this level of dilution are not detectable (van der Putten *et al.* 1988). Factors as decomposition, nutrient uptake and mineralization are not species specific and therefore not diluted but merely averaged.

Both the conspecific and heterospecific soils have been used in the previous growth phase and nutrients have been taken up by the plants growing in these soils. Therefore nutrients were added to both soils during this growth phase to correct for this nutrient uptake in the conditioning phase. From the fourth week until harvest 25 ml of a nutrient solution (KNO_3 12.5 mmol/l; $Ca(NO_3)_2$ 6.50 mmol/l; $MgSO_4$ 3.75 mmol/l; $NH_4H_2PO_4$ 7.50 mmol/l; FeEDTA 0.21 mmol/l) was added weekly.

Additionally the soil that had been stored in the cold storage during the conditioning phase was used to test the effect of non-conditioned inocula on plant performance simultaneously with the conditioned soils. New inoculated and sterilized treatments were made with the cold stored material in the same manner as in the conditioning experiment.

Pots with a diameter of 14 cm ø and 13 cm height were filled with 1000 g of soil. The seeds were treated in the same manner as in the conditioning phase, with the distinction that one seedling was planted per pot instead of three. Pots were placed in randomized complete blocks in the same greenhouses. As there are twelve plant species, four soil treatments (conspecific, heterospecific, unconditioned and

sterilized), four greenhouses over two temperatures, and three replicates per greenhouse this resulted in 576 plants. After 12 weeks all plants were harvested, roots were washed to remove the soil and both root and shoot biomass was dried at 70°C for minimally 48 hours and weighed.

Data analyses

The conditioning and feedback phases of the experiment were analysed separately as they were not performed simultaneously. For the feedback phase the non-conditioned and sterilized soils were analysed separately from the conspecific and heterospecific soil treatment; these treatments test different hypotheses (effect of inoculation and plant-soil feedback, respectively) and both have their own control. For all three analyses a mixed model was used with biomass as dependent factor (only aboveground for the conditioning phase as roots were not harvested). The effect of temperature was tested with greenhouse as a random factor. In this way the effect of temperature is tested with greenhouses and not pots as replicates (resulting in two replicates per temperature). The effect of plant origin (native or non-native) was tested with species as error term; species was entered as a random factor nested within origin. Normality and homogeneity of variance were assessed by visual inspection of the residuals and assumptions for ANOVA analyses were confirmed.

Additionally, soil feedback was analysed based on total biomass by calculating (conspecificsoil-heterospecific soil)/heterospecific soil, resulting in a proportional reduction (indicating negative feedback) or increase (indicating positive feedback) in biomass in conspecific soil compared to the heterospecific control. Replicates were obtained by pairing plant biomass originating from the conspecific and heterospecific soil from the same block. As the residuals were not normally distributed in this analysis, Mann-Whitney U tests were used to test if the effect of feedback was affected by temperature and/or nativeness.
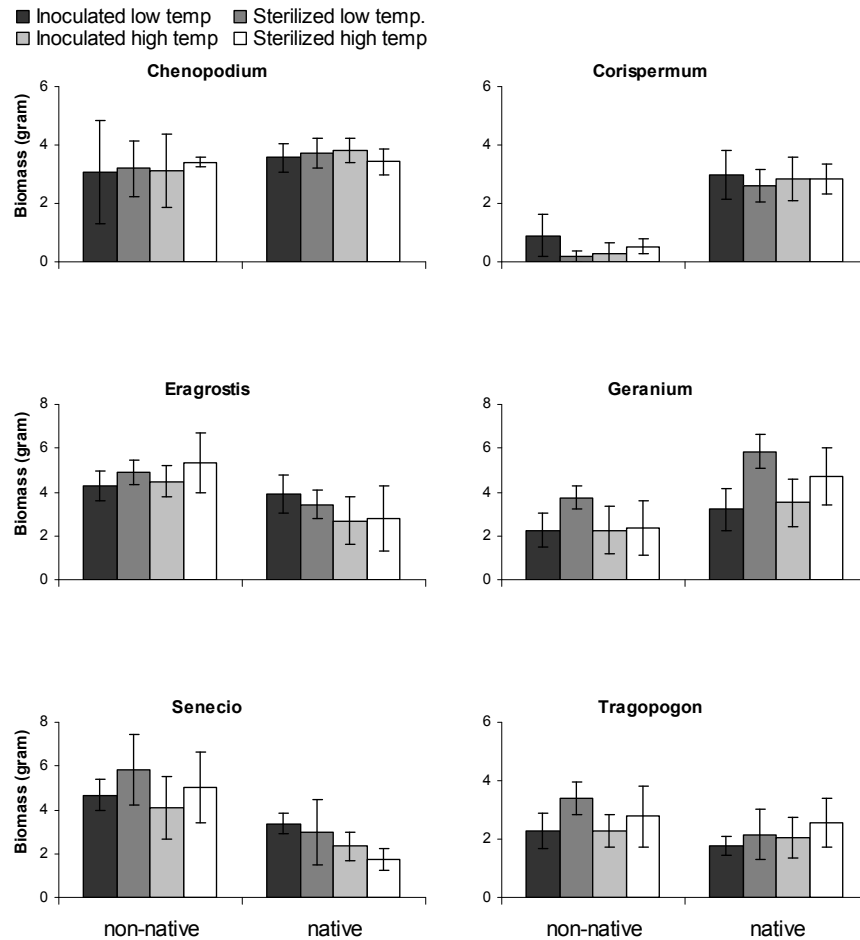
Figure 4.1: Mean (± SD) biomass of plants grown with a general inoculum or its sterilized control at low (20/15°C) or high (25/20°C) ambient temperature. Each panel contains a pair of a non-native and native plant species.

Results

Conditioning phase: comparing sterilized soil and non-conditioned inoculum

As the comparison between unconditioned soil and the sterilized control has been performed in the conditioning phase, as well as in the feedback phase, we present the statistics of both results. The effect of inoculation on biomass production did not depend on origin ($F_{1,242} = 1.524$, P = 0.245 and $F_{1,240} = 0.580$, P = 0.464 for the first and second run, respectively), or on temperature ($F_{1,242} = 0.604$, P = 0.438; $F_{1,240} = 0.937$, P = 0.334). Despite differences in setup (e.g. pot size), as described in

materials and methods, the results of both conditioning experiments are very alike (table 4.2, fig. 4.1). Individual species responded differently to soil inoculation ($F_{10,242}$ = 5.623, P < 0.001; $F_{10,240}$ = 3.531, P < 0.001 for run 1 and 2, respectively) (table 2). Some plant species produced more biomass in inoculated soils (e.g. *Poa annua* in run 1: $F_{1,20}$ = 11.607, P = 0.003) while other species produced less biomass in inoculated soil (e.g. *Tragopogon dubius* in run 1: $F_{1,20}$ = 16.395, P = 0.001). Root/shoot ratio only depended on plant species and was not affected by temperature nor by inoculation (table 4.3)

Table 4.2: Nested mixed model ANOVA results for the effect of soil inoculation with an unconditioned inoculum on aboveground (conditioning phase) or total biomass (feedback phase), temperature is tested over greenhouses and origin (native or non-native) is tested over species.

		DF	Conditioning phase			Feedback phase		
			Error DF	F	P	Error DF	F	P
Temperature	Fixed	1	3.8	6.082	0.073	2.8	1.368	0.332
Greenhouse(temp)	Random	2	242	1.111	0.331	240.0	4.146	0.017
Origin	Fixed	1	10.0	0.133	0.723	10.0	0.001	0.973
Species(origin)	Random	10	11.7	12.110	<0.001	12.0	9.287	<0.001
Inoculation	Fixed	1	10.0	7.939	0.018	10.1	3.530	0.089
Temp*spec(origin)	Random	10	242.0	1.761	0.068	240.0	1.828	0.057
Temp*inoc	Fixed	1	242.0	0.604	0.438	240.0	0.937	0.334
Temp*origin	Fixed	1	10.1	0.176	0.683	10.2	0.201	0.663
Origin*inoc	Fixed	1	10.0	1.524	0.245	10.1	0.580	0.464
Spec(origin)*inoc	Random	10	242.0	5.623	<0.001	240.0	3.531	<0.001
Temp*origin*inoc	Fixed	1	242.0	0.009	0.925	240.0	0.037	0.847

Feedback phase: comparing con- and heterospecific conditioned soil

Temperature had a significant effect on biomass production and the effect of temperature depended on plant species ($F_{10,238}$ = 1.975, P = 0.037) (table 4.4, fig. 4.2). However, the effect of temperature did not differ between native and non-native plant species ($F_{1,10.1}$ = 0.026, P = 0.875). The effect of soil conditioning on plant biomass depended on the origin of the plant species ($F_{1,238}$ = 14.157, P = 0.004), but not on temperature ($F_{1,238}$ = 0.001, P=0.976). The native species on average produced 10% less biomass in conspecific than in heterospecific soil ($F_{1,3.01}$ = 51.837, P = 0.005). The non-native species, on the other hand, produced 10% more biomass in conspecific than in heterospecific conditioned soil ($F_{1,3.00}$ = 9.610, P =

0.053). Therefore, native plants experienced growth reduction and non-native plants growth enhancement in their own soil, independent of the ambient temperature. The soil feedback effect, analyzed as the relative difference between biomass produced in conspecific and heterospecific soil, showed similar results: soil feedback depended on species origin (Mann-Whitney U = 1731, P = 0.003) and was not influenced by ambient temperature (Mann-Whitney U = 2091, P = 0.218). Root/shoot ratio was again only dependent upon plant-species and did not depend on temperature or conditioning (table 4.3).

Table 4.3: Nested mixed model ANOVA results for unconditioned soil (sterilized or inoculated) and conditioned soil (con- or heterospecific). Dependent is root/shoot ratio (LN transformed). Temperature is analysed over greenhouses and origin (native or non-native) over species to prevent pseudoreplication.

a) Root/shoot ratio Inoculated and sterilized soil

		DF	Error DF	F	P
Temperature	Fixed	1	3.3	0.130	0.741
Greenhouse (temp)	Random	2	233.0	2.149	0.119
Origin	Fixed	1	15.9	0.056	0.816
Species(origin)	Random	10	13.1	31.419	<0.001
Inoculation	Fixed	1	10.1	0.490	0.500
Temp*species(origin)	Random	10	233.0	1.765	0.068
Temp*inoc	Fixed	1	233.0	0.125	0.724
Temp*origin	Fixed	1	10.0	2.116	0.176
Origin*inoc	Fixed	1	10.1	2.075	0.180
Spec(origin)*inoc	Random	10	233.0	1.383	0.189
Temp*origin*inoc	Fixed	1	233.0	1.174	0.280

b) Root/shoot ratio Conditioned and mixed soil

		DF	Error DF	F	P
Temperature	Fixed	1	2.6	0.799	0.447
Greenhouse (temp)	Random	2	235.0	1.394	0.250
Origin	Fixed	1	10.0	0.296	0.598
Species(origin)	Random	10	3.5	70.964	0.001
Inoculation	Fixed	1	10.3	0.325	0.581
Temp*species(origin)	Random	10	235.0	1.286	0.239
Temp*inoc	Fixed	1	235.0	0.005	0.944
Temp*origin	Fixed	1	10.1	0.000	1.000
Origin*inoc	Fixed	1	10.2	2.008	0.186
Spec(origin)*inoc	Random	10	235.0	0.559	0.847
Temp*origin*inoc	Fixed	1	235.0	0.762	0.384
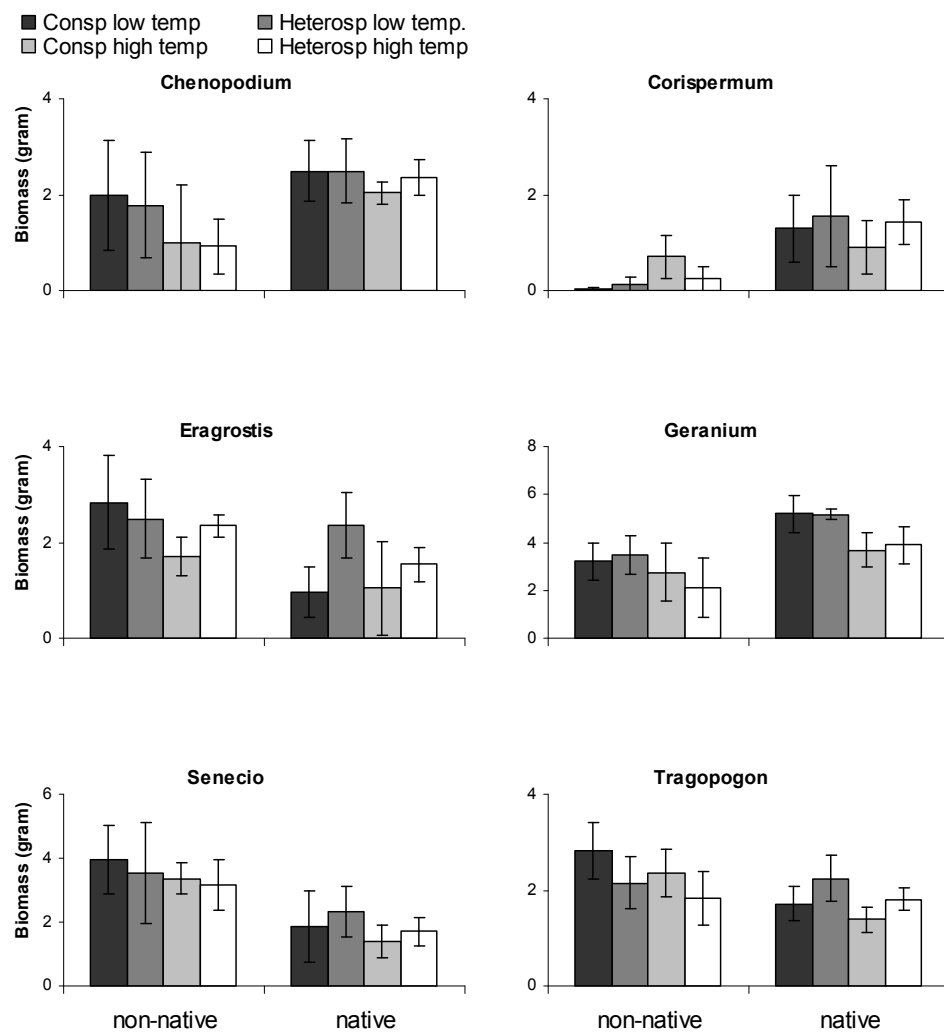
Figure 4.2: Mean (± SD) biomass of plants grown in conditioned conspecific soil or a heterospecific control at low (20/15°C) or high (25/20°C) ambient temperature. Each panel contains a pair of a non-native and native plant species.

Table 4.4: Nested mixed model ANOVA results for the effect of conspecifically conditioned soil and a heterospecific control (a mix of twelve conditioned soils) on total biomass. Temperature is analysed over greenhouses and origin (native or non-native) over species.

		DF	Error DF	F	P
Temperature	Fixed	1	3.1	7.872	0.064
Greenhouse(temp)	Random	2	238.0	3.376	0.036
Origin	Fixed	1	10.0	0.017	0.899
Species(origin)	Random	10	7.1	29.563	<0.001
Inoculums	Fixed	1	10.3	1.499	0.248
Temp*spec(origin)	Random	10	238.0	1.975	0.037
Temp*inoc	Fixed	1	238.0	0.001	0.976
Temp*origin	Fixed	1	10.1	0.026	0.875
Origin*inoc	Fixed	1	10.3	14.157	0.004
Spec(origin)*inoc	Random	10	238.0	0.852	0.579
Temp*origin*inoc	Fixed	1	238.0	0.084	0.773

Discussion

Although temperature has an impact on virtually all ecosystem processes (e.g. Bakkenes *et al.* 2002; Hickling *et al.* 2006; Brooker *et al.* 2007), an ambient temperature difference of 5ºC did not influence net plant-soil feedback in the greenhouse. Temperature had an effect on plant biomass production, but it did not influence native plants differently from plants originating from a warmer climate region. Nor did it change the effect of inoculation or conditioning. Therefore, we conclude that enhanced ambient temperature alone does not have a direct effect on plant-soil feedback patterns. In the present study, we did not include changes in precipitation, length of growing season, frequency of extreme events and direct effects of increased CO_2. These may all have effects on plant-soil interaction (van der Putten & Peters 1997; Chakraborty & Datta 2003; Ainsworth & Long 2005; Suttle *et al.* 2007; Kreyling *et al.* 2008) that may co-vary with increased temperatures and need to be investigated in order to get a more complete picture of the effects of climate change.

As micro-organisms tend to be more active at higher temperatures it would be likely that their impact on plant performance would increase with temperature (e.g. Bekal & Becker 2000; Allen *et al.* 2005) as has been found for aboveground pathogens and herbivores (Roy *et al.* 2004). Thus our results seem counterintuitive,

however, similar results, i.e. a decreased pathogenic effect with increased temperature, have also been reported (Smiley & Uddin 1993; Allen *et al.* 2005; Matheron & Porchas 2005). Furthermore, interactions in the rhizosphere community might compensate for the direct effect of temperature (e.g. Gavito *et al.* 2003; Piśkiewicz *et al.* 2007).

Native and non-native species differed in their plant-soil feedback but not in the effect of a general inoculum during soil conditioning. This indicates that the difference found with conditioned soil has been developed during the conditioning phase. In theory, this effect could have been due to changed abiotic conditions, but other studies have ruled out nutrient depletion as a factor in such feedback experiments (e.g. Kardol *et al.* 2006). Moreover, inoculation of soil fauna (De Deyn *et al.* 2003) and microbial components (Kardol *et al.* 2007; van der Putten *et al.* 2007) to sterilized soil have confirmed the biotic origin of these soil conditioning effects. As the non-natives have colonized The Netherlands relatively recently (table 4.1) the difference between native and non-native species can be explained as a release from their specific pathogens during the range expansion of the non-natives, whereas local soil communities have not yet developed pathogenicity to these exotic plants (Reinhart *et al.* 2003).

Nutrients unavailable in non-sterilized soil can become available through autoclaving (Troelstra *et al.* 2001) resulting in a higher nutrient availability in the sterilized control. Because we used relatively small sterilized inocula this effect is reduced in the present experiment. We also used the same inocula for all species, so that this effect is the same for all plant species tested and cannot have confounded the conclusions. Another potentially confounding effect is that plant uptake may differ; plants that grow strongly in the conditioning part of the experiment may have taken up more nutrients from the soil than plants that show less growth, resulting in less nutrient availability in the feedback phase. This potential difference was corrected by nutrient addition. Moreover, biomass production, and thus nutrient uptake, in the conditioning part was compared between native and exotic species and this did not differ. Therefore, nutrient availability cannot explain the observed results.

We conclude from our study that the net effect of plant-soil interactions appears to be insensitive to increases in ambient temperature. However, we did find that the non-native plant species that have recently colonized The Netherlands have

a less negative plant-soil feedback than the native species, which confirms previous findings (van Grunsven *et al.* 2007). As a result of climate change many plant species are expected to expand their range into new areas (Bakkenes *et al.* 2002). This can result in a large number of non-native plant species that have a reduced plant-soil feedback compared to native plants promoting their dominance (Bever 2003). Future studies should consider how these predicted changes in plant species composition will influence the functioning of affected ecosytems. Furthermore, both the role of specific soil organisms in plant-soil interactions and aspects of global climate change besides temperature should be explored to a greater extent.

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A poleward shifting plant species outruns its soil-borne enemies.

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Abstract

Climate warming causes many species to shift their range towards higher latitudes and altitudes. However, range shifts do not necessarily proceed at equal rates among species that used to be trophically connected. Here, we examined if a plant species that shows rapid northward range shift in Europe, may have become released from soil-borne pathogens by moving up north. We compared *Tragopogon dubius*, which is shifting northwards, with *T. pratensis*, which has a natural occurrence along the original and new ranges of *T. dubius*. First, we collected soil from the rhizospheres of populations of both plant species across a south-north gradient in Europe, including the natural and the expansion range of *T. dubius*. We then inoculated rhizophere soil into sterilized soil and compared plant performance in that inoculated soil with that in a sterilized control. This gives a measure of the effect of the inoculated soil community on plant performance. Inoculation with the rhizosphere communities from *T. dubius* populations in the native range had a more negative effect on plant biomass production than inoculation with rhizosphere communities from the new range, whereas *T. pratensis* experienced a net negative plant-soil feedback throughout its range. Therefore, we conclude that *T. dubius* has a less negative plant-soil interaction in the invaded than in the native range as a result of its range expansion. We discuss consequences for future abundance of these rapidly range-shifting plant species. Our results suggest that the effects of range shifts on biotic interactions need to be included in analyses of the impacts of climate change on plant species. When assessing the effect of climate change on plant species distribution, biotic interactions, such as between range-shifting plant species and the soil community, need to be considered as these can alter the performance of a range expanding plant species.

Keywords: enemy release, climate change, range shift, plant-soil feedback, local adaptation, *Tragopogon dubius*, *pratensis*.

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Introduction

Species arriving in new areas often become much more dominant than in their native range and show the ability to outcompete native, long established species (Elton 1958). Exotic invasive plant species can form dense and permanent monocultures in the new area, while they are not dominant in their native range. One of the main hypotheses explaining this process is the enemy release hypothesis. This hypothesis states that an exotic species is often released from its specialist enemies resulting in an increased competitive ability, increased abundance and widespread distribution in the new range (Elton 1958). This is not necessarily limited to introduction but can also be facilitated by other changes in distribution, e.g. for the range expanding butterfly *Aricia agestis* northern, newly colonized, populations show reduced parasitism compared to southern, long established populations (Menéndez *et al.* 2008). Recently it has been suggested that plant species that expand their range as a result of climate change might also experience enemy release (Voigt *et al.* 2003; van Grunsven *et al.* 2007). However studies testing this, for instance by comparing the interactions between plants and natural enemies along a gradient from the native to the newly colonized range, are unknown to us.

Many organisms show range shifts towards higher latitudes and altitudes due to climate change (Parmesan & Yohe 2003; Hickling *et al.* 2006). However, the rates of these range shifts can differ among species (Hickling *et al.* 2006). For example, many soil pathogens and root herbivores are poor dispersers and they lack targeted dispersal (van der Putten *et al.* 2001). It is therefore unlikely that they can keep up with their host plant, in contrast to most aboveground enemies, such as herbivorous insects. Plants that can expand their range rapidly might outrun their enemies and find themselves, at least temporarily, released from enemies in the newly occupied range. This principle also has been shown for successional gradients (van der Putten *et al.* 1993). The escape from soil-borne natural enemies is of particular interest when regarding the effects of range shifts, both because of the poor dispersal ability of soil-borne natural enemies, and their strong impact on plant performance.

Plant-soil interactions play an important role in determining species dominance through regulation of local density (Packer & Clay 2000; Klironomos 2002; Reinhart *et al.* 2003; Callaway *et al.* 2004; Levine *et al.* 2006). Plant-soil

interaction also drive vegetation dynamics (van der Putten *et al.* 2003), leading to both cyclic (Olff *et al.* 2000) as well as successional changes in plant community composition (De Deyn *et al.* 2003; van der Putten *et al.* 2003; Kardol *et al.* 2006). Changes in these key biotic interactions can therefore result in a difference in performance between the invaded and the native range, similar to introduced species that have escaped soil-borne pathogens (Reinhart *et al.* 2003; Reinhart & Callaway 2004). In a comparison with related native species, plant species that have expanded their range had a less negative plant-soil feedback than native species in the invaded range (van Grunsven *et al.* 2007). However, to assess whether this difference is a result of the range shift, such comparisons need to be carried out along latitudinal gradients comparing the plant-soil interactions in the native and the non-native range of the range expanding plant species (Liu & Stiling 2006).

Effects of the soil community on plants result from both positive interactions (e.g. with symbiotic mutualists) and negative interactions (e.g. with pathogens) and can have three net effects. If the effect of mutualists is stronger than the effect of pathogens and herbivores this will result in a net positive effect of the soil community on plant performance. If the effect of these two groups equals out, the net effect will be neutral and when the negative effects are strongest the net effect is negative for plant performance. Separating the positive and negative components of the soil community is nearly impossible as the effects of soil organisms depends on many biotic and abiotic factors (Borowicz 2001; Klironomos 2003) and soil biota strongly interact (Borowicz 2001; Wardle *et al.* 2004; Wolfe *et al.* 2006; Maherali & Klironomos 2007; Piśkiewicz *et al.* 2007). We therefore chose to confront plants with entire soil communities to include as many positive and negative interactions as possible (van der Putten *et al.* 1993; Bever 1994).

We used *Tragopogon dubius* to test the hypothesis that a range expanding plant is released from soil-borne enemies in its new range. This hypothesis assumes that in its native range, a plant is more exposed to soil-borne enemies than in its non-native range. Originally, *T. dubius* occurred in southern Europe, south of the Ardennes, and is indigenous to France and Luxembourg (Allorge 1922; Richardson 1976; Petit 1978). From the middle of the 20th century, *T. dubius* expanded its range into Belgium and The Netherlands (Weeda *et al.* 1991; Verloove 2002; Tamis *et al.* 2004). At present *T. dubius* occurs throughout most of The Netherlands as far north as Amsterdam, although it is still uncommon in most regions of The Netherlands

(Tamis *et al.* 2004). In our analysis, we included the related *T. pratensis*, which is native along the whole considered latitudinal gradient. We used this species to test the hypothesis that the effects of soil communities do not differ between the southern and northern part of its latitudinal gradient. The two plant species are expected not to differ in the area where they are both native but the effect of the soil community is expected to be more positive for the recent invader *T. dubius* than for the native *T. pratensis* in the new range. We also correlated latitude with the effect of inoculation within the southern range to test for a trend from south to north irrespective of nativeness.

Material and Methods

Tragopogon dubius Scop. and *Tragopogon pratensis* L. are biannual monocarpic Asteracaea that occur in grasslands and ruderal areas. The species are very alike both in appearance and ecology and although they are known to hybridize this appears to be rare in Europe (Clements *et al.* 1999). *Tragopogon dubius* was originally a species distributed in Central and Southern Europe. Until 1950, *T. dubius* was not reported from Flanders and The Netherlands. Therefore its current range can be separated in a part where it is native, i.e. roughly south of the Ardennes (approximately 50.5° N), and where it has recently established, i.e. north of the Ardennes. *Tragopogon pratensis*, on the other hand, is widespread across Western Europe (Weeda *et al.* 1991).

We identified 14 populations of *T. dubius* and 16 populations of *T. pratensis* along a transect from near Marseille, France (34.2ºN, 5.3ºE) to Amsterdam, The Netherlands (52.2ºN, 5.4ºE) (fig.5.1). All populations were well established with many well developed flowering plants and seedlings. Plants normally flower in the third calendar year (Gross 1981) and the presence of seedlings signified flowering in the previous year indicating that all populations had been present at least 4 years prior to sampling. Rhizosphere soil within 8 cm of the centre of the rosettes or flowering stalks was collected to a depth of 0-15 cm below the soil surface. From each sampling site we collected soil from 10 plants, resulting in a total of up to 7 kg per site. This was aggregated, packed in double plastic bags that were kept closed. Soil sampling equipment was sterilized with 95% ethanol between sampling sites.
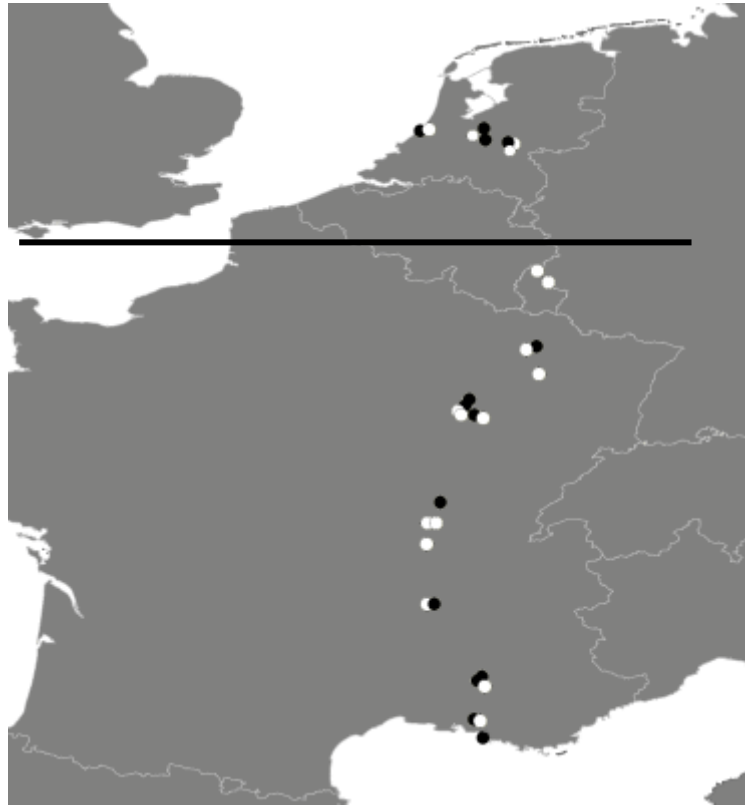
Figure 5.1: Locations of the sampled sites. The horizontal line represents 50.5° North, the border between the original and new part of the range for *T. dubius*. Black dots are populations of *T. dubius* from which inocula are collected and white dots are *T. pratensis* populations.

Watery extracts of the soils were prepared to be used as inocula; three liters of water were added to 3 kg of rhizosphere soil, mixed by stirring for 5 minutes and left to stand for 5 minutes. The supernatant was sieved through a 5.6 mm sieve to remove stones and coarse material. The filtrate was left to settle for 20 minutes after which the supernatant was sieved through a 1.7 and a 1.0 mm sieve. The resulting solution was thoroughly mixed and divided over two flasks. One flask was stored at 4°C and the other was sterilized by autoclaving (121.5°C for 20 minutes) resulting in an inoculum and a sterilized control. This was repeated for every sampled population. All equipment was cleaned and sterilized with 95% ethanol between populations.

Seeds of two native location outside the transect (Voitsau, Austria for *T. dubius* and Groningen, The Netherlands for *T. pratensis*) were used for all inocula to prevent confounding effects of local adaptation between populations and their soil communities. Seeds were germinated in sterilized coarse river sand and selected

seedlings of similar size were transplanted into 1.1 liter pots filled with 100 g of soil (12.8% moisture). This soil was a mixture of sandy loam soil and river sand (1:5 w/w, sterilized by autoclaving at 121.5°C for 2 hours). On the day of planting 50 ml of the inoculum or a sterilized control was added to the soil using a sterilized syringe and another 50 ml was added after two days. Pots were placed in 5 randomized complete blocks in a greenhouse with 70% relative humidity, 16/8 hour light/dark and 20/15°C. Philips SOL-T armatures were used for additional lighting, providing 80 µmol m^{-2} s^{-1} when sunlight was insufficient. Pots were watered daily to compensate for evaporation and plant uptake. With five replicates, 30 sampled populations and the sterilized control this resulted in 300 plants.

Plants were harvested after two months and separated into shoots and roots. Roots were rinsed to remove clinging soil and shoots and roots were dried at 70°C for 48 hours before weighing.

Statistical analyses

Biomass was averaged over the five replicates of every treatment to prevent pseudo-replication. As a result of this sampled populations and not individual plants are used as replicates. The average biomass was analysed using a general linear model with population as random factor and species, range (north or south) and inoculation as factors. By inclusion of population as random factor the main effects of range and species became redundant, every population represented either *T. dubius* or *T. pratensis* and in either in the southern or northern part of the range. Therefore inclusion of species and range did not add information to the statistical model. Interactions with inoculation could however be tested in this model. Homogeneity of variance and normality of residuals were assessed visually and found to be in agreement with assumptions for ANOVA. To test the main effects of species and range on biomass Monte Carlo analyses with 10000 iterations were used.

The effect of inoculation was proportionalized by calculating the effect of inoculation (inoculated soil-sterilized soil)/sterilized soil). This gave the proportional difference in produced biomass between plants grown in inoculated soil as compared to the sterilized control. This value is negative when inoculation reduces biomass production compared to the sterilized control and positive when it increases biomass production. Negative values are due to net dominance of pathogenic effects

and positive values are due to net dominance of mutualistic effects. These values were square root transformed but preserving their positive or negative sign (Loreau & Hector 2001; van Ruijven & Berendse 2005). Replicates were averaged per treatment and tested for significant effects with a general linear model and t-tests. A possible correlation between latitude and effect of inoculation within the southern part of the range where both plant species were native was tested with a Pearson correlation for both *T. dubius* and *T. pratensis*.

Data was analysed using SPSS 12.03 (SPSS Inc.) except for the Monte Carlo analyses, for these Poptools 2.7 (Csiro) was used.

Table 5.1: ANOVA table for the analysis of biomass. Dependent is the average biomass of the five replicates per treatment. Fixed factors are species (*T. dubius* or *T. pratensis*), origin of inoculation (south or north representing the original and new part of the range for *T. dubius*) and inoculation (non-sterilized rhizosphere soil inoculum or a sterilized control). Population is included as random factor. By inclusion of population the main effects of range and species become redundant (every population represents only one species and is in one part of the range) and therefore not testable in this model.

Biomass

	df	F	P
Population	26	0.56	0.93
Inoculation	1	6.62	0.02
Species*inoc	1	4.51	0.04
Range*inoc	1	1.27	0.27
Range * inoc * spec	1	13.78	0.01
Total	60		

Results

The two plant species differ in biomass, *T. dubius* produced a dry weight of approximately 1.4 gram per plant as compared to 1.0 gram per plant for *T. pratensis* (P < 0.001). Inoculation with soil from the northern or southern part of the transect did not affect the average biomass (P = 0.35). More biomass was produced with a sterilized than with a non sterilized inoculum, indicating that on average the negative effects of the soil community dominated. Species, range and inoculation showed an interaction as hypothesized (table 5.1). As we are interested in the effect of the soil community and not in the differences between the species and populations, we will focus on the relative effect of inoculation (fig. 5.2, fig. 5.3, table 5.2). The effect of inoculation differed between species, with a less negative effect of inoculation for *T. dubius* than for *T. pratensis* but this interacted with range (table 5.2). The proportional effect of inoculation was more positive for *T. dubius* in the invaded (+9%) than in its native range (-6%) ($t_{11.2}$ = 2.27, P = 0.044) as predicted. *Tragopogon pratensis*, native in both parts, experiences a more negative effect of inoculation in the northern than in the southern part of the transect (-37% and +3% respectively) ($t_{13.3}$ = -7.68, P < 0.001). Comparing the plant species within the two parts of the transect the effect of inoculation was similar in the southern part (-6% for *T. dubius* and +3% for *T. pratensis*) ($t_{14.6}$ = -1.36, P = 0.196) but in the northern part of the transect *T. dubius* experienced a more positive effect of inoculation than *T. pratensis* (+9 and -37% respectively) ($t_{6.0}$ = 11.292, P < 0.001).

The variation in the effect (the standard deviations) of the soil community between populations was lower in the newly invaded range (SD = 0.05) than in the native range of *T. dubius* (SD = 0.21). The standard deviation in the native range of *T. dubius* was similar to that of *T. pratensis* (SD = 0.16 and SD = 0.18 for the southern and northern part respectively).
There was no correlation between latitude and effect of inoculation in the southern part of the range of both species together (ρ = 0.203, P = 0.364) or for both species separately (ρ = -0.118, P = 0.745 for *T. dubius* and ρ = 0.354, P = 0.259 for *T. pratensis*). Nor was there a correlation between latitude and effect of inoculation within the northern part of the range (ρ = 0.191, P = 0.650 for both species together, ρ = -0.631, P = 0.369 for *T. dubius* and ρ = -0.563, P = 0.437 for *T. pratensis*).

Table 5.2: ANOVA table for the analysis of the proportional effect of inoculation, relative to a sterilized control, on biomass production for *T. dubius* and *T. pratensis* with soil from the native or invaded range of the first species (square root transformed).
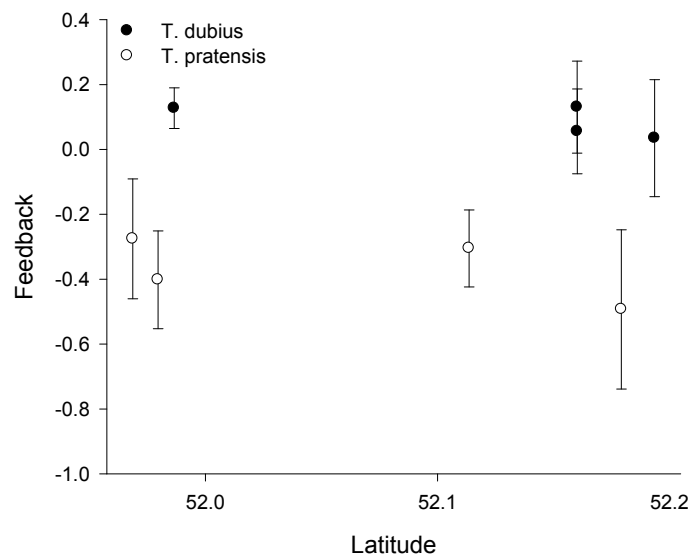
Inoculation effect

	df	F	P
Species	1	5.93	0.02
Original vs. new range	1	2.29	0.14
Species*range	1	16.67	<0.01
Total	30		

Discussion

The range expander *Tragopogon dubius* shows a clear difference in the effect of the soil community between the southern native and the northern non-native range. In the native range, the average soil community effects to *T. dubius* were negative, whereas in the non-native range, soil community effects were less negative, or more neutral. This pattern differs from that of *T. pratensis*, which is native across the entire range and had a negative soil community effect both in the southern and in the northern part of the range. In a previous study, we have shown that a number of range-shifting plant species has less negative soil feedback in their new range than related native species (van Grunsven *et al.* 2007). The current study supports the hypothesis that this is a result of the range expansion. Such enemy release is known from traditional biological invaders that artificially crossed geographical boundaries (Elton 1958; Maron & Vila 2001; Keane & Crawley 2002; Reinhart *et al.* 2003) and has recently been reported for a range expanding butterfly (Menéndez *et al.* 2008). Here, we provide novel evidence that such enemy release may also be due to climate warming-induced range expansion in plants.
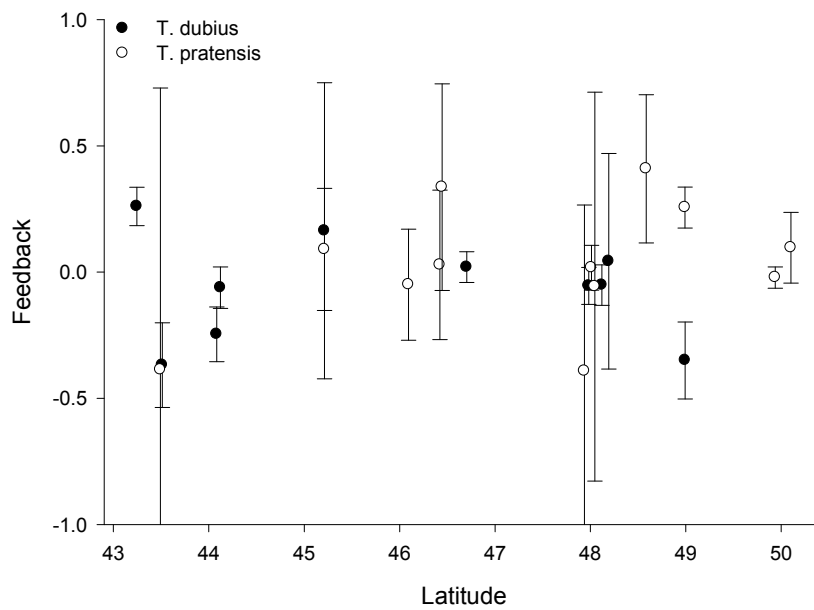
Figure 5.2: The effect of inoculation depicted as the fraction increase (positive values) or decrease (negative values) in biomass production in the inoculated soil relative to the sterilized control. Points are the mean of the five replicates per population (and whiskers represent standard error). X-axis gives the latitude of every sampled population from Marseille (43.2°N) north to The Ardennes (50.2°N).

Figure. 5.3: The effect of inoculation depicted as the fraction increase (positive values) or decrease (negative values) in biomass production in the inoculated soil relative to the sterilized control. Points are the mean of the five replicates per population (and whiskers represent standard error). X-axis gives the latitude of every sampled population from The Ardennes (52.0°N) north to Amsterdam (52.2°N).

In the native range of both *Tragopogon* species, we observed strong variation in the effect of inoculation, ranging from negative to neutral or positive (fig. 5.2, 5.3). Such variation has been observed in other native species experiencing strong effects of soil pathogens (Olff *et al.* 2000; Knevel *et al.* 2004; Reinhart *et al.* 2005). This large variation emphasizes the need to incorporate a relatively large number of replicate populations, especially for the native range. When only a small number of populations is sampled (Reinhart *et al.* 2003) differences found might be a result of sampling effect (Reinhart *et al.* 2005). Apparently, populations of plants may be in different states of a build-up of soil pathogenicity. In the new range of *T. dubius* the specialist enemies are thought to be absent from all populations (fig. 5.3). As this removed an important source of variation this did not only result in a more positive average effect of the soil community but also in a lower variation between populations as compared to the native range of *T. pratensis* and *T. dubius*.

Tragopogon dubius and *T. pratensis* differed significantly in the effect of inoculation in the northern but not in the southern part of the range. This difference was a result of a more neutral effect of inoculation on *T. dubius* in the northern range but also of a more negative effect of inoculation on *T. pratensis* in the northern range. This native species was included to control for a general trend in the effect from the soil community from south to north. We did not anticipate such a pattern in *T. pratensis*. However, the pattern observed in *T. dubius* was clearly absent in *T. pratensis*. Furthermore within both the southern and northern part of the range there was no relation between latitude and feedback effect for either *T. dubius* or *T. pratensis* or the two species combined. Therefore a general trend in plant soil interaction, or pathogen load of soils, from south to north as an alternative explanation for the pattern found in *T. dubius* could be rejected. Why *T. pratensis* had a more negative effect in the northern part of the transect is unknown to us and needs further investigation.

Plant-soil interaction is the net effect of many components of the soil community, some of which positively affect plant performance while other negatively affect plant performance. Therefore a more positive plant-soil interaction can be the result of a decreased pathogenic component or an increased mutualistic component. Besides natural enemies the soil community contains mutualists of which arbuscular mycorrhizal fungi are considered the most important for *Tragopogon*. Arbuscular mycorrhizal fungi are highly generalistic and able to form mutualistic relations with

many different plant species (Richardson *et al.* 2000). It is unlikely that the found effect is caused by a stronger mutualistic effect in the non-native than in the native range. Therefore the absence of strongly pathogenic soil organisms in the invaded range is the most likely explanation for the observed results. Which organisms are responsible for the found results can be established by analysis of the soil community in the native and invaded range of this and other range-expanding plant species.

A more positive plant-soil interaction through range shifts can have strong effects on the ecology of the released plant. Local abundance is likely to increase (Klironomos 2002) but possibly not spread, as this also depends on long distance dispersal through vectors (Levine *et al.* 2006). In climate envelope modeling, the future range of a plant species under a climate scenario is based on its current distribution and expected temperature changes along latitudinal gradients (Bakkenes *et al.* 2002; Brooker *et al.* 2007). But if biotic interactions change as a result of range shift the predictions made using climate envelopes might be unsound (Davis *et al.* 1998; Brooker *et al.* 2007). Models that focus on environmental characters fail to appreciate changes in biotic interactions that will affect the realized niche of the species.

We conclude that *T. dubius* has a less negative plant-soil interaction in the invaded than in the native range as a result of its range expansion resulting in an advantage over a similar native species. This can have strong implications for abundance in its future range and range limits which appear less constrained by biotic interactions. As a result of atmospheric warming many plant species are expected to expand their range into new areas (Bakkenes *et al.* 2002; Tamis *et al.* 2005) as *T. dubius* has done in the past decades. By 2050 more than 50% of plant species are predicted to be recent additions in almost 40% of the land area of Europe (Bakkenes *et al.* 2002). It is likely that these plant species will show a similar shift in plant-soil feedback as found for *T. dubius*. Considering the importance of plant-soil feedback in succession, species competition and dynamics of communities (van der Putten *et al.* 1993; Bever 1994; Olff *et al.* 2000; Klironomos 2002; Kardol *et al.* 2006), these newly arrived species are likely to become more dominant than in their native range and have a strong impact on the invaded ecosystems.

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The poleward shifting *Tragopogon dubius* is just as effective in forming mycorrhizal associations as the native *T. pratensis*.

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Abstract

Many plant species, and other organisms, are shifting their range polewards. As not all species shift their range at the same speed, this may modify biotic interactions. Plants might become released from their natural enemies but could also lose mutualists. One of the most important groups of plant mutualists is the arbuscular mycorrhizal fungi (AMF). Since AMF are thought to be poor dispersers, poleward expansion of plant ranges may result in uncoupling between plants at their co-adapted AMF-taxa. We used greenhouse experiments to test if *Tragopogon dubius*, a species that has recently colonized The Netherlands, differs from the native *T. pratensis* in their association with arbuscular mycorrhiza. We compared the density of infective propagules associated with for both plant species in soils collected at four locations, as well as root-length colonization. The AMF community structure in the roots of these species was analyzed using PCR-DGGE techniques. *T. dubius* and *T. pratensis* do not differ in any of these characteristics. We therefore conclude that *T. dubius* and *T. pratensis* do not differ in their associations with AMF.

Introduction

As a result of climate change many species are shifting their distributions towards the poles (Bakkenes *et al.* 2002; Parmesan & Yohe 2003; Hickling *et al.* 2006) but do this at different rates. Some species can expand their range quite rapidly while other species or groups of species appear much slower (Hickling *et al.* 2006). Although dispersal rates of AMF are poorly studied, they appear to be poor dispersers compared to many plant species (Reddell *et al.* 1997; Seres *et al.* 2007). It is therefore likely that some plants will encounter AMF communities that are highly disparate from their native, co-adapted assemblages as they expand their ranges toward the poles (Klironomos 2003).

Plants colonizing new areas, as a result of an introduction or a range shift, often experience new biotic interactions compared to the area of origin (Mitchell *et al.* 2006). This can have profound effects on the performance of this species, for instance through release from natural enemies (Elton 1958; Blair & Wolfe 2004; Genton *et al.* 2005) including pathogens (Reinhart *et al.* 2003; DeWalt *et al.* 2004).

Simultaneously species may lose their mutualists (Richardson *et al.* 2000; Parker *et al.* 2006; Reinhart & Callaway 2006). The role of mutualists in mediating the success of invasive plants has been acknowledged for quite some time but has received little experimental attention. Many plant species depend on mutualists for services such as pollination and seed dispersal, as well as nutrient uptake and nitrogen fixation. If species are introduced into a new area, where mutualists are lacking or less effective, a reduced performance in comparison to the native range can be expected (Richardson *et al.* 2000). If this effect is strong, the likelihood that a species becomes invasive in this area is reduced. On the other hand, highly invasive species are unlikely to experience a severe loss of mutualist benefits. As most attention in invasion ecology has been on these highly invasive species the role of mutualist has only recently gained more attention (Parker 2001; Downs & Radford 2005; Fumanal *et al.* 2006; van der Putten *et al.* 2007).

Arbuscular mycorrhizal fungi (AMF; Phylum Glomeromycota) are among the most important mutualists in many ecosystems (Kiers & van der Heijden 2006). AMF can have highly disparate effects on host plants, from highly positive to neutral and negative (Klironomos 2003). There is also evidence that AMF infection directly or

indirectly affects surrounding plants. Taken together these interactions can have major implications on plant competition, community succession and plant diversity. (a.o. van der Heijden *et al.* 1998; Klironomos *et al.* 2000; Hart *et al.* 2003). As AMF are thought to be generalistic and cosmopolitan, it is often assumed that invasive species will have no problem forming sufficient mycorrhizal relationships in the invaded range (Richardson *et al.* 2000). However, recent evidence suggests that this is an oversimplification, with formation and effectiveness of mycorrhiza dependent on the identity of both the AMF and plant partners (van der Heijden *et al.* 1998; Kiers *et al.* 2000; van der Putten *et al.* 2007). First, Klironomos (2003) has shown that local adaptation is significant in AMF. Similar but exotic genotypes of the same AMF taxa affect plants to a lesser degree than locally co-adapted strains, and this is true for both positive and negative effects on plants performance. Second, even though AMF propagules might be almost omnipresent, the composition of AMF communities varies among habitats (Öpik *et al.* 2006) and depends on abiotic conditions such as nutrient availability, disturbance regime, pH etc. (e.g. Bever *et al.* 1996; Bever *et al.* 1997; Oehl *et al.* 2003). Third, several studies have also shown that different coexisting plant species associate with different AMF communities (Helgason *et al.* 2002; Scheublin *et al.* 2004) and it has been proposed that plants can select specific AMF (Kiers & van der Heijden 2006). Hence, local adaptation and specialization are likely to occur in AMF, and we can not a priori assume that an invasive species will always be able to form sufficient and effective mycorrhiza as suggested by Richardson *et al.* (2000).

The effect of an invasive species on the local AMF community is well studied and can be significant (Johnson & Wedin 1997; Callaway *et al.* 2003; Carey *et al.* 2004; Batten *et al.* 2006; Greipsson & DiTommaso 2006; Stinson *et al.* 2006; Zhang *et al.* 2007). However the association between the local AMF community and the invasive species has received much less attention. In the available studies comparing invasive and native species, results are mixed. Batten *et al.* (2006) reported greater abundance of AMF in invasive versus native species, although AMF diversity and effects on plant performance were not examined. In contrast, Van der Putten *et al.* (2007) found that an invasive grass was predominantly colonized by a single species of AMF despite the presence of several other AMF-taxa in co-occurring native grasses. As the exotic plant species did not show a reduced performance as a result, it illustrates that the presence of many AMF taxa does not

mean that there are many suitable mutualists for an introduced, not co-adapted, species. It is currently unknown if this is also true for plant species expanding their range as a result of climate change

In this study, we compared the AMF communities associated with a plant species that has recently spread from Southern Europe into The Netherlands, *Tragopogon dubius,* with those associated with a congeneric native to this area, *Tragopogon pratensis*. *Tragopogon dubius* is known to be less negatively affected by the soil community in the invaded range than the native *T. pratensis* (van Grunsven *et al.* 2007). But as a net effect of the soil community was measured, mutualistic and pathogenic effects were not separated. It is therefore unknown if *T. dubius* experiences reduced mutualism in comparison to *T. pratensis.*

Here, we test if *T. pratensis*. and *T. dubius* differ in the association with mycorrhizal taxa in soil from an invaded site in The Netherlands. The association between plants and mycorrhiza does not only depend on the selectivity of the plant species, but also on the community of AMF propagules present in the soil. Therefore, soil samples from four different sites are used for both species. We tested if the density of AMF propagules able to colonize these host plants and the percentage of root length colonized by AMF (both as measures for plant-fungal affinity) differs between the two plant species. In addition, we performed molecular community profiling experiments to allow comparison of the mycorrhizal communities colonizing the two plant species.

As *T. dubius* is a recent arrival, we expected this species to have a less adapted relationship with local AMF species. We therefore hypothesized that this will result in (i) a lower number propagule infection rate in soils tested with *T. dubius* than with *T. pratensis*, i.e. AMF are less able to colonize *T. dubius*, (ii) a lower percentage of root length colonized for *T. dubius* than for *T. pratensis* and (iii) different mycorrhizal communities in the roots of the two plant hosts, with a species poor community in the roots of *T. dubius*.

Materials and methods

Tragopogon dubius Scop. and *Tragopogon pratensis* L. are biannual monocarpic Asteracaea that occur in grasslands and ruderal areas. The species are very alike,

and are known to hybridize (Clements *et al.* 1999), although this is rare in Europe. *Tragopogon dubius* was previously a species of Central and Southern Europe north to Luxembourg, but was not found in Flanders or The Netherlands. Since the 1950's, it has spread north (Verloove 2002; Tamis *et al.* 2004). In contrast, *Tragopogon pratensis* is widespread in Europe and native to Western Europe, and The Netherlands (Weeda *et al.* 1991). Soil was collected from four areas in The Netherlands, a population of *T. dubius* (51º59'11N 5º39'38E), a population of *T. pratensis* (51º58'48N 5º40'48E), a site where both species grow together (52º10'46N 4º29'51E) and a grassland where no *Tragopogon* species occur (51º59'11N 5º40'48E). These soils will be referred to as "Dubius", "Pratensis", "Both" and "None" soil respectively. "Both" soil is a mixture of clay and sand relatively rich in nutrients while the other soils are sandy and nutrient poor. "Pratensis" and "None" soils are *Holcus lanatus* dominated grasslands, the second being richer in herbs. The sites where "Both" and "Dubius" soils are collected are more ruderal. Soil samples were collected within 8 cm of the centre of the rosettes or flowering stalks to a depth of 15 cm using a sterilized garden trowel.

Most probable number determination of AMF propagules

The number of infective propagules in these soils was analyzed using a Most Probable Number analysis as described in Sieverding (1991). This method uses presence and absence data in a stepwise dilution to calculate the number of propagules in a soil sample. Approximately 10 kg of field soils were collected from each of the four locations. The soil was homogenized and divided into two parts, one of two and one of 8 kg. The larger subsamples were sterilized by autoclaving (2 hours at 121.5ºC) A mixture of commercial potting soil and river sand (1:9 w/w, sterilized by autoclaving at 121.5 °C for 2 hours) was used as a bulk soil. Pots were filled with 150 g of the bulk soil, on top of this 50 g of rhizosphere soil was applied and covered with an additional 50 g of bulk soil. The rhizosphere soil was a mixture of non-sterilized and sterilized soil with ten steps of four-fold dilution. Per level of dilution, 4 replicate pots were used. Both plant species were used for all four soils, resulting in a total of 320 pots. Pots were placed in randomized complete blocks in a green house (20ºC/15ºC, 70%RH) and watered every other day. After 10 weeks plants were harvested. The entire soil volume was taken from the pot and was cut

one centimeter below and above the layer of rhizosphere soil. Roots in were rinsed from this layer and stained with ink (Vierheilig *et al.* 1998). The presence or absence of mycorrhizal infection in the roots in this layer was assessed under a microscope (40X to 100X magnification). Roots were searched until presence of AMF was confirmed or when presence of AMF was not confirmed all roots were searched. Only presence or absence in a root sample was noted. Data was analyzed as described in (Sieverding 1991).

Infection rates

To determine the amount of mycorrhizal hyphae and other mycorrhizal structures, in the roots of both plant species when inoculated, the same plant species and soils as above were used. Pots were filled with a homogenized mixture of the aforementioned sterilized bulk soil and non-sterilized rhizosphere soils (1:5 W/W). Pots were planted with four seedlings of either plant species or with two seedlings of each species. Per treatment 5 replicates were made and placed in 5 complete randomized blocks, resulting in a total of 60 pots, 4 soil types 3 plant combinations and 5 replicates. Plants were grown in a green house for 10 weeks, at 20/15°C, 70% RH. Pots were brought to the same weight by watering three times a week.

After 10 weeks, plants were harvested soil was rinsed from the roots and root samples were taken. These samples where thoroughly washed to remove clinging soil. Part was stored in FAA (formalin-aceto-alcohol) and part was stained using ink (Vierheilig *et al.* 1998). In the ink-stained roots, percentage colonization was assessed using the Gridline Intersect Method as described in (Brundrett *et al.* 1996). The rest of the plants were separated in roots and shoots and dried at 70°C for 48 hours after which dry weight was measured.

Molecular community profiling analysis

The root samples stored in FAA were ground individually in liquid nitrogen using a mortar and pestle. A 0.25 g subsample of the resulting powder was used for DNA extraction with a Power Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA). PCR was used to amplify fragments of the nuclear ribosomal small subunit (SSU) RNA gene using the Expand DNA polymerase system (Roche, Basel,

Switserland) and the fungal primers NS31 and NS41 (Simon *et al.* 1992) (annealing at 50ºC, 25 cycles) in a Gene Amp PCR System 2400 (Perkin-Elmer, Foster City, CA). The PCR-product was diluted 25-fold and subjected to a second round of amplification using the AMF-specific primer pair AM1R (Helgason *et al.* 1998) and NS31GC (annealing at 58ºC, 40 cycles). The product of this amplification was digested with ERh1 at 37ºC for 3 hours. This removes a highly variable but uninformative part of the SSU rDNA resulting in better definition of the bands and thus a more accurate analysis (pers. com. Wilfred Roling).

The digested product was separated using Denaturing Gradient Gel Electrophoresis (DGGE). DGGE was performed using a D-Code Universal Mutation Detection System (Bio-Rad, Hercules, CA) with a denaturing gradient in the polyacrylamide gel which ranged from 25% to 30% denaturant (100% denaturant is defined as 7M urea and 40%, v/v, formamide). DGGE was performed in a 0.5_TAE buffer (1_TAE = 40mM Tri-acetate, 20 mM sodium acetate, 1 mM EDTA, pH 8.0). Electrophoresis was performed at 200 V for 15 min followed by 60 V for an additional 16 h. Gels were stained with ethidium bromide and photographed on a Gel Doc 2000 UV transluminator (BIO RAD laboratories). Images were analyzed using Image Master Elite (version 4.20) (Amersham Bioscience, Roosendaal, The Netherlands). Only presence or absence of bands was registered as extraction and PCR amplification is known to be quantitatively biased. Bands represent different ribosomal types and can be used as a proxy for AMF taxa. However, some taxa might not be separated using this method while other taxa can show multiple bands (van der Putten *et al.* 2007). We will use the term taxa here for convenience but these points should be considered.

Statistical analysis

Propagule densities were considered significantly different if the 95% confidence intervals did not overlap. The two plant species were compared using a pairwise t-test with the four soils as replicates.

Colonized root length was analyzed using a general linear model with plant-species, soil origin and mix- versus monoculture and all their interactions included as independent fixed factors and block as a random factor. Normality and homogeneity of variance met the assumptions of a general linear model. Tukey posthoc tests were performed to separate the effects of the different soil origins.

To analyze the effect on biomass production a general linear model with plant-species, soil origin and mix- versus monoculture and all their interactions included as independent fixed factors and biomass as dependent was used. Normality and homogeneity of variance met the assumptions of a general linear model. Tukey posthoc tests were performed to separate the effects of the different soil origins.

Data from the molecular analysis was examined in two ways. Firstly, we tested for differences in the number of AMF taxa using a general linear model with plant-species and soil origin and their interaction as independent fixed factors and number of bands as dependent. Normality and homogeneity of variance met the assumptions of a general linear model. Tukey posthoc tests were performed to separate the effects of the different soil origins. In order to test if the number of occurrences of a band was correlated between the two plant species a Pearson correlation was performed.

Composition of the mycorrhizal community in the roots was analyzed using a canonic correspondence analysis with the four soil inocula and the two plant species as environmental variables. To test for significant effects of these variables Monte Carlo tests with 4999 iterations have been used.

Data was analysed using SPSS 12.03 (SPSS Inc.) and Canoco 4.5 (Plant Research International).

Results

Most probable number

The number of infective propagules per 100g of soil did not differ between the two species *(*pairwise t = 1.462, P = 0.240), indicating that *T. dubius* is as effective as *T. pratensis* in forming mycorrhizal associations under these conditions. Even though the different combinations of plant species and soil origin showed marked differences in propagule density, ranging from 51 (*T. dubius* in "both"-soil) to 9506 propagules per 100 g of soil (*T. dubius* in "pratensis" soil) (fig. 6.1).

Table 6.1a: Mean biomass and standard deviation of *T. dubius* and *T. pratensis* in different soils in monoculture or grown together.

	Soil		Biomass	SD
T. dubius	Dubius	mix	0.82	0.10
		mono	0.75	0.06
	Pratensis	mix	0.71	0.09
		mono	0.63	0.07
	Both	mix	0.90	0.12
		mono	0.66	0.09
	None	mix	0.66	0.29
		mono	0.55	0.12
T. pratensis	Dubius	mix	0.68	0.14
		mono	0.72	0.07
	Pratensis	mix	0.65	0.13
		mono	0.66	0.10
	Both	mix	0.51	0.08
		mono	0.70	0.14
	None	mix	0.28	0.13
		mono	0.45	0.08

**b:** ANOVA results of the analysis of produced biomass with species, soil and mixed/monoculture and all interactions

|	df	F	P
Species	1	21.99	0.00
Soil	3	16.18	0.00
Mix/mono	1	0.11	0.74
Spec * soil	3	3.22	0.03
Spec * mixmono	1	16.25	0.00
Soil * mixmono	3	0.30	0.83
Spec * soil * mixmono	3	2.15	0.10
Error	64		

Infection rates

In the mixed cultures both plant species were grown together. The percentage root that was colonized by AMF was however not correlated between plants grown together (Pearsons r = 0.327, P = 0.30). Therefore these samples were considered independent and were analyzed using a general linear model. The percentage colonized root was not dependent upon plant species ($F_{1,60}$ = 1.77, P = 0.19, fig. 6.2) but did depend on soil inocula ($F_{3,60}$ = 3.68, P = 0.017), with a significantly higher infection in "Dubius" and "Both" soil as compared to "None" soil (Tukey post hoc test, P < 0.05). "Pratensis" soil had intermediate infections. There was however an interaction between monoculture/mixculture and soiltype ($F_{3,60}$ = 2.90, P = 0.042). In

mixed culture, the percentage colonization differed between soils ($F_{3,28}$ = 4.83, P = 0.008), while there was no significant effect of soil in the monocultures ($F_{3,28}$ = 2.42, P = 0.087). There was an effect of soil, at least in monocultures, but the two species tested did not differ in the amount of hyphae in their roots.

Biomass production was lower in "None" soil compared to the other three soils (Tukey-B, α = 0.05), but this effect was stronger in *T. pratensis* than in *T. dubius* resulting in a significant interaction between soil and species (table 6.1a,b). Biomass also differed between plant species but this interacted with monoculture/mixed culture. In monoculture there was no difference between the two species ($F_{1,39}$ = 0.374, P = 0.545), but in mixed culture *T. dubius* produced more biomass than *T. pratensis* ($F_{1,39}$ = 26.767, P < 0.001).
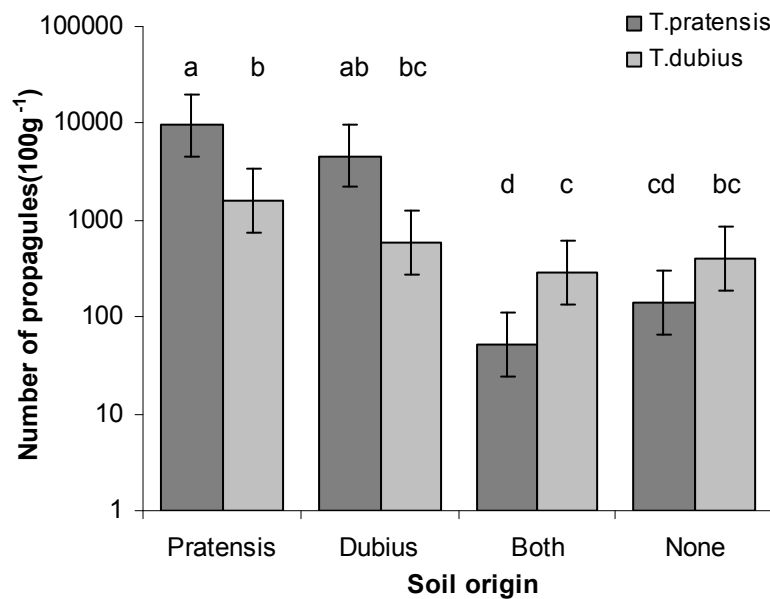
Figure 6.1: Density of infective arbuscular mycorrhizal propagules in four soils as determined by a most probable number analysis. Soils used are rhizosphere soil from a population of *T. pratensis*, *T. dubius*, a mixed population or a grassland without *Tragopogon* species. Soils are tested for both *T. pratensis* and *T. dubius*. Letters indicate significant difference (non-overlapping 95% confidence intervals). (Mean and 95% interval)
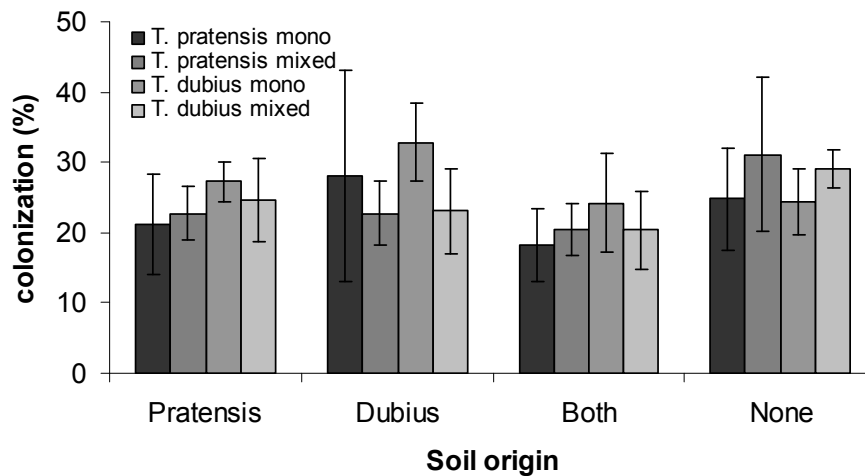
Figure 6.2: Percentage root length colonized by AMF examined by grid intersect counting after ink staining for *T.pratensis* and *T. dubius* grown in four different soil in either monoculture or together. (Mean and SD)

Molecular AMF community profiling analysis

The number of bands did not differ between the two plant species ($F_{1,28}$ = 1.74, P = 0.197) but did differ between soils ($F_{3,28}$ = 4.08, P = 0.016). Bands that were rare in one host were also rare in the other and the same was true for often occurring bands. The number of times a specific band was found in T. dubius samples was highly correlated with the number of samples of T. pratensis in which it was found (R^2 = 0.81, P < 0.001, fig. 6.3).

Plant species did not have a significant effect, (P = 0.245, fig 6.4) for *T. dubius* or (P = 0.237) for *T. pratensis*. Soils, however, did have a significant effect (P < 0.001) and this effect explained 17.5% of the variation. "None" soil explained most of the variation (P < 0.001; accounting for 10.6%) followed by "Both" soil (P < 0.001; explaining 7.2%) and "Dubius" soil (P = 0.002; explaining 6.7%). "Pratensis" soil did not differ significantly from the other three combined (P = 0.505).

There number of bands varied from 1 to 8 with an average of 3.4 and a standard deviation of 1.6. There were no highly dominant bands, being present in the majority of samples and none of the common bands were restricted to a single soil.
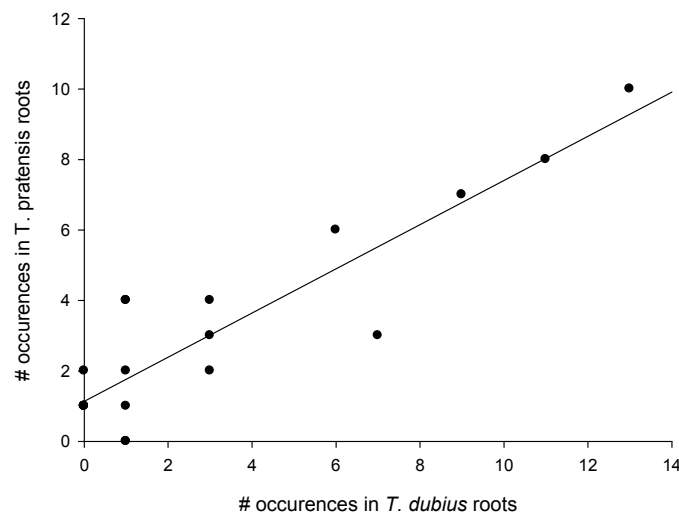
Figure 6.3: The number of root samples of *T. dubius* in which bands, i.e. AMF-taxa, occurred (x-axis) and the number of occurrences of that band in root samples of *T. pratensis* (Y-axis) is highly correlated (R^2 = 0.81, P < 0.001). Each dot represents a band.

Discussion

T. dubius and *T. pratensis* did not differ in the density of suitable AMF propaguls in the tested soils, nor in the resulting percentage root colonization. The community structure of AMF in the roots of these tow species did not differ either. The affinity for and association with arbuscular mycorrhizal fungi does not differ between these two plant species even though they are known to differ in their interaction with the soil community as a whole (van Grunsven *et al.* 2007). We found that the ability of the plants to form associations with mycorrhiza was similar for both species in the four soils tested as measured by the number of infective propagules in the soil and the percentage root length colonized by AMF. The AMF community in the roots also did not differ significantly between the two species, as determined by molecular community profiling of the 18S rRNA gene. The number of bands was the same and the relative abundance of bands was highly correlated. These data suggest that the affinity of *T. dubius* and *T. pratensis* for thes AMF-taxa is the same; they are just as effective forming mycorrhiza with the two species behaving identical in this respect.

Therefore the invasive species is unlikely to experience a negative effect from reduced affinity due to loss of co-adapted AMF during range expansion.

The used primer pair, NS31 and AM1 has been used extensively to selectively amplify AMF SSU rRNA gene fragments (Helgason *et al.* 1998; Kowalchuk *et al.* 2002). However, it has recently been demonstrated that these are not completely specific to AMF, with amplification of some Ascomycetes and Basidiomycetes species (Douhan *et al.* 2005; Ma *et al.* 2005; Rodríguez-Echeverría & Freitas 2006). Non-AMF fungi were very rare in the microscopically examined roots and roots were cleaned to prevent contamination with fungi clinging to the root.

The used primers fail to amplify some AMF lineages, most notably the *Archaeosporaceae*, *Ambisporaceae* and *Paraglomaceae* (Lee *et al.* 2008). Therefore these families are not considered in this study. As most studies use AM1 as specific primer it is unknown how common these families are.

In contrast to van der Putten *et al.* (2007), who found a reduced AMF community in the roots of the introduced grass species *Cenchrus biflorus*, we observed no significant difference between the infections density of the native versus exotic plant species. It should be noted however, that the study of van der Putten (2007) examined grasses across different subfamilies, and the AMF communities of different native grasses were also shown to differ. *C. biflorus* is an annual or short lived perennial species in contrast to the native species which were perennials. The reduced AMF community may thus have been more a result of different plant characteristics and life-history strategies than of nativeness (Veenendaal *et al.* 1992). *T. pratensis* and *T. dubius* are closely related, similar in life history, ecology and morphology.
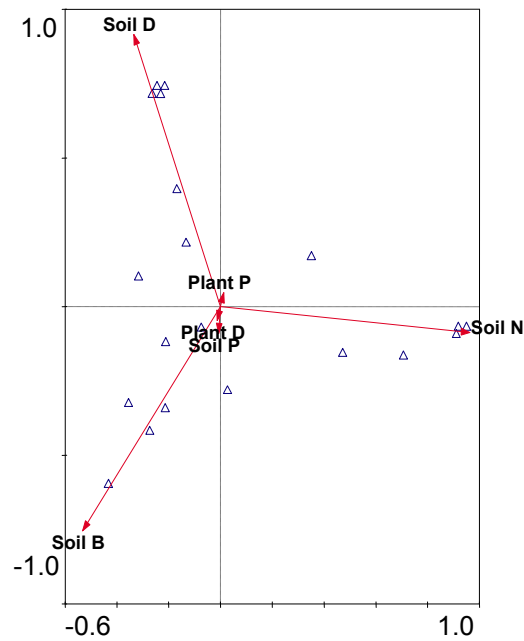
Figure 6.4: Canonical correspondence analysis (CCA) biplot of the arbuscular mycorrhizal fungi banding patterns (indicated as absence or presence) in roots of the native *T. pratensis* and the exotic *T. dubius*. Four soils from different origins within The Netherlands were used as replicates. Effects of soil origin and plant species are depicted as arrows. Overlapping datapoints have been offset

T. dubius and *T. pratensis* have been shown to differ in their interaction with the soil community in the tested range (van Grunsven *et al.* 2007). But they do not differ in their affinity for AMF, in the percentage of root length colonized, nor in the composition of the AMF community. Therefore we can conclude that the association with AMF is not reduced in the range expanding plant species *T. dubius* as compared to the native *T. pratensis*. We have not tested to what extend the found AMF provide mutualistics services to the two studied plant species. To test this inoculation studies quantifying the effects of AMF on plant performance under a diversity of conditions are needed.

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Synthesis

Over the last 15 years, the importance of soil organisms in vegetation dynamics has received increasing attention (van der Putten *et al.* 1993; Bever 1994; Callaway *et al.* 2004; Wardle *et al.* 2004; Kardol *et al.* 2006). The impact of the soil community on plant performance has proven to be potentially very strong and can vary from very negative to positive. Direction and strength of the effect of the soil community on plant performance can, to a certain extent, be used to explain the performance of a plant or plant species (Klironomos 2002; Reinhart & Callaway 2006). The fact that plant species can become released from their natural enemies when introduced into new areas (Elton 1958), has long been acknowledged but the evidence that this is also true for soil-borne natural enemies has up to now been limited. Release from soil-borne natural enemies has been tested for a few tree species, one grass and one forb (Reinhart & Callaway 2006). I have shown that this is likely to be also true in a perennial, non-mycorrhizal succulent (chapter 2). This experiment also illustrates the possible impact of the soil community on plant performance. I found a substantial decrease of 32% in biomass production of plants grown in inoculated soil compared to sterilized soil from the native range. There was no significant difference between biomass production in sterilized and non-sterilized soil when soil from the invasive range was used. The impact of the soil community can thus vary strongly between locations, even within a plant species.

Plant-soil interaction does not only change after introductions but also after natural colonizationof a new habitat. Colonization of new sites that have become suitable within the native range, e.g. through succession, can be viewed as a similar process on a smaller scale. Temporary release of natural enemies has repeatedly been found on this scale as well. Immediately after colonization specialist natural enemies are absent. With time plant-soil interaction becomes more negative to the plant through the accumulation of pathogens (van der Putten *et al.* 1993; Kardol *et*

al. 2006). As a result of climate change many plant species are expanding their range polewards (Bakkenes *et al.* 2002; Parmesan & Yohe 2003). These plant species leave there native range without anthropogenic introductions.

Plants that have recently colonized The Netherlands experience a less negative plant-soil feedback than native related species in this area (chapter 3, 4, 5). The difference between the native species and the range expanding species is a result of the range shift. The range expanding species experience a less negative plant-soil feedback in the non-native than in their native range (chapter 5). The changes in plant-soil interaction of range-expanding plant species can change the performance and ecological profile of these plant species (Klironomos 2002; Kardol *et al.* 2006). They are likely to become more dominant than in their native range. Interestingly, a comparable result has recently been found for the butterfly *Aricia agestis*. This species is also expanding its range polewards and experiences less parasitism in the newly colonized sites than in the long established populations (Menéndez *et al.* 2008). Furthermore, it also experiences less parasitism than the long established *Polyommatus icarus* at the same location. The present and the aforementioned study are the only studies on enemy release through range expansion known to us. The fact that, despite their obvious differences, enemy release has been found in both cases makes it likely that this phenomenon is rather common in range expanding organisms.

The effect of the soil community on plants is a net effect. Many biotic and abiotic factors affect and are affected by both the plant and soil organisms and these interactions can be positive or negative. The net effect of plant-soil feedback is negative for most plant species (Kulmatiski *et al.* 2008) indicating that negative, pathogenic effects are dominant over positive, mutualistic effects. However, the absence of suitable mutualists can prevent invasions while foreign mutualists with strong positive effects on plant performance can promote invasions (Reinhart & Callaway 2006). The less negative net plant-soil feedback found for plants that have recently expanded their range can therefore either be caused by a reduction of negative factors or an increase in positive factors. Furthermore, the loss of soil-borne mutualists can have negative effects on plant performance, reducing the net effect of enemy release. Plants can lose mutualistic interactions in the same manner as they can escape natural enemies (Richardson *et al.* 2000; Parker *et al.* 2006; van der Putten *et al.* 2007). This does not seem to be the case for arbuscular mycorrhizal

fungi (AMF) in *Tragopogon dubius*. As AMF are one of the most important groups of mutualists for most plant species I have focused on this group. The non-native plant species, *T. dubius,* did not differ from the native plant species, *T. pratensis,* in its affinity for arbuscular mycorrhiza (chapter 6). Neither did the mycorrhizal communities in the roots of these two plant species differ. It is therefore unlikely that *T. dubius* experiences a loss of mutualistic services from AMF in its newly colonized range. Nor is it likely that a more positive interaction can explain the less negative net plant-soil feedback.

The fact that closely related species were already present in the invaded range might have resulted in an AMF community also suitable for *T. dubius*. It is likely that AMF with a high affinity for a plant species will also have a high affinity for closely related and physiological similar species. Plant species that colonize areas where no related species are present might experience difficulty in associating with AMF. This can however not be tested with the methods used in chapter 6 as I used a native related species as yardstick. Comparisons with a number of native, unrelated plant species might be used instead. Other soil-borne mutualists such as rhizobia and ectomycorrhizal fungi might show different patterns as these are often highly host specific (Alexander 1989; Bruns *et al.* 2002; Fauvart & Michiels 2008). For plants depending on these groups of soil-borne mutualists the absence of suitable mutualist species significantly hinders invasion (Richardson *et al.* 2000). If this is the case, plant species dependent on more specialized mutualist might show a slower spread polewards. *Tragopogon sp.* do not form associations with these mutualists, rhizobia and ectomycorrhizal fungi are of no concern in this study.

The net plant-soil feedback is less negative for the range expanding plants than for the native plants, but their affinity for AMF does not differ. The difference in plant-soil feedback between the range expanding and native species is therefore likely to be the result of a reduction of negative effects, i.e. a release from soil-borne natural enemies. It is currently unknown which species of soil-borne natural enemies these species are released from. In order to clarify this, a comparison of the soil community in the native and invaded range of range expanding plant species has to be made, followed by inoculation experiments to test the effect of the herbivorous and pathogenic members of the found community. A major point of concern is that the effect of natural enemies in isolation may not represent their effect as member of a complex soil community (Piśkiewicz *et al.* 2007). Furthermore, it is likely that each

plant species will have its own set of dominant natural enemies. Two well studied plant species in this context are *Prunus serotina* and *Ammophila arenaria*. The first has escaped from *Pythium spp.* when introduced into Europe (Packer & Clay 2000; Reinhart *et al.* 2003); the second has been released from specialist nematodes (Beckstead & Parker 2003) when it establishes in wind-blown sand. The key players in these two cases are two very different organisms. This will make extrapolation to other plant species difficult.

Plant-soil feedback has not only been affected by climate change through range shifts. Global climate change may also have direct effects on plant-soil interactions through e.g. changes in the local temperature. The increase in ambient temperature as a result of global climate change can affect soil processes such as decomposition (Cornelissen *et al.* 2007). However it does not seem to influence plant-soil feedback (chapter 5). The absence of a net effect of temperature on plant-soil feedback might be a result of the complex interactions in soil communities dampening direct effects of a temperature increase (Reinhart & Callaway 2006; Piśkiewicz *et al.* 2007). Predictions on the effect of climate change are often based on the direct effects on organisms or processes, in this case increased activity of pathogens at higher temperatures. But there are many feedbacks and interactions in natural systems, both biotic and abiotic. As the result of these interactions the ultimate effects can be different from the direct effects of climate change. A few examples of this are known. The increased decomposition as a result of warming in the arctic can be counterbalanced by an increase of plant species with recalcitrant litter (Cornelissen *et al.* 2007). Effects of climate change can even be reversed. In grasslands with summer droughts, additional water in late spring is beneficial for species of all trophic levels. However, the subsequent changes in the vegetation in the long run result in a dominance of early season grasses and a diminished species richness of plants and invertebrates (Suttle *et al.* 2007). The fact that such interactions can compensate the direct effects might explain why no effect of temperature on plant-soil feedback was found. Global climate change encompasses more effects than a mere temperature change. CO_2 concentrations are increasing, the number of rain events is likely to increase in some areas and decrease in others and an increase in frequency of extreme events, such as flooding and droughts, is expected. These changes might have their effect on plants and plant-soil interactions

but the studies currently available have conflicting results (Sadowsky & Schortemeyer 1997; Ainsworth & Long 2005; Suttle *et al.* 2007; Kreyling *et al.* 2008).

The effects of climate change on the distribution of plant species are enhanced by changing interactions between plants and the soil community. Although it has been stated that a positive plant-soil feedback does not result in faster spread in theoretical homogenous landscapes, it can result in increased dispersal in heterogeneous landscapes (Levine *et al.* 2006). Rare, long-distance dispersal events determine the rate of range expansion in a heterogeneous landscape with pockets of suitable habitat in an unsuitable matrix, as is typical for North-Western Europe. These rare events increase in likelihood with the number of seeds produced and the height at which they are released (Soons *et al.* 2004). As both plant performance and plant density, and thus seed production, are partly determined by plant-soil feedback (Klironomos 2002), a less negative plant-soil feedback will result in an increased rate of these long-distance dispersal events. This will result in faster range expansions in a heterogeneous landscape. A comparable prediction has been has been made for the forementioned butterfly *Aricia agestis*, (Menéndez et al. 2008). Species expanding their range into areas that have recently become suitable may escape from their natural enemies. This in turn increases their rate of expansion. Organisms with a dispersal ability that exceeds that of their natural enemies, enabling them to escape these natural enemies, thereby further increase their dispersal ability.

In the long run natural enemies are likely to expand their range as well and undo the enemy release of the range expanding organism, similar to temporary enemy release within successional systems (van der Putten *et al.* 1993; Kardol *et al.* 2006). Dependent on the dispersal abilities of both the host and the natural enemy, the enemy release can be sustained for a shorter or longer time period. The natural enemies might in turn be released from their own natural enemy while expanding its range (Menéndez *et al.* 2008). Furthermore organisms present in the colonized range might switch host to the range expanding organism thus undoing the enemy release (Prokopy *et al.* 1988).

Climate change will be one of the most important issues in ecology in the coming decades (Thomas *et al.* 2004; Thuiller 2007). It is vital to be able to make better predictions on the effects of climate change, so conservation efforts can be imposed where most effective. Currently, these predictions are mainly based on

bioclimatic envelopes. This method describes the current distribution of a species in terms of climate and other conditions, the so called envelope. With predictions of the future climate, the future range can be assessed using these limits. These predictions have for instance been made for the distribution of plant species in Europe in the year 2050 (Bakkenes *et al.* 2002). There are several weaknesses in these predictions. Species might have limited dispersal and will not be able to reach some of the available sites. The decrease of migration (Wilcove & Wikelski 2008), and thus long distance dispersal through migratory animals (Whelan *et al.* 2008), and increasing habitat fragmentation further limit dispersal. The current state of the landscape is unprecedented and therefore historical data, e.g. based on post-glacial dispersal, may proof unreliable. Furthermore, interactions with other organisms are not considered in these model predictions (Davis *et al.* 1998; Araújo & Luoto 2007; Brooker *et al.* 2007). I have shown that the range shifts result in a change in one of the key biotic interactions of plants, i.e. plant-soil feedback. The range expansion can cause a positive feedback through a release from natural enemies, and in effect change the characteristics, the envelope, of a species. The usage of ensemble models is being advocated recently (Araújo & New 2007). This method is thought to generate more accurate forecasts and uncertainty values. However, these uncertainty values do not represent true uncertainty on future distribution, but merely the disagreement between models. As all models lack many important ecological aspects, an agreement between models does not mean they are correct. Future studies should make a stronger effort to encompass the complexity of natural systems, including biotic interactions of the species. In order to achieve this we need more insight in the reciprocal effects of climate change on ecological systems.

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Summary

The global climate has changed over the last decades. Temperature in Western Europe has risen significantly. The current temperature in The Netherlands is comparable with the temperature in central France in 1960-1990. As a result of this recent climate change, areas that have previously been climatically unsuitable for species have now become suitable. Many species are expanding their range polewards, colonizing these newly available areas. We currently find both animal and plant species in The Netherlands that used to be restricted to warmer areas but that have been able to expand their range. If a species can expand its range rapidly, it might be able to become released from its natural enemies in the newly colonized part of its range, even though there is not an actual barrier that prevents natural enemies to follow. Soil-borne natural enemies are often poor dispersers and are therefore likely to be released from. This would result in a less negative net effect of the soil community for plants that have recently colonized The Netherlands than for native species.

   Plants interact with many other organisms, both above- and belowground. Most of these organisms depend on the plants with which they interact. Some interact directly with the plant while others interact only indirectly, e.g. by feeding on herbivores or litter formed by the plant. The soil community also affects the plant either through direct interactions such as herbivory, or through indirect interactions such as mineralization of organic matter. A plant growing in soil will change both the abiotic conditions in the soil, e.g. by nutrient uptake, and the community in this soil. These changes in the soil will in turn affect the plants' performance. The effect a plant has on its own performance through its effect on the soil is known as plant-soil feedback, and is considered a significant factor in explaining local dominance of plants and succession. Plant-soil feedback is often negative for the plant as it accumulates pathogens and depletes resources. However, this is not necessarily the

case. Plants that have been introduced into another continent can be released from soil-borne natural enemies. If their natural enemies are not introduced with them and the native species have less impact, a plant can be released from an important limiting factor. This has been shown to result in a less negative plant-soil interaction in the invaded than in the native range. However, only a few plant species have been previously tested.

I tested if this was also valid for *Carpobrotus edulis s.l..* This succulent plant originates from South Africa and is invasive in e.g.California, Australia and the Mediterranean basin. The impact of the soil community on the plant is assessed by comparing the biomass production of plants in soils inoculated soil with a sterilized control. As inoculum rhizosphere soil collected in a population of *Carpobrotus* was used. When the inoculum was collected from the native range, there was a strong negative effect of inoculation on plant performance but no effect of inoculation when an inoculum from the invaded range was used. This indicates that these plants are indeed released from natural enemies in the invaded range. A release from natural enemies makes it possible to reduce the investments in defense and increases competitive ability. I did not find any evidence for this in *Carpobrotus edulis s.l..* Biomass production did not differ between plants from the native and invaded range.

However, enemy release is not only restricted to anthropogenic introductions. Plants that colonize new sites are also released from natural enemies, be it temporarily. I tested this by comparing the plant-soil feedback of three plant species that had recently extended their range into The Netherlands with related native species. Soils were conditioned by growing one of the six plant species in soil after inoculating with rhizosphere soil from a conspecific plant. In the second growing phase, pots were inoculated with this conditioned soil or a sterilized control, and plants of the same species were grown in these pots for 12 weeks. The difference in produced biomass between a plant grown in inoculated soil and the sterilized control is used as a measure for plant-soil feedback. The plant-soil feedback of the non-native species was significantly less negative than that of the native species.

The effect of climate change also includes changes in local temperature. Ecosystems that are invaded by range-expanding plant species simultaneously experience an increase in ambient temperature. As temperature itself might affect plant-soil feedback, I performed a similar experiment at two temperatures, i.e. 20°C/15°C and 25°C/20°C day/night temperature. In this experiment, I used six plant

species that had recently extended their range into The Netherlands, and compared them pairwise with six related native species. In order to create species-specific conditioned soils, these twelve plant species were grown in soils inoculated with a rhizosphere community that was collected from a Dutch grassland. In the second phase of this experiment, the plants were grown in soil that was conditioned by a conspecific in the first phase and in a mixture of the 12 species-specific conditioned soils. This last treatment can be used as a control, as the effects on plant performance at this level of dilution are not detectable. The difference in biomass produced in these two treatments is a measure of plant-soil feedback. Again, the non-native species experienced a less negative plant-soil feedback than the native species. There was however no effect of the temperature treatment on this plant-soil feedback.

These two experiments demonstrated a difference between the species native to The Netherlands and the non-native species. However, these experiments did not show whether these plant species have indeed escaped their natural soil-borne enemies while expanding their range. This requires a comparison between the native and invaded range of a plant species that has recently expanded its range. I did this by assessing the impact of the soil community in the native and invaded range of the range expanding plant species *Tragopogon dubius*. A gradient of plant soil interaction from south to north irrespective of the plants nativeness, e.g as a result of the climatic gradient, cannot be excluded a priori. To control for such gradients I also tested a related species that is native to the entire transect, *Tragopogon pratensis*. Rhizosphere soil from both species was collected in populations along a transect from Marseille, in the native range of *T. dubius*, to Amsterdam, in the invaded range of *T. dubius*. I compared growth of plants in soil inoculated with an extract of a conspecific rhizosphere soil, with a sterilized control. This is a measure of the impact of the soil community on plant performance. The range expanding species *T. dubius*, did experience a less negative effect of inoculation in the invaded part of the range than in the native part of the range, indicating enemy release. In *T. pratensis* this pattern was absent; it showed the opposite with a more negative effect of inoculation in the northern than in the southern part of the range. In the northern part of the transect, where *T. dubius* is non-native, the effect of inoculation was significantly more negative for *T. pratensis* than for *T. dubius*.

I therefore conclude that *T. dubius* was released from negative plant-soil feedback while expanding its range northwards.

The effect of the soil community is a net effect. The soil community consists of many different organisms, some of which affect plant performance negatively, such as pathogens and herbivores, while other organisms enhance plant performance, so called mutualists. Loss of mutualist would have a negative effect on net plant-soil interactions. Contrastingly, the presence of foreign mutualists with strong positive effects can result in a more positive net plant-soil interaction. One of the most important groups of soil-borne mutualists is arbuscular mycorrhizal fungi, or AMF. These fungi can enhance plant performance by providing phosphorous, reduce herbivory and increase drought resistance. In return the fungi receive a carbon source, such as sugars, from the plant. AMF are poor dispersers and therefore plant species might lose their co-adapted mutalists as well as their enemies, while expanding their range. I tested if *T. dubius*, which has recently colonized The Netherlands, is less successful in associating with mycorrhiza than the native related *T. pratensis*. Several measures for plant-fungal affinity were compared between the two plant species; the number of AMF propagules that are able to colonize the plant, the percentage of root length colonized by arbuscular mycorrhiza, and the composition of the resulting AMF community in the roots. As these factors also depend on the inoculum, four soil replicates from different origins were used as inocula. The number of infective propagules, the percentage colonized root and the composition of the AMF community did differ between soil inocula but not between plant species. *Tragopogon dubius* and *T. pratensis* are as effective in associating with mycorrhiza, and it is unlikely that *T. dubius* experiences a negative effect through the loss of AMF mutualists during range expansion. The net effect of the soil community on the performance of range expanding plants is more negative in the native than in the non-native range, but there are no differences in the association with the most important mutualist. Therefore the changes in net plant-soil feedback most likely reflect a reduction of negative components during range expansion, namely release from natural enemies.

The soil community potentially has large negative effects on plant performance, but these effects are not constant. *Carpobrotus edulis s.l.* experiences a strong negative effect in the native but not in the invaded range, indicating that it is released from soil-borne pathogens.

Plant species that expand their range polewards experience a less negative plant-soil interaction in the non-native range than native, related species. This result was confirmed in three independent experiments. Plant-soil interaction appears to be insensitive to temperature change; a 5°C difference in ambient temperature did not have a significant effect.

Alterations in biotic interactions through climate change, such as those we described in this thesis, can have significant effects on the outcome of climate change over the coming decades. In order to be able to prepare for these changes and focus conservation efforts where they are most effective, it is essential to have a more thorough understanding of the potential effects of climate change on the interactions between plant and soil communities.

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Samenvatting

Het wereldwijde klimaat is de laatste decennia veranderd, zo is de temperatuur in West-Europa aanzienlijk gestegen. De gemiddelde temperatuur in Nederland over de laatste jaren is vergelijkbaar met de temperatuur in Frankrijk in 1960-1990. Ten gevolge van deze klimaatverandering zijn gebieden, die voorheen klimatologisch ongeschikt waren, voor veel soorten geschikt geworden. Veel plant- en diersoorten breiden dan ook recentelijk hun verspreidingsgebied uit en koloniseren de gebieden die door klimaatverandering geschikter geworden zijn. De laatste jaren worden in Nederland plant- en diersoorten aangetroffen die voorheen voorkwamen in zuidelijker gebieden.

Natuurlijke vijanden, die gespecialiseerd zijn in een van deze soorten, zullen niet aanwezig zijn in het nieuw gekoloniseerd gebied omdat de plant waarvan ze afhankelijk zijn daar nog niet aanwezig was. Als een plantensoort zijn verspreidingsgebied snel uit kan breiden kunnen deze natuurlijke vijanden mogelijk niet volgen en kan de plant ontsnappen aan zijn natuurlijke vijanden. Dit ondanks het ontbreken van fysieke barrières. Natuurlijke vijanden die in de bodem leven hebben vaak zeer beperkte dispersiecapaciteiten en zijn vaak specialisten. Dit kan ertoe leiden dat planten die nieuwe gebieden koloniseren makkelijker aan deze bodembewonende vijanden kunnen ontsnappen. Hierdoor kunnen planten die nieuwe gebieden koloniseren waarschijnlijk aan deze vijanden ontsnappen. Daarom ondervinden plantensoorten die Nederland recentelijk gekoloniseerd hebben een minder negatief effect van de bodemgemeenschap dan plantensoorten die hier van oudsher voorkomen.

Planten beïnvloeden en worden beïnvloed door vele andere organismen, zowel boven- als ondergronds. De meeste van deze organismen zijn afhankelijk van de plant waarmee ze samenleven. Sommige soorten zijn direct afhankelijk van de plant waarmee ze samenleven, zoals herbivoren, andere alleen indirect, bijvoorbeeld doordat ze herbivoren of dood plantenmateriaal eten. De bodemgemeenschap op zijn beurt beïnvloedt de plant door bijvoorbeeld herbivorie maar ook door indirecte

interacties zoals het afbreken van organisch, waardoor voedingsstoffen voor de plant vrijkomen. Een plant verandert hierdoor zowel de abiotische aspecten van de bodem waar hij in groeit als de bodemgemeenschap. Deze veranderingen hebben vervolgens ook een effect op de plant. Deze terugkoppeling staat bekend als plant-bodem interactie en wordt gezien als een belangrijke factor bij het verklaren van lokale dominantie van plantensoorten en successie. Plant–bodem interactie is vaak negatief voor de plant omdat natuurlijke vijanden toenemen in aantal en hulpbronnen uitgeput raken, dit is echter niet noodzakelijkerwijs het geval. Plantensoorten die geïntroduceerd worden waar ze niet van nature voorkomen kunnen ontsnappen aan hun natuurlijke (bodem-)vijanden. Als de natuurlijke vijanden niet met de plant geïntroduceerd worden en de inheemse soorten een minder negatief effect hebben, is de plant bevrijd van een belangrijke beperkende factor. Het is aangetoond dat invasieve planten in het gebied waar ze invasief zijn een minder negatieve plant-bodem interactie hebben dan in het gebied waar ze inheems zijn. Dit is echter maar bij een zeer beperkt aantal plantensoorten onderzocht.

Ik heb getest of dit ook waar is voor *Carpobrotus edulis s.l.*. Deze succulent komt oorspronkelijk uit Zuid-Afrika maar is invasief in o.a. Californië, Australië en in het Middellandse Zeegebied. De impact van de bodemgemeenschap op deze plant is bepaald door de biomassa productie van planten te vergelijken tussen geïnoculeerde en gesteriliseerde bodems (steriele grond waaraan respectievelijk wel en geen bodemgemeenschap is toegevoegd). Als inoculum is grond uit de wortelzone van *Carpobrotus* planten gebruikt. Inoculatie met grond uit het natuurlijke verspreidingsgebied had een sterk negatief effect op de biomassa productie van de plant. Inoculatie met grond uit het gekoloniseerde gebied had echter geen aantoonbaar effect op de biomassa productie. Dit wijst erop dat deze plant in het gekoloniseerde gebied inderdaad is ontsnapt aan zijn natuurlijke vijanden. Wanneer een plant is ontsnapt aan zijn natuurlijke vijanden kan deze investeringen in afweer verminderen en meer investeren in groei en competitieve kwaliteiten. Hiervoor heb ik echter geen aanwijzingen gevonden in *Carpobrotus edulis s.l.*. Biomassa productie verschilde niet tussen planten uit het oorspronkelijk verspreidingsgebied en die uit het gekoloniseerde gebied.

Het ontsnappen aan natuurlijke vijanden is echter niet beperkt tot introducties. Planten die nieuwe gebieden koloniseren kunnen ook ontsnappen aan hun natuurlijke vijanden, zij het tijdelijk. Ik heb dit getest door de plant-bodem interactie

van drie plantensoorten die recent Nederland gekoloniseerd hebben, te vergelijken met die van drie verwante inheemse soorten. Steriele grond werd geïnoculeerd met grond uit de wortelzone van een van deze zes plantensoorten. Hierna werd deze grond geconditioneerd door de zes planten soorten er in te laten groeien, elk met hun eigen inoculum. In de tweede groeifase werd grond geïnoculeerd met de geconditioneerde bodem uit de eerste groeifase of een gesteriliseerde controle. Planten van dezelfde soort groeide voor 12 weken in deze bodems. Het verschil in biomassa tussen de planten in geïnoculeerde bodems en gesteriliseerde bodems is een maat voor plant-bodem interactie. Plant-bodem interactie bleek minder negatief voor de soorten die recent Nederland gekoloniseerd hebben dan voor de inheemse soorten.

Het effect van klimaatverandering omvat ook veranderingen in lokale temperaturen. Ecosystemen die geïnvadeerd zijn door planten die hun areaal uitbreiden ondergaan tegelijkertijd een verhoging van de omgevingstemperatuur. Omdat temperatuur zelf een effect op plant-bodem interacties kan hebben, heb ik een vergelijkbaar experiment uitgevoerd bij twee temperaturen, 20°C/15°C en 25°C/20°C, dag/nacht temperatuur. In dit experiment heb ik zes plantensoorten gebruikt die recent Nederland gekoloniseerd hebben en deze paarsgewijs vergeleken met zes inheemse verwante soorten. Om soortspecifieke geconditioneerde bodems te krijgen zijn deze twaalf plantensoorten gegroeid in bodems geïnoculeerd met de bodemgemeenschap van een Nederlands grasland. In de tweede fase van dit experiment groeide de planten in de bodem geconditioneerd door een plant van dezelfde soort en in een mengsel van de 12 geconditioneerde bodems. Deze laatste behandeling is gebruikt als controle, bij deze mate van verdunning zijn geen effecten op plantengroei aantoonbaar. Het verschil in biomassa productie tussen deze twee behandelingen is een maat voor plant-bodem interactie. Wederom hadden de soorten die Nederland recent gekoloniseerd hebben een minder negatieve plant-bodem interactie dan de inheemse soorten. Er was echter geen effect van de temperatuur behandeling op de plant-bodem interactie.

In de twee voorgaande experimenten is aangetoond dat planten die recent Nederland gekoloniseerd hebben een minder negatieve plant-bodem interactie hebben dan inheemse plantensoorten. Het is echter niet aangetoond dat deze plantensoorten ontsnapt zijn aan hun bodembewonende natuurlijke vijanden tijdens het uitbreiden van hun areaal. Hiervoor is een vergelijking tussen het oorspronkelijke

en nieuwe deel van het verspreidingsgebied noodzakelijk. Dit heb ik gedaan door de impact van de bodemgemeenschap in het oorspronkelijke en nieuwe verspreidingsgebied van *Tragopogon dubius* te bepalen. Deze plantensoort heeft recent zijn areaal noordwaarts uitgebreid en Nederland gekoloniseerd. Een gradiënt in plant-bodem interactie van zuid naar noord, bijvoorbeeld ten gevolge van het verschil in klimaat, is niet a-priori uit te sluiten. Om te controleren voor een dergelijke gradiënt heb ik ook *Tragopogon pratensis* ,een nauw verwante soort die inheems is over het gehele transect, meegenomen in het experiment. Langs een transect van Marseille tot Amsterdam heb ik in populaties van beide soorten grond verzameld. Hierna heb ik de groei van planten vergeleken tussen bodems geïnoculeerd met een waterig extract van deze grond en een steriele controle. Dit is een maat voor het effect van de bodemgemeenschap op de plant. Inoculatie met grond uit het recent gekoloniseerde gebied had een minder negatief effect op de groei van *T. dubius* dan inoculatie met grond uit het oorspronkelijke verspreidingsgebied. Dit indiceert een bevrijding van natuurlijke vijanden. Dit patroon ontbrak in *T. pratensis* die overal inheems is. Deze soort liet zelfs een tegenovergesteld patroon zien met een meer negatief effect van inoculatie met grond uit het noordelijk dan uit het zuidelijk gebied. Voor het noordelijke deel, waar *T. pratensis* wel en *T. dubius* niet inheems is, ondervond de eerste een groter negatief effect van inoculatie dan de laatste soort. Daarom concludeer ik dat *T. dubius* inderdaad gedeeltelijk is bevrijd van negatieve plant-bodem interacties tijdens het uitbreiden van zijn verspreidingsgebied.

Het effect van de bodemgemeenschap is een netto effect. De bodem gemeenschap bestaat uit veel verschillende organismen, sommige met een negatief effect op planten, zoals pathogenen en herbivoren, andere met een positief effect op planten, de zogenaamde mutualisten. Het verlies van mutualisten heeft een negatief effect op plant-bodem interacties. De aanwezigheid van mutualisten met een sterk positief effect in het nieuw gekoloniseerd gebied kan daarentegen resulteren in een meer positieve plant-bodem interacties. Een van de belangrijkste bodem mutualisten zijn arbusculaire mycorrhiza schimmels (AMF). Deze schimmels kunnen plantengroei bevorderen door fosfor aan de plant te geven, herbivorie te verminderen of droogteresistentie te verbeteren. In ruil hiervoor krijgt de schimmel van de plant een koolstofbron, bijvoorbeeld suikers. AMF verspreiden zich slecht en planten die een nieuw gebied koloniseren kunnen deze mutualisten verliezen net zoals ze ontsnappen aan hun vijanden. Ik heb getest of *T. dubius*, die recent

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Nederland heeft gekoloniseerd, minder succesvol is in zijn associaties met mycorrhiza dan de inheemse verwante *T. pratensis*. Verschillende aspecten van plant-schimmel affiniteit zijn vergeleken tussen deze twee plantensoorten; het aantal infectieve eenheden dat de plant kan koloniseren, het percentage wortellengte dat gekoloniseerd wordt en de compositie van de AMF gemeenschap in de wortels. Omdat deze factoren ook afhankelijk zijn van het gebruikte inoculum zijn vier inocula van verschillende locaties gebruikt. Het aantal infectieve eenheden, het percentage wortel dat gekoloniseerd was, noch de compositie van de AMF gemeenschap verschilde tussen de twee plantensoorten. *Tragopogon dubius* en *T. pratensis* zijn even effectief in hun associatie met AMF en het is onwaarschijnlijk dat *T. dubius* een negatief effect ervaart door het verlies van AMF mutualisten tijdens het uitbreiden van zijn areaal.

Als we de resultaten van de verschillende experimenten bekijken worden een aantal trends zichtbaar. We kunnen stellen dat het netto effect van de bodem gemeenschap op de planten die een gebied recentelijk gekoloniseerd hebben minder negatief is dan voor inheemse soorten. Dit resultaat is bevestigd in drie onafhankelijke experimenten. Verder blijken plant-bodem interacties ongevoelig te zijn voor temperatuur verandering; een verschil van 5°C in omgevingstemperatuur had geen significant effect. Bovendien ervaren plantensoorten die hun areaal naar het noorden uitbreiden een minder negatieve plant-bodem interactie in het nieuwe verspreidingsgebied dan in hun oorspronkelijk verspreidingsgebied. Ze verschillen echter niet van inheemse soorten in hun interactie met arbusculaire mycorrhiza. Deze twee laatste punten wijzen er op dat de veranderingen in plant-bodem interactie waarschijnlijk een reductie van negatieve componenten weerspiegelen, zoals bijvoorbeeld een bevrijding van natuurlijke vijanden. De bodemgemeenschap heeft potentieel grote negatieve effecten op planten, maar deze effecten zijn niet altijd hetzelfde. *Carpobrotus edulis s.l.* wordt bijvoorbeeld sterk negatief beïnvloed door de bodemgemeenschap in het oorspronkelijke verspreidingsgebied maar niet in het nieuwe verspreidingsgebied, wat een bevrijding van natuurlijke vijanden indiceert.

Veranderingen in plant-bodem interacties ten gevolge van klimaatverandering, zoals beschreven in dit proefschrift, kunnen belangrijke consequenties hebben. Wanneer plantensoorten die hun areaal uitbreiden ontsnappen aan hun natuurlijke vijanden zal dit ook effecten hebben op de ecologie

van deze plant. Hier zijn parallellen te trekken met geïntroduceerde invasieve plantensoorten. De eigenschappen van de plantensoort zoals we die kennen uit het oorspronkelijk verspreidingsgebied zijn mogelijk niet representatief voor de nieuwe situatie.Zo kan een plant in het nieuw gekoloniseerde gebied dominanter worden dan in het oorspronkelijke verspreidingsgebied. Dit bemoeilijkt het bepalen van het potentiële areaal en impact van de plantensoort. Om ons voor te kunnen bereiden op de komende veranderingen ten gevolge van klimaatverandering en tevens inspanningen in natuurbescherming te richten daar waar deze het meest effect zullen hebben, is het essentieel een beter inzicht te hebben in de potentiële effecten van klimaatverandering op de interactie tussen planten en de bodemgemeenschap.

Dankwoord

Een groot aantal mensen is betrokken geweest bij het tot stand komen van dit proefschrift, sommige zeer direct, andere meer op afstand. Nu het boekje er bijna ligt is het een mooi moment om een aantal van deze mensen te bedanken.

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Curriculum vitae

De auteur werd op 7 juli 1978 te 's-Hertogenbosch geboren. In 1990 werd begonnen aan het Gymnasium Bernrode te Heeswijk waarna na twee jaar werd overgeschakeld op het Atheneum aan het Rodenborgh college in Rosmalen. Deze opleiding werd in 1997 afgerond waarna de studie biologie aan Universiteit Leiden begon. Hier koos hij voor de richting populatiebiologie en de specialisatie ecologie. Tijdens deze studie zijn twee stages uitgevoerd. De eerste stage was een studie naar plant bestuiver interactie bij de vakgroep Plantenecologie. Hierbij werd gekeken naar dichtheidsafhankelijke discriminatie van afwijkende kleurvormen in Slangenkruid door hommels en naar de functie van de bloemkleurverandering zoals die optreed in Hondstong. De tweede stage was bij de vakgroep Evolutiebiologie tevens in Leiden. Het veldwerk van deze stage vond plaats in het Nationaal Park Kibale in Uganda. Tijdens deze stage werd gekeken naar de relatie tussen het fourageren op aas en mest door fruitetende vlinders en hun morfologie en paargedrag. In 2002 is deze studie afgesloten met het cum laude behalen van het doctoraaldiploma. In 2003 werd begonnnen aan het promotietraject aan de vakgrope Natuurbeheer en Plantenecologie van Wageningen UR, dit heeft geresulteerd in dit proefschrift.

Sinds mei 2008 is Roy werkzaam als Plantenecoloog bij Bureau Waardenburg, een Ecologisch adviesbureau.

List of publications

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Is male puddling behaviour of tropical butterflies targeted at sodium for nuptial gifts or activity? Biological Journal of the Linnean Society, 86(3), 345-361

In press:
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Release from soil pathogens plays an important role in the success of invasive Carpobrotus in the Mediterranean. South African Journal of Botany, doi:10.1016/j.sajb.2008.09.003

Submitted:
Grunsven, R.H.A. van, W.H. van der Putten, T.M. Bezemer, E.M. Veenendaal
Plant-soil feedback of native and range expanding plant species is insensitive to temperature

Grunsven R.H.A. van, W.H. van der Putten, T.M. Bezemer, F. Berendse, E.M. Veenendaal
A poleward shifting plant species outruns its soil-borne enemies

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- Understanding Global Environmental Change: Processes, Compartments and Interactions

Other Phd courses:

- Functioning of Boreal Forest Ecosystems

Oral Presentations:

- Sense Summer symposium "Kyoto and Beyond", 23 June 2005, Ede, The Netherlands
- KNAW Opportunities in Global Change Research, 7 February 2006, Amsterdam, The Netherlands
- Necov wintersymposium, 16 February 2006, Brussels, Belgium
- Gesellschaft für Ökologie, 11 September 2007, Marburg, Germany

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