Newcomers in plant communities

Interactions with soil and climate change



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This research was conducted under the auspices of the C.T. de Wit Graduate School for Production Ecology & Resource Conservation (PE&RC)

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Thesis

submitted in fulfilment of the requirements for the degree of doctor at Wageningen University by the authority of the Rector Magnificus Prof. dr. M.J. Kropff, in the presence of the Thesis Committee appointed by the Academic Board to be defended in public on Wednesday 5 October 2011 at 1.30 p.m. in the Aula.

Annelein Meisner Newcomers in plant communities: interactions with soil and climate change 136 pages

Thesis, Wageningen University, Wageningen NL (2011) With references, with summaries in Dutch and English

ISBN 978-90-8585-982-6

The story is in the soil, keep your ear to the ground -Bright Eyes-

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Summary

Human activities are the cause of the spread of many plant species around the world. Propagules of these newcomers in plant communities, who are named exotic plant species in this thesis, are not only introduced by current human travel and trade, but also by current climate and land-use change. Climate and land-use change remove inhibiting factors for these propagules to colonize and establish into new habitats. Therefore, many of the recently established exotic plant species in the Netherlands originate from warmer climate regions. Exotic species may become very dominant and decrease the performance of native species in the newly colonized ecosystems. The main aim of my thesis research was to study how exotic plant species interact with soil processes and how these plant-soil interactions are influenced by abiotic stresses that are a consequence of climate change.

There is no agreement in the literature if exotic plant species speed up nitrogen (N) cycling for their own benefit. In a literature survey, I did not observe a general pattern suggesting that exotic plant species speed up N cycling. Instead, the effects of exotic plant species are very likely context depended. The identity of the exotic plant species under study, the comparions with natives species as well as site conditions may be important for the effects of exotic plant species on N cycling. Moreover, most of the studies in literature were field surveys that cannot rule out that other factors besides the presence of exotic plant species can interact with altered N cycling. In addition, it has not been examined in literature if altered N cycling stimulate exotic plant species by positive feedbacks, whereas these inhibit natives. Therefore, I propose that we are lost in translation if we use the literature data to find general patterns for exotic plant species effects on soil processes. More manipulative experimental studies and fewer correlative studies are needed to disentangle the effect of exotic plant species on soil processes. To this end, I performed a series of experiments to identify if recent established exotic plant species in the Netherlands can alter soil processes to increase their productivity.

I studied if recent established exotic plant species from riverine habitats in the Netherlands could increase their nutrient uptake via enhanced rhizosphere nutrient mineralization. I studied this in a greenhouse experiment where five recently established exotic plant species were grown in monocultures and compared them with five phylogenetically related native plant species. The amount of nutrient uptake, rhizosphere nutrient mineralization and microbial biomass was determined for all plant species. Exotic and related native plant species did not consistently differ in their uptake of N and phosphorus (P), or in their effect on rhizosphere nutrient mineralization and microbial biomass. However, exotic plant species had more often higher shoot N concentrations than native congeners.

A higher shoot N concentration suggests that litter inputs of exotic species

may be of a higher quality and decompose faster. Therefore, I tested the hypothesis that exotic plant species may speed up soil processes and nutrient availability via litter inputs, thereby increasing their own performance while inhibiting native plant species. In order to test that hypothesis, litter and plant propagules of three plant pairs were collected from the field and a series of experiments was performed under controlled conditions in a climate chamber. All three exotics speeded up cumulative soil respiration in comparison with native congeners. In addition, exotics increased N availability in the soil in comparison with their native congeners when their litter inputs had a higher N content. However, litter from exotic plant species did not inhibit germination rates and biomass production of natives. Instead, if the litter from exotic plant species had a positive feedback, it had also a positive effect on the related native plant species. Therefore, exotic plant species that have a higher quality litter than the related native plant species can not only create a positive feedback to its own performance, but can also stimulate a related native plant species. These results suggest that litter feedbacks can contribute to the productivity of exotic plant species only when there are interactions with other mechanisms that promote the productivity of exotic plant species.

Ecosystems may not only be affected by the presence of exotic plant species, but also by stressful climate events. These stresses are expected to increase the dominance of exotic plant species in future plant communities. However, drought and flood can occur before or during the growing season. When these stresses occur before the growing season, they may affect the dominance of exotic plant species indirectly via altered soil legacies. However, when the stresses occur during the growing season they may affect the dominance of exotic plant species directly. I studied this in a mesocosm experiment with a full-factorial experimental design. Soils were pre-exposed to drought followed by flooding. Then a plant-community was planted in these soils and exposed to drought during the growing season. We observed that it depended on the type and combination of stresses if exotic plant species could increase in dominance. A single drought stress before or during the growing season increased the dominance of exotic plant species. However, the natives remained as dominant as in control soils when the mesocosms were exposed to two drought stresses in a row, one before and one during the growing season.

Soil legacies may have been important for determining the dominance of exotic plant species in the plant community exposed to abiotic stresses. In a second experiment, I tested, therefore, if soil biotic legacies affected the dominance of exotic plant species. Soil biota had a positive effect to exotic plant species and a negative effect to native plant species in soil pre-exposed to drought and/ or flooding, but the response was the opposite in control soils unexposed to water stresses. However, in the first experiment, the exotics became more dominant only in soil pre-exposed

to drought stresses. Other soil processes, e.g. ammonification and soil respiration, may have been important as well. These altered soil processes may have resulted in highest inorganic N concentrations in soil pre-exposed to drought and lowest in soil pre-exposed to flood. Increased N concentrations benefitted both exotic and native plant species. Therefore, the net effect of legacies in soil processes and biota can have played a role for the dominance of exotic plant species in future plant communities.

I conclude that interactions between exotic plant species and soil processes will not necessarily increase the productivity of exotic plant species more than natives. Moreover, soil legacies developed by climate-warming induced water stress may be very important for the dominance of exotic plant species in future plant communities.

Chapter 1

Introduction



Introduction

Plant species are being intentionally and accidentally transported across the world by human trade and travel. Recent climate warming and land use change contributed to range-expansion within continents. This all has resulted in species introductions into novel environments (Vitousek et al. 1997). These newcomers in plant communities are named exotic plant species throughout this thesis. Their interactions with soil processes are suggested to increase their dominance in the new communities (Ehrenfeld 2003; Liao et al. 2008a). However, it is unclear if and how exotic plant species can affect these soil processes and if altered soil processes are involved in feedback processes that benefit the exotic plant species over natives. Exotic plant species may not only promote themselves via altered soil processes, but are also expected to benefit from abiotic stresses resulting from climate changes (Dukes and Mooney 1999). However, there is little experimental evidence that climate change will increase the dominance of exotic plant species in plant communities. Therefore, the main aim of this thesis is to study how exotic plant species interact with soil and how plant-soil interactions are influenced by abiotic stresses resulting from climate change.

The process of exotic plant invasions

Introduction of propagules

Exotic plant species that are introduced into a new habitat may become very dominant via a phased process (Fig. 1.1). The first step is the transport of propagules to a new area. Transport of exotic propagules often takes place accidentally by long-distance transport by humans or intentionally by introducing novel species for horticulture use (Hodkinson and Thompson 1997; Vitousek *et al.* 1997; Mack *et al.* 2000). However, long-distance range expansion by plant species may also introduce novel species into new habitats. For example, the European river system of canals and rivers form an excellent migration route for many propagules (Galil *et al.* 2007) and can be a pathway for propagules into new riparian habitats (Pyšek and Prach 1993; Jacquemyn *et al.* 2010).

Invasion process

The second step of this phased process is the introduction of propagules into nonnative habitats. The more propagules introduced into a new area, the greater the chance that one of those propagules survives during the next steps of plant invasion (Lockwood *et al.* 2005; but see Nuñez *et al.* 2011), because there are many filters that control exotic plant species within each step of the invasion process (Theoharides and Dukes 2007). For example, exotics may be inhibited to establish by competing native plant species, interactions with enemies and control by herbivores. However, biotic interactions are often not resisting the colonization of successful exotic plant species in the new habitats (Levine *et al.* 2004). In the colonization phase, an individual propagule needs to germinate and survive. In the establishment phase, the exotic plant species can reproduce and form sustainable populations that survive in the new habitat (Catford *et al.* 2009). When exotic plant species are able to overcome the biotic and abiotic filters, spread into other areas, produce offspring, and become abundant, they can be defined as invasive exotic plant species (Richardson *et al.* 2000). This definition is irrespective of the impact the species has in the new range, because impact is subjective to human perception. Nevertheless, about 0.1% of all introduced species will have an ecological or economical impact in the new range (Williamson and Fitter 1996; Richardson *et al.* 2000). Therefore, invasive exotics are identified as one of the five most important human-induced global changes (Millenium Ecosystem Assessment 2005).

Climate change may promote thermophylic exotics

Climatic conditions are a major determinant for propagules to become established in new habitats. Modeling studies suggest that the climate niche of many plant species will move pole wards due to climate warming (Bakkenes *et al.* 2002; Walther *et al.* 2002; Parmesan and Yohe 2003). Therefore, many plant species from warmer regions are expected to colonize novel habitats, which have increased in temperatures due to recent climate warming. Indeed, observations suggest that the number of exotic plant species that originated from warmer climate regions have increased in number in the previous century in the Netherlands (Tamis *et al.* 2005a). The increase in



Fig. 1.1 Stages of plant invasion. A propagule is first transported to a new range, after which it is introduced. After introduction, the propagule needs to colonize, establish and create a self-sustainable population. Individual propagules of the established population may spread to a new range. Established populations may have an impact on the whole ecosystems (Figure is adapted from Catford *et al.* (2009) and Theodaris and Dukes (2007))

abundance of a number of these species coincides with climate warming. These intra-continental exotic plant species, which can also be called range-expanders, may use similar mechanisms to invade novel ecosystems as exotic plant species from inter-continental origin (Morriën *et al.* 2010; Van der Putten *et al.* 2010).

Plant-soil interactions

Individual exotic and native plant species can affect their own performance via direct and indirect interactions with soil biota (Fig. 1.2) (Wardle *et al.* 2004; Van der Putten *et al.* 2007a). These interactions are called plant-soil feedbacks (Bever *et al.* 1997), which are defined as 'a sequence of events or interactions, in which the result of a process affects the conditions that initially generate the process' (Ehrenfeld *et al.* 2005). Altered inputs of compounds in litter, root and shoots of exotic plant species can be a starting point for these feedbacks, because these plant traits can alter soil processes and interactions with soil biota (Ehrenfeld 2004).

Plant traits

Exotic plant species in general can have traits associated with enhanced performance, such as higher shoot nutrient allocation and higher growth rates than native plant species (e.g. Liao et al. 2008a; Van Kleunen et al. 2010). However, many studies in those meta-analyses compared exotic plant species with the natives they have displaced or non-related natives in the invaded range. These comparisons have as advantage that for a particular ecosystem the trait inputs may differ from the species that is displaced. However, the outcomes of the comparisons may be dependent on plant functional group identity or plant growth form instead of the origin of the exotics and native species. For example, N-fixing exotic invaders have traits that differ more from native species than non-N fixing invaders (Liao et al. 2008a). Furthermore, herbaceous species in Argentina have similar traits as natives whereas woody species show traits associated with higher nutrient acquisition (Tecco et al. 2010). Therefore, interpretations of comparisons where exotics and natives are from different lifeforms have been suggested to have minor explanatory power to find a universal suit of plant traits associated with invasiveness (Tecco et al. 2010). Therefore, when possible, it may be better to use related plant pairs to identify traits that are in general associated with exotic invasive plant species (Pyšek and Richardson 2007). These plant traits can result in an altered composition of compounds with rhizosphere and litter inputs, which can alter soil processes (Ehrenfeld 2003; Levine et al. 2003). These altered soil processes are often hypothesized to promote exotic plant species via a positive plant-soil feedback (e.g. Allison and Vitousek 2004; Ehrenfeld 2004; Liao et al. 2008a) However, little is known about how altered rhizosphere nutrient cycling or soil processes by litter inputs will promote exotic plant species.

Rhizosphere nutrient mineralization

Exotic plant species may influence soil processes in the rhizosphere via carbon sources released by roots (Ehrenfeld 2004). These rhizosphere inputs can contain root exudates, cells from root turnover and compounds that are actively excreted into the rhizosphere. These inputs are a carbon source for the carbon-limited microbes in the rhizosphere (Arrow 1, Fig. 1.2) (Buée et al. 2009). Priming theory suggests that plant species can increase mineralization of nutrients locked up in soil organic matter (SOM) via easily available carbon inputs in the rhizosphere (Kuzyakov et al. 2000; Kuzyakov 2010). The carbon (C) inputs can stimulate the rhizosphere microbes to increase their activity and biomass production and are increasing the mineralization of nutrients locked up in SOM (Arrows 2 + 3, Fig. 1.2) (e.g. Cheng et al. 2003; Dijkstra et al. 2009). Plants can use the nutrients released by this mineralization for their own nutrient uptake (Arrow 4, Fig. 1.2) (Dijkstra et al. 2009). However, microbes may also immobilize nutrients in their biomass. These nutrients locked up in the microbial biomass can be released for plant uptake when microbes are grazed by soil fauna (Clarholm 1985; Ingham et al. 1985; Bardgett and Chan 1999: Bonkowski 2004). Therefore, an increased stimulation of microbes in the rhizosphere may increase nutrients available for uptake by plant species.

Exotic plant species have been observed to alter soil processes via rhizosphere inputs in comparison with natives in the invaded range. For example, exotic grass *Microstegium vimineum*, but not exotic shrub *Berberis thunbergii* can increase the nitrification potential, the activity of some extracellular enzymes and respiration response to substrates in comparison with native shrubs (*Vaccinium* spp.) (Kourtev *et al.* 2003). However, the question remains if altered rhizosphere nutrient mineralization can increase the nutrient uptake and therewith increase the performance of exotic plant species in the new range.

Interactions with rhizosphere biota

Root exudates not only stimulate rhizosphere nutrient mineralization, but are also important for the plant-specific rhizosphere communities (Kowalchuk *et al.* 2002; Scheublin *et al.* 2004; Broeckling *et al.* 2008; Viketoft 2008). These rhizosphere communities are composed of microbes, symbionts, pathogens and herbivores (Fig 1.2) and can have a direct positive or negative effect to the performance of their host plant (Bever *et al.* 1997). Mutualistic symbionts as mycorrhizal fungi and rhizobia give the plant access to respectively phosphorus (P) and nitrogen (N) in return for C whereas soil pathogens and herbivores reduce the performance of their host plants. Exotic plant species have often been observed to have a less negative effect from their rhizosphere community compared with plant species that are native in the new range (Klironomos 2002; Van Grunsven *et al.* 2007; Engelkes *et al.* 2008). Plant species in



Fig. 1.2 direct and indirect plant-soil interactions (solid arrows) as affected by interactions with climate change stresses (dotted arrows). Direct interactions (right part) between plants and soil biota are mediated directly between the roots and biota. Root pathogens and herbivores can decrease the performance of individual plant species whereas mutualists can promote the access of plant to nutrients in the soil. Indirect interactions (left part) between plants and soil can be mediated via the excretion of root exudates into the rhizosphere (1). These exudates can stimulate soil microbes to mineralize nutrients in the soil from the organic nutrient pool (2), which increase inorganic nutrient concentrations in the rhizosphere (3). Plants can take up these inorganic nutrients from the soil (4). Plant species can also affect indirect interactions when leafs fall on the ground after leaf senescence (5). The recalcitrant nutrients in the litter are being added up by the soil organic nutrient pool (6) whereas other components are being decomposed by soil microbes and biota (7). Decomposition can than increase the concentration of inorganic nutrients for plant species (4) via increased mineralization of organic nutrients in the litter (7). Climate change can alter precipitation amounts by increasing rainfall or drought periods (8). These will affect the water content in the soil (9), which may affect the plant directly (10), but also indirectly via soil microbes (11) and soil biota (12) (This figure is adapted from Wardle et al. (2004))

the new range may also have a less negative effect than the same native species from their native range as has been observed for *Prunus serotina* (Reinhart *et al.* 2003). The release from soil pathogens and the ability to associate with mycorrhizal fungi in the new range may both contribute to a less negative rhizosphere feedback of the exotic plant species (Van der Putten *et al.* 2007b). Therefore, exotic plant species may benefit from their rhizosphere community in the invaded range by increasing their performance in comparison with native plant species (Van der Putten *et al.* 2007a).

Interactions between plant litter and soil

Exotic plant species may also affect soil processes via differences with native species in litter chemistry (Arrows 2-7; Fig. 1.2). Litter chemistry is important for the rate and processes involved in every step of litter breakdown (Wardle *et al.* 1998; Meier and Bowman 2008). First, the easily available soluble compounds, like sugars, are metabolized by opportunistic microbes. When these easily available substrates are consumed, cellulolytic or lignolytic microbes take over. They produce extracellular enzymes, like cellulases, that break down the more recalcitrant compounds in the litter. Then, only very recalcitrant lignin-containing compounds are left for microbes to decompose. The mass loss of the litter is very low in this final phase (Linkins *et al.* 1990; Sinsabaugh *et al.* 2002; Berg and McClaugherty 2008). The decomposition of lignin requires specialist lignolytic fungi for degradation and can shield the more easily available components (e.g. cellulose) from decomposers in the earlier phases (De Boer *et al.* 2005; Osono 2007).

Exotic plant species with lower quality litter than native species can slow down litter breakdown (e.g. Godoy et al. 2010) whereas exotic plant species with higher quality litter than native species can speed up litter breakdown (e.g. Allison and Vitousek 2004). Exotic plant species may increase nutrient availability (Ehrenfeld 2003; Ashton et al. 2005) via nutrient mineralization (Rothstein et al. 2004; Petsikos et al. 2007) when their litter is of higher quality than the native that is being replaced. This litter effect is suggested to have a positive feedback to the exotic plant species while inhibiting natives (e.g. Raizada et al. 2008; Farrer and Goldberg 2009). However, litter-feedback mechanisms have been studied only in few cases. For example, Myrica fava invasion into Hawaii has transformed an entire ecosystem, because it is able to form a symbiosis with N-fixing bacteria in a nutrient poor ecosystem, whereas none of the native species could form such a symbiosis. This mutualistic relationship increased the N available in the invaded ecosystem and might have contributed to promote fast-growing invaders instead of slow-growing native species (Vitousek et al. 1987; Vitousek and Walker 1989). Many other N-fixing plant species that became invasive after their introduction could increase soil N

availability (Scherer-Lorenzen *et al.* 2007), which has been suggested to affect the performance of natives via the mechanism of *M. faya* in Hawaii (Levine *et al.* 2003). However, also non-N fixing plant species can become invasive and alter soil nutrient cycling (Ehrenfeld 2003), but the effects on N cycling are less pronounced for non-N fixing species than for N fixing-species (Liao *et al.* 2008a). Therefore, the question still remains how important enhanced nutrient cycling may be for litter feedbacks of non-N fixing exotic plant species.

Climate change and plant invasions

The increase in the release of greenhouse gases in the atmosphere since the industrial revolution has very likely resulted in higher air temperatures, more extreme rainfall and longer periods with drought, which are expected to be more pronounced in future (IPCC 2007; KNMI 2009). Therefore, plant communities will very likely be exposed to increased abiotic stresses in the future. It is suggested that exotic plant species may better deal with such stresses (Dukes and Mooney 1999; Vilà et al. 2007). Moreover, as many recent established exotic plant species in the Netherlands are originating from warmer climate regions (Tamis et al. 2005a), they may be better able to deal with such climate-induced stresses than native plant species. Indeed, experimental warming has been observed to reduce the performance of individual native species, but not that of individual exotic plant species (Verlinden and Nijs 2010). Furthermore, these exotic plant species experience a less negative effect from their soil biota than related native plants under current and future temperatures (Van Grunsven et al. 2010b). Thus, increased temperatures may increase the dominance of exotic plant species in future plant communities. However, climate stresses that affect precipitation patterns may be more severe to the plant community than changes in air temperature. Moreover, altered precipitation patterns may not only exert directly on plant communities, but also indirectly when they occur before the growing season and changes the soil conditions.

Soil biotic and abiotic conditions are expected to change when soil are exposed to altered precipitation patterns before the growing seasons (Arrows 9 and 11 in Fig. 1.2). Drought often decreases the activity of soil microbial communities and upon rewetting there is a short-term increase in microbial activity after which activity decreases again to control situation (e.g. Birch 1958; Fierer and Schimel 2002; Butterly *et al.* 2009). This phenomenon may be caused by an immediate mineralization of previously unavailable organic substrates or dead microbial biomass (Schimel *et al.* 2007; Borken and Matzner 2009). Flooding events can make soils anaerobic and may inhibit microbes that need aerobic conditions. Instead, processes that require anaerobic conditions, such as denitrification can be stimulated by flooding (Priemé and Christensen 2001). Drought and rewetting stresses may also

alter the composition and functional diversity of soil microbes (Degens *et al.* 2001; Fierer *et al.* 2003; Butterly *et al.* 2009). These may involve changes in soil organisms that directly affect plant growth (line 10, Fig. 1.2). Soil microbial communities may not be resistant and may not recover from stresses (Allison and Martiny 2008). Therefore, it is uncertain how these changes in soil work out for the exotic plant species in plant communities.

When plant communities are exposed to altered precipitation patterns during the growing season, plant species may be affected directly by altered water levels in soils (Fig 1.2, line 10) (Wu *et al.* 2011). Furthermore, individual plant species within a plant community can respond differently to altered precipitation patterns. For example, drought has been observed to increase the evenness of the biomass distribution of a plant community, because biomass of sub-dominant species could increase whereas biomass of the dominant species decreased (Kardol *et al.* 2010). Furthermore, the timing of extreme events can affect plant species differently. For example, summer drought and heat had a greater effect on the biomass reduction of the plant community than spring drought and heat (De Boeck *et al.* 2011). Disturbance that reduced the dominant native species has been observed to give a priority to the exotic plant species to dominate the plant community in California (Seabloom *et al.* 2003). In my thesis, I will examine effects of abiotic water stress before and during the growing season on the dominance of native and exotic plant species in an invaded plant community.

Research objectives and thesis outline

The main aim of my thesis is to study if exotic plant species alter nutrient cycling by a general direction, how recent established exotic plant species in the Netherlands can alter ecosystem processes, how altered soil processes feedbacks to these exotic plant species, and if they can dominate in plant communities under future climate change stresses. My four research objectives are:

- 1. To identify if exotic plant species can have a general impact on nutrient cycling
- 2. To study if recent established exotic plant species can increase their nutrient uptake via enhanced nutrient mineralization in the rhizosphere nutrient
- 3. To study if litter inputs of recently established exotic plant species can alter soil processes that can promote their own performance while inhibiting native species

4. To study if the dominance of recent established exotic plant species is influenced by climate-related abiotic stresses

In Chapter 2, I study if there is a general pattern of the effects of invasive exotic plant species on the cycling on nitrogen. Thereto, I performed a small literature survey. The intermezzo at the end of chapter 2 illustrates that there is a debate whether or not invasive exotic plant species increase the cycling of N. One of the main conclusions of chapter 2 is that only few studies manipulated exotic plant invasions experimentally. Therefore, I performed a series of experiments to test if recent established exotic plant species in the Netherlands will affect the cycling of nutrients.

In chapter 3, I study if exotic plant species can increase their nutrient uptake via effects on nutrient mineralization and microbes in the rhizosphere. In a greenhouse experiment, exotic and related native plant species have been grown in monocultures in Dutch soil. I calculate the total amount of N and P the plants could take up and I measure the cycling of nutrients and microbial biomass in the rhizosphere. Although exotic plant species did not consistently increase or decrease total nutrient uptake, they had higher shoot N concentrations than native species. Therefore, I wanted to know if exotic plant species may increase soil nutrient cycling via litter inputs and thereby create a positive feedback for themselves while inhibiting native congeners. I study this in chapter 4 by collecting litter, propagules and soil in the field of one nature reserve with which three experiments are performed. In two microcosm experiments, I study if exotic plant species may increase nutrient cycling and availability. In a third experiment, I study if decomposed litter may reciprocally affect the performance of native and exotic plant species.

Ecosystems may not only be affected by exotic plant species, but also by extreme climate stresses, like drought and flood, which are expected to occur more frequently in the future. These climate stresses may interact with the dominance of exotic plant species in plant communities. Furthermore, drought and flood are often observed to affect soil functions and biota (Schimel *et al.* 2007; Borken and Matzner 2009). Therefore, when drought or flood occurs before the growing season, this may have a soil effect on the performance of plant species during the growing season. Therefore I study if abiotic stresses before or during the growing season may affect the dominance of exotic plant species in a plant community in chapter 5. Finally, I will synthesize and discuss the findings of chapters 2-5 in chapter 6 and make recommendations for future research directions.

Chapter 2

Plant invasion and soil N cycling: are we lost in translation?



Annelein Meisner, Wietse De Boer, W. H. Gera Hol, Jennifer Adams Krumins, Wim H. Van der Putten

Abstract

A major question in ecology is why some species become so invasive when introduced into a new ecosystem. It has been suggested that invasive exotic plant species promote their own abundance by increasing nitrogen (N) cycling in invaded habitats. However, the question remains if this pattern can be generalized. We argue that this is not the case. We performed a literature survey to check if there are consistent patterns of invasive exotic plant species on N cycling. We observed that N cycling under invasive exotic plant species is highly variable when compared with native plant species and that there is no general pattern of increased N cycling. Most of the studies we surveyed used a field sampling approach and did not investigate other factors that may explain N cycling differences. For example, fertile sites might be more easily invaded than infertile sites. Some published observations may be caused by plant species-specific traits like the capacity to fix nitrogen through microbial symbioses. Efforts to find generalized patterns that explain plant invasions and their effects on nitrogen cycling will be lost in translation as long as we limit our data to that from field observations. We propose that experimental tests are needed in order to be more conclusive about causes and consequences of invasiveness.

Introduction

Unraveling the mechanisms involved in biological invasions is important for understanding why some exotic species thrive so well in new environments. For exotic plants, many hypotheses have been described to explain their disproportionate abundance and invasive spread in new habitats (Catford, et al. 2009). One key explanation is that exotic plant species increase soil nitrogen (N) cycling in their new ecosystem (Rout and Callaway 2009). This proposed positive effect of invasive exotic plant species on their nutrient supply is in line with the increasing number of reports describing a positive relationship between invasive plant species and soil N cycling (Ehrenfeld et al. 2001; Rodgers et al. 2008; Sharma and Raghubanshi 2009). However, invasive exotic plants can also have negative or neutral effects on soil N cycling (Ehrenfeld 2003). The question therefore is if effects of higher N cycling are more prominent than neutral or negative effects. In order to address this question, we performed a literature survey. In this survey, we paid special attention to whether invasive exotic plant species were N-fixing or not, because such plants may increase N cycling more than non-N-fixing plant species (Liao et al. 2008a). We discuss if the apparent effects of invasive exotic plant species on soil N cycling may be caused by plant-species specific differences, initial site differences, or other factors that may explain establishment and abundance of exotic plants.

Literature survey

To assess whether a general pattern exists in effects of invasive exotic plants on soil N cycling, we performed a literature survey using studies published from 2003 - 2009 and combined our analyses with the studies published before 2003 as reviewed by Ehrenfeld (2003). We searched for papers in Web of science with keyword-combinations: exotic plant or plant invasi* and N cycling, nitrogen, soil funct* or soil processes. We also selected manuscript based on references cited in other manuscripts as well as manuscripts that cited papers. Data were taken from papers that compared invasive exotic plant species with natives in the invaded range. We identified data from three different experimental approaches: manipulative experiments were exotics and natives were planted in similar soils (Table S2.1), field experiments where established exotic and native species were manipulated (Table S2.2) and observational studies where soil was sampled under exotic and native plant species (Table S2.3). The data available resulted from a variety of experiments with different study set-ups, native-exotic plant species comparisons and methods to estimate plant and soil parameters related to N cycling. This variation made the data-set less suitable for a quantitative meta-analysis and instead a more robust votecounting approach was chosen to detect whether a trend existed within these data. This approach is essentially the same approach as used previous on data-

Ecosystem property	response	all invaders	all minus N-fixing invaders
Soil total N concentration	Invasive >native	20	14
	Invasive <native< td=""><td>10</td><td>10</td></native<>	10	10
	Invasive =native	27	26
	Mixed results	8	7
Soil inorganic N	Invasive >native	15	11
concentrations	Invasive <native< td=""><td>6</td><td>6</td></native<>	6	6
	Invasive =native	15	12
	Mixed results	13	12
N mineralization and	Invasive >native	14	11
nitrification	Invasive <native< td=""><td>5</td><td>5</td></native<>	5	5
	Invasive =native	9	7
	Mixed results	11	10
Litter N	Invasive >native	13	7
	Invasive <native< td=""><td>6</td><td>6</td></native<>	6	6
	Invasive =native	3	3
	Mixed results	1	1
Litter decomposition	Invasive >native	16	15
	Invasive <native< td=""><td>5</td><td>4</td></native<>	5	4
	Invasive =native	10	9
	Mixed results	5	4

Table 2.1 Number of studies with a positive (>), negative (<), neutral (=) or mixed invader response to ecosystem properties related to N cycling for all study types combined. Statistics are described in the text. (For details on mixed results see supplement table S2.1-S2.3 and Ehrenfeld 2003)</td>

sets that were also very heterogeneous, because as they claim 'the use of metaanalytical approaches would be incautious on such heterogeneous data sets' (Ives and Carpenter 2007; Tylianakis *et al.* 2008). Data were assigned to one of four categories: (1) N-cycling was higher for invasive exotic than native plants; (2) lower for invasive exotic plants than native plants; (3) not different between invasive exotic and native plants; and (4) when the results differed within a study, we called them mixed. We performed a binominal test (in SPSS version 17.0) to determine if there were more studies in the category 'invasive higher than native' than the total sum of studies in all other three categories. We performed the same test on the dataset excluding studies on N-fixing plants in order to isolate the effects of N-fixing plants.

Invasive plants and soil N cycling

There were no more studies showing higher N-cycling than all other results (Table 2.1; P > 0.05). Instead, total N was higher in 31 % for all studies and in 25 % of all studies minus studies with N-fixing invaders (P < 0.01). Moreover, inorganic N concentrations in soil were higher in 31 % of all studies and in 27 % of all studies

minus studies with N-fixing invaders (P < 0.01). Therefore the hypothesis that invasive exotic plants enhance N cycling when compared with native plants was rejected. Actually, invasive exotic plants differ in their effect on the N cycle because N cycling could be higher, lower or not different than N cycling for native plants (Table 2.1).

In 81 % of all studies, the results were based on a field sampling approach. To determine if invasive exotic plants increase N cycling, experimental studies are needed that control for all other factors except the presence or absence of the invasive exotic plants. Although ecosystem properties related to N cycling can be different between invasive exotic plants and native plants (Wedin and Tilman 1990; Wedin and Pastor 1993; Ehrenfeld *et al.* 2001; Kourtev *et al.* 2003; Hawkes *et al.* 2005; Diallo *et al.* 2006; Petsikos *et al.* 2007; Rodgers *et al.* 2008; Scharfy 2009), no consistent pattern emerges from the few experimental studies that have been done so far (Table S2.1 and Ehrenfeld 2003). More experiments that compare invasive exotic plants with native plants are required to determine if the enhanced N-cycling of invasive exotic plants indeed is a general phenomenon.

Plant-species identity

Invasive exotic plant species that have an association with symbiotic N-fixing bacteria may increase total N pools in the ecosystem (Vitousek and Walker 1989). However, mechanistically it is difficult to imagine that non-N-fixing invasive exotic plants can increase the total amount of N present in the ecosystem. Moreover, if non-N-fixing invaders are capable of increasing N pools in soil, then why is this not also a benefit to fast-growing native plants or soil microbes? We propose that invasive exotic plants may use similar mechanisms for N uptake as native plants use. Native plants can increase or decrease soil N cycling in species-specific ways (Binkley and Giardina 1998; Aerts and Chapin 2000; Chapman et al. 2006; Manning et al. 2008), which favors or inhibits the coexistence with neighbor plant species (Daufresne and Hedin 2005). Therefore, invasive exotic plants may alter soil N cycling simply because their traits differ from native plants in the invaded community (Ehrenfeld et al. 2001). However, such processes are also known from natural plant communities where one plant species replaces another (Berendse 1998). In addition, although it is often suggested that exotic plant species can create a positive feedback via altered soil processes (e.g. Ehrenfeld 2003; Allison and Vitousek 2004; Liao et al. 2008a), it is to our knowledge only studied for the famous N-fixing invasive in Hawaii (Vitousek and Walker 1989). Thus, invasive exotic plants can alter soil N cycling in species-specific ways, which is often suggested to inhibit the growth of native plant species. However, it is unknown if increased soil processes will exclusively promote invasive exotic plant species (Fig. 2.1).



Fig. 2.1 Different plant species can have different N use strategies that drive N cycling (Laungani and Knops 2009). Therefore, plant-species specific differences between invasive exotic and native plant species may determine the direction in which invasive exotic plant species alter N cycling when they invade into a new ecosystem

Initial site-differences

Plant species-specific differences between native and invasive exotic plant species may cause differences in soil N cycling, but these differences can only emerge after the establishment of invasive exotic plants. However, sites often differ in nutrient availability due to a patchy distribution of resources. Therefore, soil N concentrations and fluxes can be higher or lower at different sites under established invasive exotic plants (Stock et al. 1995; Dassonville et al. 2008). As invasive exotic plants have a higher abundance and frequency at habitats disturbed by past human activities like agriculture (Lundgren et al. 2004), soil conditions at the start of plant invasion may already have provided the initial conditions promoting the establishment of invasive exotic plants. This context-dependence may also play a hidden role in the studies used for our literature review, because most results were based on field sampling of established stands of invasive exotic plants at single field-sites. It is known that sites with high N availability can be more vulnerable to plant invasion than sites with low N availability (e.g. Huenneke et al. 1990; Weiss 1999; Vinton and Goergen 2006). As a consequence of the higher N availability, invasive exotic plants may become established and outcompete natives better at N richer sites than N poor sites. High N availability may result in more N uptake from soil (Chapman et al. 2006) leading to enhanced N concentrations in leafs and litter (Aerts and Chapin 2000; Lowe et al. 2002). This can feedback to enhanced N cycling via increased litter decomposition (Chapman et al. 2006; Cornwell et al. 2008). However, increased N concentrations in litter from invasive exotic plants do not necessarily result in increased litter decomposition rates, because other nutrients like P may have been limiting litter decomposition (Kurokawa *et al.* 2010). Therefore, if other nutrients are not limiting, it is possible that initial increases in soil N availability result in increased N cycling under invasive exotic plants (Fig. 2.2a and 2.2b).

Causes for establishment

Whether or not invasive exotic plants enhance N cycling, the question remains if altered N cycling is a cause or a consequence of plant invasion. There are many hypotheses that may explain plant invasions, and it is likely that not one hypothesis is exclusively explaining the invasion of all exotic species. More likely a number of factors are contributing to the successful establishment of invasive exotic plants (Catford *et al.* 2009). For example, invasive exotic plant species that originate from nutrient richer sites with higher enemy pressure may take up more nutrients for growth than native species in the new range when they are released from enemies and establish at nutrient rich sites (Blumenthal *et al.* 2009). Therefore, the combination of increased resource availability and enemy release may interact. Both resource availability and pathogen pressure may determine individual species abundance (Leibold 1996). Resource availability determines the growth rate of plants and their



Fig. 2.2 Differences in nitrogen (N) availability between sites. These site differences may determine the cycling of nutrients between two sites with native and exotic plant species. If exotic plant species establishes at sites with higher N availability, then this may increase the cycling of N in comparison with native that establishes at sites with lower N availability (a). The opposite may be the case when exotic species establishes at low N availability and natives at high N availability (b)

(a) enemy release

(b) no enemy release



Fig. 2.3 High resource environments. For exotic plants, adapted to nutrient richer habitats, enemy release may increase their nutrient uptake when compared with native plant species. These invasive exotic plants are less controlled by pathogens and can therefore, take up more N than native plants (a). However, if invasive exotic plants are not released from enemies, they have equal chances to establish when compared with native plants and they may, therefore, on average not alter N cycling (b)

investment in defense to enemies. As pathogen pressure is more expensive for the plant when growth is limited, low resource availability selects for low growth rates and higher defense whereas high resource availability selects for fast growth rates and low defense (Coley *et al.* 1985). Although many invasive exotic plants have some degree of enemy release (Keane and Crawley 2002; Mitchell and Power 2003; Van Grunsven *et al.* 2010a), enemy release alone cannot explain the abundance of all invasive exotic plants (Colautti *et al.* 2004). Therefore, exotics may benefit from enemy release only if enough resources are available for their growth. This high N availability may then increase N uptake of exotics more than natives. However, a higher N cycling may thus be caused by previous environmental conditions and not by the exotic plant species self (Fig. 2.3a and 2.3b).

Lost in translation

We conclude that the effects on N cycling by invasive exotic plants are so inconclusive that no general pattern yet emerges. Most studies have used a field sampling approach to observe differences in soil N cycling under established invasive exotic plants and native plants. Therefore, one may wonder whether we are lost in translating the general patterns of invasive exotic plants on N cycling if we only consider the outcomes of field studies without being aware that in the field patterns of N cycling can be a consequence, as well as a cause of plant invasion. We propose that more rigorous experimental tests are needed before being able to conclude that a key mechanism of plant invasiveness is that invasive exotic plants in general alter soil N cycling for their own benefit only.

Acknowledgements

We thank Tim Engelkes, Fernando Monroy-Martinez, and Elly Morriën for discussion.

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Table S2.1 experimental studies where invasive exotic and native plants were planted in soils. + indicate that measurement is higher in exotic invasive plant than native, - indicate that the measurement is lower in exotic than native plant; = indicate that exotic plant equals native plant mixed indicated that it can be higher, lower or equal in exotic plant compared with native plant

	ò				-		
Invasive species	Functional	Total N	Inorganic N	N fluxes	Litter N	Decomposition	Reference
	group					rate	
Avena barbate, Bromus hordeaceous	grass			gross nitrification increased in monocultures, but not in mixed. N			Hawkes <i>et al.</i> 2005
Allaria petiolata	forb		II	mineralization =			Rodgers et al. 2008
Berberis thunbergii	shrub		II	11			Kourtev et al. 2003
Microstegium vimineum	grass		II	NH4 mineralization =; NO3 mineralization higher			Kourtev <i>et al.</i> 2003
Oxalis pes-caprae	forb					+	Petsikos <i>et al.</i> 2007
mixed	forb					11	Scharfy et. al. 2010
mixed trees (three)	tree		mixed ¹				Diallo <i>et al.</i> 2006
							•

 NH_{a}^{4} was higher in soil from 1 invasive, but other 2 similar concentrations. At higher concentrations NH_{a}^{4} was similar or lower than naitves. NO₃ was higher for 1 exotic but other 2 had similar concentrations. At higher concentrations exotic were lower or similar than natives

Table S2.2 studies w	/here established	l invasive ex	xotic plants wer	e manipulated in the fie	ld. + indicate tha	t measurem	ent is higher in exotic
invasive plant than na	ative, - indicate t	that the meas	surement is lowe	er in exotic than native p	lant; = indicate th	at exotic pla	int equals native plant;
mixed indicated that	it can be higher,	lower or equ	ual in exotic plan	it compared with native J	olant		
Invasive species	Functional	Total N	Inorganic N	N fluxes Litte	r N Decompo	ition Ref	ference
	group				rate		
Bromus inermis	grass				+	Vir 20(nton and Goergen)6
Andropogon bladhi	grass			- 0r =	ı	Ree	ed <i>et al.</i> 2005
Euphorbia esula, Cirsium	forb		II		II	Pri	tekel <i>et al.</i> 2006
arvense							
Andropogon virginicus and	grass	II		+	II	Ma	ck <i>et al.</i> 2001
melinis minutiflora							

Table S2.3 observatioplant; = exotic plant ec	nal studies. + quals native pl	- measurement i lant; mixed indi	s higher in exoti cated that it can b	c invasive plant th be higher, lower on	an native, c equal in exo	measurement is lov tic plant compared	ver in exotic than native with native plant
Invasive species	Functional	Total N	Inorganic N	N fluxes	Litter N	Decomposition	References
	group					rate	
Aegilops triuncialis	grass	ı					Drenovsky et al. 2007
Agropyron cristatum	grass	11					Henderson and Naeth 2005
Andropogon gayanus	grass		NO ³ - and NH ⁴ +				Rossiter-rachor 2009
Bromus tectorum	grass	+ than historical or not invaded	11	11			Belnap <i>et al.</i> 2005
Bromus tectorum	grass	+	$NO_{3}^{-} + and$ $NH_{4}^{+} =$	gros nitrification =; PNR +; gross mineralziation +			Booth <i>et al.</i> 2003
Bromus tectorum	grass		ı	ı			Rimer and Evans 2006
Bromus tectorum	grass	recently invaded +; invaded/ disturbed -	NO ³ = and NH ⁴ + in recently invaded and invaded				Sperry <i>et al.</i> 2006
Cortaderia selloana	grass	- 0f =					Domenech et al. 2006
Spartina alterniflora	grass	+			ı	- for	Liao <i>et al.</i> 2007
						belowground litter; + for arial	
Snartina alterniflora	orass	ı			ı	and surface - for	Liao <i>et al</i> 2008
	2					belowground litter: + or - for	
						aboveground litter	

Angeloni <i>et al.</i> 2006	Farrer and Goldberg 2009	Niu <i>et al.</i> 2009	Rodgers et al. 2008	Dassonville <i>et al</i> .	/ 007	Vanderhoeven <i>et al.</i> 2005	Duda <i>et al.</i> 2003	Fink 2005		Vanderhoeven <i>et al.</i> 2005	Chacon <i>et al</i> . 2009	Fickbohm and Zhu 2006
			+					- or - +	5			
		nt: 					id led	=				+
+	$\mathrm{NH}_4^+ =$	depends c status pla Heaviliy invaded +; newly invaded -	5 +		on		NO ³ + an NH ⁴ - in just invad and = in					II
		+		mixed	direction depended site	II				II	- N fixing native; = shrub	
grass	grass	forb	forb	forb		forb	forb	forh		forb	forb	forb
Typha angustifolia x T. latifolia	Typha angustifolia x T. latifolia	Ageratina adenophora	Allaria petiolata	Fallopia japonica		Fallopia japonica	Halogeton glomeratus	Hedvchium	gardnerianum	Heracleum mantegazzianum	Kalanchoe daigremontiana	Lythrum salicaria

				6003	t al.	al. 2005	n <i>et al.</i>	2009	2009	. 2004	004	2009	96
	Reference		Li <i>et al.</i> 2006	Zhang <i>et al.</i> 2	Dassonville <i>e</i> 2008	Guesewell et	Vanderhoevel 2005	Scharfy et al.	Chacon <i>et al</i> .	Standish <i>et al</i>	Hook <i>et al.</i> 2(Sharma and Raghubanshi	Vilà <i>et al.</i> 200
	Decomposition	rate								= but litter decomposed faster as invasive site		+	
	Litter N											+	
	N fluxes										= (but direction different within sites)	+	
	Inorganic N			NO ₃ and NH4+ +				NO_3^{-} - and NH_4^{+} =	,	$NO_{3}^{-} + and$ $NH_{4}^{+} =$		+	
	Total N		+ (but higher in recently invaded than invaded plot)	II			II	11	- N fixing native, = shrub		= (but direction different within sites)	+	= , but
	Functional	group	forb	forb	forb	forb	forb	forb	forb	forb	forb	forb	forb
Table S2.3 continued	Invasive species		Mikania micrantha	Solidago canadensis	Solidago gigantea	Solidago gigantea	Solidago gigantea	Solidago gigantea	Stapelia gigantea	Tradescantia fluminensis	Centaurea maculosa	Lantana camara	Oxalis pes-caprae

Solidago gigantea	forb		NO_3^{-} - and NH_4^{+} =	mixed (direction depended on site)		Vanderhoeven <i>et al.</i> 2006	
Sorghum halepense	N fixing grass		+			Rout and Chrzanowski 2009	
Elaegnus umbellata	N fixing shrub	II	$NO_{3}^{-} + and$ $NH_{4}^{+} =$	nitrification and N mineralization +;		Baer <i>et al.</i> 2006	
Cystisus scoparius	N fixing shrub (leoume)	+		ammonification =		Caldwell 2006	
Acacia longifolia	N fixing tree	+	11	+	+	Marchante <i>et al</i> . 2008	
Falcataria moluccana	N fixing tree				+ =(for both native and invasive	Hughes and Uowolo 2006	
Falcataria moluccana	N fixing tree	+ or = in oldest site			invasive sites)	Allison <i>et al.</i> 2006	
Falcataria moluccana	N fixing tree		+		+	Hughes and denslow 2005	
Robinia pseudoacacia	N fixing tree			Π	•	Castro-diez et al. 2009	
Elaeagnus angustifolia	N fixing tree or shrub				 + depend on habitat; 2 faster 1 = 	Harner <i>et al.</i> 2009	
Carpobrotus spp.	shrub	=, but differed at sites				Vilà <i>et al.</i> 2006	
Lonicera morrowii	shrub	II			+	Ashton et al. 2005	
Rhamnus cathartica	shrub	+	II	mixed (direction depend on site; - or =)		Heneghan <i>et al.</i> 2006	
Table S2.3 continued							
------------------------------	------------	------------	-------------	----------	----------	---------------	-------------------------
Invasive species	Functional	Total N	Inorganic N	N fluxes	Litter N	Decomposition	Reference
	group					rate	
Rosa multiflora	shrub					11	Ashton et al. 2005
Rosa rugosa	shrub	+					Vanderhoeven et al.
							2005
Chrysanthemoides	shrub	+	+		11		Lindsay and French
monilifera spp. Rotundata							2005
Prunus serotina	shrub or						Vanderhoeven et al.
	tree						2005
Acer platanoides	tree					11	Ashton et al. 2005
Ailanthus altissima	tree			11	11	+	Castro-Diez et al.
							2009
Ailanthus atissima	tree	1+, 4 =					Vilà <i>et al.</i> 2006
Fraxinus udhei	tree				+	+	Rothstein et al. 2004
Melaleuca	tree	toplayer=,		11	,		Martin et al. 2009
quinquenervia		deeper -					
Prunus serotina	tree						Chabrerie et al. 2008
Ampelopsis	vine	11				11	Ashton et al. 2005
brevipedunculata							

Intermezzo

No paradox for invasive plants



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Science 325 (5942): 814

The Perspective "An invasive plant paradox" by M. Rout and R. Callaway (2009) overgeneralizes the effect of invasive plants on the nitrogen cycle. An invasive plant's impact on nitrogen cycling is based on plant identity rather than origin. Invasive nitrogen-fixing plants can increase nitrogen cycling in a newly invaded ecosystem, but this does not apply to all functional groups of invasive plants. Mechanistically, it is difficult to imagine how non-nitrogen-fixing plants could enhance total nitrogen pools in the ecosystem, unless they did so by affecting free-living nitrogen-fixing microbes. Furthermore, the effects of invasive plants on nitrogen fluxes are site dependent (Stock *et al.* 1995; Dassonville *et al.* 2008). In order to avoid the confounding effects caused by site, we need experimental studies that can unequivocally separate causes from consequences. We agree with Sax and Brown (Sax and Brown 2000) that there is no paradox of invasion. Indeed, there are underlying mechanistic explanations for each species in its new environment. A general pattern of enhanced nitrogen cycling does not exist for plant invaders.

Chapter 3

Comparison of nutrient acquisition in exotic plant species and congeneric natives



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Accepted for publication in Journal of Ecology

Summary

The ability of exotic plant species to establish and expand in new areas may be enhanced by a relatively high ability to acquire soil nutrients. To test this hypothesis, we predicted that the capacity for nutrient acquisition would be higher in seedlings of exotic species than in seedlings of native congeners.

We selected the five exotic species that had recently increased in abundance in a riverine habitat in the Netherlands and that had a native congener that was common in the same habitat. We grew seedlings of each of these ten species singly in pots of soil from this habitat in a glasshouse. After two months, we measured the final dry mass and N and P content of each plant and components of microbial biomass and nutrient mineralization in the soil. We also measured these soil characteristics in pots that had been left unplanted.

Exotic and native congeners did not differ consistently in the uptake of N or P or in effects on components of soil mineralization. Within a genus, values of these measurements were sometimes higher, sometimes lower and sometimes similar for the exotic when compared to the native species.

Depending upon the statistical analysis used, biomarker-based biomass of arbuscular mycorrhizal fungi was generally higher in soil planted with exotic than with native species. Most measures of microbial biomass and soil mineralization were higher in pots that had been planted with plants than in pots with no plant.

Synthesis. Our results do not suggest that invasive, exotic plant species generally possess greater capacity for nutrient acquisition during the early establishment than native species do.

Introduction

Over many years, humans have, both intentionally and accidentally, introduced a large number of plant species into non-native habitats across much of the biosphere (Hodkinson and Thompson 1997; Vitousek *et al.* 1997). Only a small number of these introduced exotic plant species are able to establish in their new region and only a subset of those species will spread into new areas (Williamson and Fitter 1996; Richardson *et al.* 2000). Several exotic plant species have been shown to increase nutrient cycling after establishment (e.g. Duda *et al.* 2003; Chapuis-Lardy *et al.* 2006; Sharma and Raghubanshi 2009), especially when natives were displaced by plant species from different functional groups (Ehrenfeld 2003; Liao *et al.* 2008a). The impact of exotic plant species on ecosystem processes is mostly studied via comparisons of invaded and non-invaded sites. This approach has the advantage in that it includes long-term changes in litter and nutrient dynamics, but this does not indicate whether altered nutrient dynamics play a role during the establishment of exotics.

Exotic plant species may be expected to exploit soil nutrients better than natives do, given that their above-ground nutrient concentrations are often higher and resource use strategies are often more efficient than those of native species present in the invaded range (e.g. Agrawal *et al.* 2005; Funk and Vitousek 2007). Furthermore, exotic plant species are often observed to be released from natural enemies such as pathogens and herbivores (Keane and Crawley 2002; Klironomos 2002). For example, two recent studies observed that exotic plant species that invaded a riverine habitat experienced less negative effects from soil biota (Van Grunsven *et al.* 2007; Engelkes *et al.* 2008). In addition, exotic plant species, which could be released from enemies in their new range, have been observed to allocate more of their resources to plant production instead of defence compared with the species from the native range (Blair and Wolfe 2004; Zou *et al.* 2006; Feng *et al.* 2009), possibly as a result of increased uptake of soil nutrients in the new range (Zou *et al.* 2006).

Increased nutrient uptake may be accomplished by increased root growth, stimulation of microbial decomposers via rhizodepositon or allocation of carbon to arbuscular mycorrhizal fungi (AMF) (Hobbie 1992; Aerts and Chapin 2000; Chapin *et al.* 2002). Stimulation of biomass and activity of microbes may enhance plant growth via increased mineralization of nutrients in the soil organic matter (e.g. Kuzyakov, Friedel and Stahr 2000; Dijkstra *et al.* 2009). Moreover, nutrients will become released when rhizosphere microbes are grazed by soil fauna (Clarholm 1985; Ingham *et al.* 1985; Bonkowski *et al.* 2009). Plant phosphorus nutrition can also be enhanced by symbiosis with AMF, providing phosphorus to the plants in return for carbon (Smith and Read 2008). However, AMF can also 'cheat' by being

Plant Species	Origin ^{1,2,3}	Time of first introduction ^{1,2,3}
Artemisia vulgaris	Native*	
Artemisia biennis	North-Asia	1950-1975
Centaurea jacea	Native*	
Centaurea stoebe	Mid-Europe	1950-1975
Tragopogon pratensis subsp. pratensis	Native*	
Tragopogon dubius	Mid- and South Europe	1950s
Bidens tripartite	Native *	
Bidens frondosa	North-America	1929
Senecio vulgaris	Native*	
Senecio inaequidens	South-Africa	1925-1950

Table 3.1 Exotic and native plant species selected for the experiment. Plant species names follow

 Van der Meijden (2005). Bold plant names are exotic plant species

¹(Tamis et al. 2005b), ²(Weeda et al. 1991), ³(Dirkse et al. 2007), *Native to the Netherlands

parasitic instead of mutualistic to plant species (Johnson *et al.* 1997; Klironomos 2003; Kiers and van der Heijden 2006).

Here, we compare nutrient acquisition strategies of exotic plant species and phylogenetically related native congeners during their establishment phase to test the hypothesis that invasive exotic plants have relatively high nutrient acquisition during early establishment. We specifically predicted that exotic plant species would show greater uptake of N and P than native congeners. We further predicted that exotics had lower amounts of available phosphorus, greater fluxes of N, P and biomass of microbes in the rhizosphere than natives. We further expected that the uptake of N would be correlated with fluxes of N in soil and that the uptake of P would be correlated with both P mineralization and AMF biomass. We tested these predictions in a glasshouse experiment using five exotic and five phylogenetically related native plant species all co-occurring in the same habitat. This approach enabled us to control for phylogenetic differences between native and exotic plant species (Harvey and Purvis 1991), as well as for habitat-specific aspects, including physical, chemical and biological soil characteristics.

Material and methods

Plants

Five exotic plant species and their five native congeners, all members of the Asteraceae, were selected for the experiment with the following selection criteria also used by Engelkes *et. al.* (2008): (1) exotic plant species increased in frequency in the second half of the 20th century; (2) a native plant species within the same genus had to be present in the Netherlands; (3) the native plant species is abundant in the Netherlands; (4) the native and exotic plant species within a genus co-occur

within the same habitat; (5) seeds that can be germinated had to be present for both native and exotic plant species; and (6) all plant pairs had to be present in the same habitat. There were 5 pairs that fulfilled these criteria (Table 3.1). They all occur in the Geldersche Poort region (51°52'N; 6°02'E) along the river Waal (Dirkse *et al.* 2007).

Seed collection and seedling germination

Most of the plant seeds were collected along the river Waal (*Artemisia biennis, Artemisia vulgaris, Bidens tripartita, Senecio inaequidens, Senecio vulgaris* and *Tragopogon pratensis* subsp. *pratensis*). Seeds of *Tragopogon dubius* were collected in the centre of the Netherlands (52°16'N; 5°35E), whereas seeds of *Bidens frondosa, Centaurea jacea* and *Centaurea stoebe* were obtained from small seed companies that collect seeds locally in the Netherlands or Germany.

Seeds were surface-sterilized in a 0.5% sodium hypochlorite solution, germinated on glass beads, moistened with demineralized water and placed in a germination cabinet. Because not all seeds germinated at the same time, seedlings were placed in a 4 °C climate chamber with 10 h light per 24 h after germination until the start of the experiment. Seedlings from *B. frondosa* and *B. tripartita* were kept in sterilized soil after germination, because these seedlings did not survive on glass beads.

Soil sampling and preparing

In September 2007, soil was collected from five locations in the nature reserve Millingerwaard (51°52'N; 6°00'E), which is part of the Geldersche Poort region in the Netherlands. All plant species used for our experiment occur in this region and most of them co-occur in the Millingerwaard. This nature reserve is located along the river Waal, which is the southernmost branch of the river Rhine. The Millingerwaard, as other nature reserves in this region, is grazed by horses and cattle and flooded periodically during high water discharge of the river. After sampling, the soil was homogenized, sieved through a 10-mm mesh and stored outdoors for 2 $\frac{1}{2}$ weeks. The sandy soil (90.5% sand, 9.47% silt and 0.09% clay) contained 14% moisture (w/w), had a pH_{water} of 7.94, 1.97% C and a C:N ratio of 20.3.

Experimental design and set-up

In our experimental design, we had 5 exotic and 5 phylogenetically related native plant species. There were 10 replicate pots for each plant species and 10 control pots without plants. The control pots had been set up at the same time as the pots with plants and were included to determine the effects of plant presence on soil nutrient cycling, nutrient pools and microbial community structure. Before planting

the seedlings, 3-L pots were filled with moist field soil equivalent to 2500 g dry soil per pot. During the experiment all soils were kept at 50% water holding capacity which is equivalent to17.7% moisture based on dry soil. Each pot was planted with seedlings from one of the five exotic plant species or their native congeners. Three seedlings were planted per pot. We planted two individuals per pot for both native and exotic plant species within *Tragopogon* and *Bidens* pairs, because seedling availability was limited. The plants were grown in a climate-controlled glasshouse with a day temperature of 21 °C (\pm 2 °C) and a night temperature of 16 °C (\pm 1 °C). There was 16 h daylight in the greenhouse and artificial light was supplemented up to 225 µmol m⁻² s⁻¹ when natural daylight fell below this level. The experiment was started on 16 October 2007 and the plants were harvested on 10 December 2007. At harvest, soil, roots and shoots were separated.

Plant biomass, nutrient content and soil analyses

The harvested plant material was dried at 70 °C to determine shoot and root biomass. The dried roots and shoots were ground and analysed for total C, N and P content. Total C and N were determined in 3 mg of plant material using a NC analyser (Thermo flash EA 1112, Thermo Fisher Scientific Inc., Waltham, USA), which uses flash combustion in conjunction with an analytical column and a thermal detector to determine percentages of N and C in the plant samples. Total P was measured following digestion with a mixture of H₂SO₄-Se and salicylic acid (Novozamsky et al. 1984). Briefly, digestion was performed by incubating a mixture of 300 mg plant material and 2.5 mL of a mixture of H₂SO₄-Se and salicylic acid for 3 hours at 100 °C, 20 minutes at 150 °C and finally at 325 °C until liquid was transparent. Then, 48.3 mL Milli-q water was added and the mixture was filtered. Phosphorus in the filtrate was coloured with Molybdate reagens and was measured against a reference line on a plate reader (bio-tek synergy HT-1, biotek, Winooski, USA) at 720 nm. With these data the total nutrients in the plant were calculated for N and P separately using the following formula: Total N and P =(shoot concentration \times shoot biomass) + (root concentration × root biomass).

Soil sub samples were stored in plastic bags at 4 °C prior to analysis. Soil water content was determined as weight loss after overnight incubation at 105 °C. All soils reached constant mass after overnight incubation. This dry weight was used to calculate soil nutrient concentrations and mineralization activities.

Potential ammonification activity was determined using the arginine ammonification assay according to Alef and Kleiner (1987) with minor modifications. For each pot, two replicate samples of fresh soil (equivalent to 1 g dry weight) were spiked with 0.25 mL arginine solution (4.8 mg arginine mL⁻¹ milli-q water), and one control was left unspiked. After 3 h of incubation at 25 °C the reaction was

stopped by adding 4 mL 2 M KCl. Ammonium was extracted by shaking for 15 minutes. Arginine was also added to control for background colour. Samples were centrifuged at 6000 rpm for 10 minutes and dilutions (1:1 with water) were stored at -20 °C until analyses. Potential nitrifying activity was measured as accumulation of nitrite and nitrate over a 3-h period in ammonium-enriched soil slurries as described by Bodelier *et al.* (1998). N-NH₄⁺ and N-NO₃⁻ concentrations were measured on a Technicon TrAAcs 800 autoanalyser (Technicon Instruments Corp., Tarrytown, N.Y., USA).

Alkaline phosphomonoesterase is produced by soil microorganisms. This extra-cellular enzyme releases phosphate from phosphomonoesters in soil organic matter. The phosphate released from soil organic matter by alkaline phosphomonoesterase can be taken up by plants. Enzyme activity was measured according to Schinner *et al.* (1995) with the following modifications: two samples and one control were centrifuged at 6000 rpm for 10 minutes and the supernatant was diluted 5 times before the released *p*-nitrophenol was measured on a plate reader (biotek synergy HT-1, biotek, Winooski, USA) at 405 nm.

Potential plant-available inorganic P was estimated via extraction of soil subsamples in 0.5 M NaHCO₃ (P_{olsen}) (Troelstra, Wagenaar & De Boer 1990). In order to measure P_{olsen} , soil sub-samples were dried at a maximum of 40 °C for one week after harvest.

Microbial biomass

Phospholipid derived fatty acids (PLFA's) were extracted from freeze-dried soil samples with a three-step extraction protocol according to Boschker et al. (2004). This consisted of a Bligh and Dyer total lipid extraction, a fractioning of the total lipids on a silicic-acid column with chloroform, acetone and methanol, and finally mild-alkaline derivation to release the fatty acid methyl esters (FAMEs). Neutral lipids were collected in the chloroform fraction and phospholipids were collected in the methanol fraction. The FAMEs were analysed by gas-chromatography-flame ionization detector (GC FID) on a Thermo Scientific Focus GC with a Zebron ZB5 (60 m, 0.32 mm, 0.25 µm) column. Peak areas were calculated relative to an internal standard (19:0). In total, 28 different PLFA's were detected and identified based on retention time comparisons with FAME standards. The following PLFA's were chosen to represent bacterial biomass: i15:0, a15:0, 15:0, i16:0, 16:109, 16:1 007t, i17:0, a17:0, 17:0, cy17:0, 18:1 $\omega7$ and cy19:0 (Frostegård & Bååth 1996); total fungal biomass: 18:206 (Federle 1986); actinomycetes: 10Me16:0, 10Me17:0, 10Me18:0 (Frostegård, Tunlid & Bååth 1993). Fungal: bacterial ratios were based on 18:2w6:bacterial PLFA ratio (Frostegård and Bååth 1996). The PLFA-based AMF biomarker 16:1w5 was not a good indicator for arbuscular mycorrhizal fungal biomass, because this biomarker

is also present in bacteria (Olsson 1999; Hedlund 2002). Therefore, the neutral lipid fatty acid (NLFA) 16:1 ω 5 was used as an arbuscular mycorrhizal fungi biomarker (Hedlund 2002). Ergosterol was also used to measure fungal biomass. This is a specific fungal biomarker in the cell wall of fungi, but not in AMF (Olsson *et al.* 2003). This was extracted from frozen soil using an alkaline-extraction method and measured on a Dionex HPLC (Olten, Switzerland) equipped with a C 18 reverse-phase column (Alltech, Deerfield, USA) and a UV-detector set at 282 nm (De Ridder-Duine *et al.* 2006).

Data analyses

Fixed-effect models with origin, genus and their interaction were performed using Statistica version 8 (Statsoft, Inc. (2007), Tulsa, USA), following the approach of previous studies with similar experimental design (e.g. Agrawal et al. 2005). Planned comparisons were used to test for differences between species within genera. Genus was considered fixed, because plant species were selected non-randomly following the list of criteria described above, and we included all genera that met these criteria. A consequence of this approach is that the inference space for the statistical test is limited to the genera and plant species included in the experiment and does not extend to other genera that were not included in our experiment. Because experimental designs similar to ours are sometimes analysed differently, we also fitted models with genus as random factor (using the type III ANOVA implementation of PROC MIXED in SAS for windows 9.1., the SAS institute, Cary, NC, USA). A drawback of this approach is that it is underpowered with only few levels (5 in our study) of the random factor included in the study (Bart et al. 1998). Therefore, the random-factor approach is presented in the Table S3.1 in Supporting Information and is briefly discussed in the result section.

Data were transformed when necessary to meet the assumptions for ANOVA. The following transformations were used: y^2 for total plant N uptake; ln(y) for total plant P uptake, potential nitrifying activity and fungal biomass (PLFA biomarker); $y^{(-1/2)}$ for nitrate concentration in soil and biomass of actinomycetes in soil; and $y^{(-1/4)}$ for fungal : bacterial ratio in soil. Control pots and planted pots were analysed with planned comparisons after a one-way ANOVA at pot level (see Table S3.2 and S3.3). Pearson correlations between plant nutrient uptake and soil nutrient mineralization measurements were performed. For each table of results presented in the manuscript, we set a table-wide false discovery rate (FDR) threshold for significance at FDR = 0.05 to control for inflation of type I error due to multiple testing (Benjamini & Hochberg 1995; Verhoeven, Simonsen & McIntyre 2005). FDR is typically more powerful than Bonferroni approaches and has fewer false negative results (= type II errors) (Verhoeven *et al.* 2005). The FDR method evaluates ranked p values against

Table 3.2 Comparison of genus and origin differences for nutrient acquisition, microbial activity and microbial biomass using a two-way fixed factor ANOVA. Bold P values indicate that they are significant at a false discovery rate threshold of P = 0.05 (see methods). * d.f._{den} is denominator degrees of freedom (d.f.). numerator d.f.: genus = 4; origin = 1; genus x origin = 4

	Genus		Origin		Genus x	Origin	d.f.*
	F _{4,77-88} *	Р	F _{1,77-88} *	Р	F _{4,77-88} *	Р	
Total N uptake	16.3	< 0.001	1.74	0.19	29.4	< 0.001	86
Total P uptake	38.2	< 0.001	0.06	0.95	21.5	< 0.001	84
Total C in plant	2.74	0.033	8.73	0.004	48.0	< 0.001	85
Total biomass	1.06	0.38	5.51	0.02	34.2	< 0.001	88
Arginine ammonification	9.76	< 0.001	2.78	0.099	2.84	0.03	87
Potential nitrifying activity	1.13	0.27	1.72	0.19	1.29	0.28	82
Phosphomonoesterase	81.9	< 0.001	14.8	<0.001	1.87	0.12	88
Polson	4.72	0.002	2.38	0.13	0.62	0.65	88
Microbial biomass	0.05	0.99	0.27	0.6	1.5	0.21	84
Bacterial biomass	0.04	1	0.05	0.84	1.14	0.43	84
Fungal biomass (PLFA)	2.46	0.051	6.92	0.01	3.03	0.02	84
Fungal biomass (ergosterol)	2.74	0.04	1.55	0.22	1.31	0.27	77
Actinomycetes	0.65	0.63	0.02	0.90	1.45	0.22	84
Fungi: bacteria (PLFA)	4.04	0.005	11.4	0.001	2.39	0.06	84
AMF	14.62	< 0.001	7.91	0.006	6.40	<0.001	86

a rank-specific significance threshold and results in a list of significant P values of which a pre-determined proportion are false discoveries. Thus, at FDR = 0.05, 5% of the significant results are expected to be false discoveries (= type I error).

Results

Plant nutrient uptake

There was no consistent difference in nutrient uptake between exotic and native congeners (Table 3.2; Fig. 3.1). Therefore, the results did not support the hypothesis that exotic plant species exploit soil nutrient pools better than congeneric native plant species. There was also no significant origin effect for total C content in plant species if analysed with genus as a random factor (Table S3.1), and lower C content in exotic plant species was only observed within three genera (Fig. S3.1). However, total C content was lower in exotic than native congeners if analysed with genus as a fixed factor (Table 3.2 and Fig. S3.1).

Soil nutrient fluxes

No origin effects were observed for N and P fluxes if genus was considered a random factor and multiple testing correction was performed (Table S3.1). However, alkaline phosphomonoesterase activity was significantly higher in soil from exotic



Fig. 3.1 a) Total N uptake and b) total P uptake. Mean \pm SE are presented for native (grey bars) and exotic (white bars) plant species. * indicate P < 0.05 and # indicates P < 0.1 for pair wise within genus comparisons

plant species than in soil from native congeners if tested with genus as a random effect without correction for multiple tests or with genus as a fixed effect (Table 3.2 and Fig. 3.2). The significant effect of origin was mainly due to differences within two genera. Origin did not affect concentration of plant-available P (P_{olsen} ; Table 3.2 and Fig S3.2) or fluxes of N as measured by arginine ammonification and potential nitrification (see Table 3.2 and Fig.3.2). Pots with plants had higher ammonification and lower nitrification than unplanted pots and generally had lower rates of alkaline phophomonoesterase (Table S3.2).

Microbial biomass in soil

Origin did not affect mass of soil microbes when genus was treated as a random factor (Table S3.1). However, PLFA-based fungal biomass and NLFA-based AMF biomass were on average higher in soil of exotic plant species than in that of native plant species if the data were analysed with a fixed-factor approach (Table 3.2, Fig. 3.3, Fig. S3.3). Microbial biomass tended to be higher in pots with plants than in unplanted pots (Table S3.3).

Correlations between plant nutrient uptake and soil nutrient fluxes and AMF Plant N uptake did not correlate with soil arginine ammonification or nitrifying activity (P>0.05). Plant P uptake did not correlate with NLFA-based AMF biomass or plant-available P concentrations. However, alkaline phosphomomoesterase activity correlated negatively with P uptake (r = -0.48, P < 0.001).



Fig. 3.2 a) arginine ammonification, b) potential nitrifying activity and c) alkaline phosphomonosterase activity in soil. Mean \pm SE are presented for native (grey bars) and exotic (white bars) plant species. * indicates P < 0.05 and # indicates P < 0.1 for pairwise within-genus comparisons



Fig. 3.3 a) fungal biomass measured with PLFA biomarker $18:2\omega6$, b) fungal biomass measured as ergosterol concentration and c) AMF biomass measured with the NLFA C16:1 ω 5 biomarker. Mean \pm SE are presented for native (grey bars) and exotic (white bars) plant species. * indicates P < 0.05 and # indicates P < 0.10 for pairwise within-genus comparisons

Discussion

Overall, our results do not support the hypothesis that exotic plant species are generally better able to exploit soil nutrient pools during their establishment than native plant species. Instead, in our study, rhizosphere nutrient fluxes and plant N and P uptake were higher, lower or did not differ between exotic and native plant species within the studied genera. These results are consistent with a study of effects of two exotic and one native plant species in the US on short-term N mineralization (Kourtev *et al.* 2003).

Plant presence increased arginine ammonification and microbial biomass, but decreased nitrification rates in comparison with control pots. This plant-induced stimulation of N-mineralizing microbes and simultaneous suppression of nitrifying bacteria points at an increased N flux from soil organic matter while soil conditions remain N-limited. Under such conditions plants are often better competitors for N-NH₄⁺ than nitrifying bacteria (Verhagen *et al.* 1994). However, plant N uptake did not correlate with potential soil N mineralization, possibly because the range of arginine ammonification rates between plant species was too narrow. Furthermore, plant species may have used additional strategies for N uptake to the ones we have measured here. For example, the turnover of soil microbes, e.g. via soil fauna, may have released N for plant uptake when they were grazing on rhizosphere microbes (Clarholm 1985; Ingham *et al.* 1985; Bardgett and Chan 1999; Bonkowski 2004).

Phosphorous uptake differed between some species within genera. However, it is not clear which processes caused this difference, because a higher P uptake did not correlate with a decrease in potential plant-available inorganic P pools (P_{olsen}) or higher organic P mineralization as indicated by alkaline phosphomonoesterase activity. Interestingly, native plant species inhibited alkaline phosphomonoesterase more than exotic plant species did, at least when analysed with genus as a fixed effect. Alkaline phosphomonoesterase is excreted by soil microbes in situations with low P availability (Bardgett 2005). However, our soil was not P-limited, because the concentrations of potential plant-available P (P_{olsen}) in our study is considered to be moderately high (26 to 45 mg P per kg soil; Sharpley & Withers 1994). Furthermore, the low N:P ratios in plant shoots (range N:P: $3.1 \pm 0.3 - 8.0 \pm 0.3$; data not shown) indicated that our plant species were N-limited rather than P-limited (Koerselman and Meuleman 1996).

Exotic plant species in our study had on average more NLFA-based AMF biomass than congeneric native plant species when analysed with genus as a fixed effect, except for the genus *Artemisia*, where an opposite pattern was observed. In other studies exotic plant species have been observed to increase (e.g. Walling & Zabinski 2004; Batten *et al.* 2006) or decrease AMF abundance (e.g. Mummey & Rillig 2006; Vogelsang and Bever 2009). Those studies used a variety of different techniques to quantify AMF-abundance. Here, we measured AMF with the NLFA-biomarker, which is present in storage lipids in external hyphae (Olsson 1999; Hedlund 2002). Therefore, exotic plant species in our study might have a higher carbon allocation to AMF hyphae than native plant species, but without a higher

uptake of P. It is possible that a reduction in the dependence on AMF of the exotic plant species studied here (Seifert *et al.* 2009) might have contributed to the observed results (Pringle *et al.* 2009) and made the AMF more parasitic to the exotic plants (Bever 2002). Interestingly, our results suggest that there was no origin effect for saprotrophic fungal biomass when using ergosterol (Fig. 3.3). It could be that fungal biomass in soil needs more time to respond, because a study on exotic *Solidago gigantea* showed that it takes more than one growing season to increase fungal biomass (Scharfy *et al.* 2010).

One of the key questions of this study was to determine if, as cause for their establishment, exotic plant species would exploit soil nutrients better than natives. At establishment, there was no pattern in our study indicating that exotic plant species would differ consistently in nutrient acquisition from native congeners. However, previous studies have shown that exotic plant species experience a less negative effect from soil biota than native congeners (Van Grunsven *et al.* 2007; Engelkes *et al.* 2008). Therefore, direct interactions between exotic plant species and soil biota, such as release from soil-borne enemies (Reinhart *et al.* 2003), could play an important role in the establishment of exotic plant species. Increased shoot N concentrations of exotics in our study (Fig. S3.4) as well as in other studies (e.g. Agrawal *et al.* 2005) may suggest that exotic plant species are able to accelerate nutrient cycling via higher-quality litter inputs (Cornwell *et al.* 2008). Therefore, altered soil nutrient cycling may be a consequence of, rather than a cause for, plant invasions (Ehrenfeld 2010).

In conclusion, our results suggest that exotic plant species do not consistently differ from native congeners in their ability to exploit soil nutrients during their early stage of establishment.

Acknowledgement

We thank Staatsbosbeheer regio Oost for giving us permission to work in Millingerwaard. We thank Wiecher Smant, Maarten Nab, Henk Duyts, Roel Wagenaar, Marco Houtekamer and Ciska Raaijmakers for technical assistance. We thank Mirka Macel, Tim Engelkes, Elly Morriën, Wil Tamis and George Kowalchuk for discussions and advice on the experiment. We thank Roy van Grunsven for help with collecting *Tragopogon dubius* seeds. We thank Jeff Harvey, two anonymous reviewers and the Handling Editor for valuable suggestions that improved the manuscript.

Supplement

Table S3.1 Effects of origin and plant genus on nutrient acquisition, microbial activities and microbial biomass were analyzed by two-way ANOVA with genus as random factor. P values in bold indicate that they are significant at false discovery rate threshold P = 0.05 (see methods). * d.f._{den} is denominator degrees of freedom for the interaction Genus x Origin

	Genus		Origin		Genus x	Origin	
	F _{4,4}	Р	F _{1,4}	Р	F4, 77-88*	Р	d.f. _{den} *
Total N uptake	0.56	0.71	0.06	0.8	29.42	< 0.001	86
Total P uptake	1.78	0.3	0.00	0.99	21.5	< 0.001	84
Total C in plant	0.00	0.99	0.18	0.70	47.96	< 0.001	85
Total biomass	0.03	1	0.16	0.71	34.19	< 0.001	88
Arginine ammonification	3.43	0.13	0.98	0.38	2.84	0.03	87
Potential nitrifying activity	1.04	0.50	1.09	0.36	1.29	0.28	82
Phosphomonoesterase	43.73	< 0.001	7.92	0.048	1.87	0.12	88
Polson	7.6	0.04	3.82	0.12	0.62	0.65	88
Microbial biomass	0.03	0.99	0.18	0.69	1.5	0.21	84
Bacterial biomass	0.04	0.99	0.04	0.86	1.14	0.34	84
Fungal biomass (PLFA)	0.81	0.58	2.29	0.20	3.03	0.02	84
Fungal biomass (ergosterol)	0.71	0.62	0.00	0.99	1.80	0.14	77
Actinomycetes	0.45	0.77	0.01	0.92	1.45	0.22	84
Fungi: bacteria (PLFA)	1.69	0.31	4.78	0.09	2.39	0.06	84
AMF	2.35	0.21	1.24	0.33	6.53	< 0.001	86

Table S3.2. Comparison of parameters related to nutrient fluxes in soil with plant species and soil without plants (mean \pm SE). Bold indicates that pots with that plant species are significantly different from pots without plants at P < 0.05. Phos is alkaline phosphomonoesterase activity (mg p-nitrophenol (kg dry soil)-1 h-1), Arginine is arginine ammonification (mg N-NH₄⁺ (kg dry soil)⁻¹ h⁻¹), PNR is potential nitrifying activity (mg N-NO₃⁻ (kg dry soil)⁻¹ h⁻¹). P_{olson} is the P fraction in soil that is potentially available to plants (mg P per kg soil)

	Phos	Arginine	PNR	Polsen
Bidens tripartita	282 ± 6.3	1.90 ± 0.05	0.36 ± 0.03	23.9 ± 4.8
Bidens frondosa	281 ± 7.6	$\textbf{1.78} \pm \textbf{0.04}$	$\textbf{0.31} \pm \textbf{0.02}$	25.3 ± 3.5
Centaurea jaceae	243 ± 5.2	$\textbf{2.12} \pm \textbf{0.06}$	$\textbf{0.34} \pm \textbf{0.02}$	24.3 ± 2.3
Centaurea stoebe	260 ± 4.8	1.93 ± 0.05	0.35 ± 0.05	26.0 ± 3.3
Senecio vulgaris	201 ± 5.0	$1.9\ 4\pm 0.05$	$\textbf{0.43} \pm \textbf{0.02}$	27.4 ± 4.3
Senecio inaequidens	209 ± 5.9	$\textbf{2.11} \pm \textbf{0.06}$	$\textbf{0.34} \pm \textbf{0.01}$	30.3 ± 5.0
Artemisia vulgaris	196 ± 4.4	$\boldsymbol{1.90 \pm 0.07}$	0.31 ± 0.03	26.0 ± 3.1
Artemisia biennis	223 ± 4.4	$\textbf{1.77} \pm \textbf{0.05}$	$\textbf{0.37} \pm \textbf{0.04}$	25.6 ± 1.9
Tragopogon pratensis	200 ± 3.7	$\textbf{2.14} \pm \textbf{0.05}$	$\textbf{0.38} \pm \textbf{0.04}$	27.6 ± 2.0
Tragopogon dubius	212 ± 4.8	$\textbf{2.08} \pm \textbf{0.07}$	0.32 ± 0.02	27.7 ± 4.0
Pots without plants	260 ± 4.9	1.58 ± 0.03	5.3 ± 0.31	28.7 ± 3.7

TADIC 33.3 Comparison of indicators of informate normass in soft with and without plant species (mean ± 3 r). Both indicates pots with plant

	Microbial	Bact	Fungi(PLFA)	Actino	Fungi:bact	AMF	Ergosterol
Bidens tripartita	36.9 ± 2.6	17.6 ± 1.3	1.04 ± 0.08	3.31 ± 0.54	0.06 ± 0.01	45.4 ± 9.6	0.62 ± 0.10
Bidens frondosa	44.2 ± 3.8	20.1 ± 1.7	1.83 ± 0.20	$\textbf{4.18} \pm \textbf{0.86}$	0.09 ± 0.01	61.2 ± 7.1	0.39 ± 0.11
Centaurea jaceae	37.2 ± 3.9	17.7 ± 1.8	0.70 ± 0.11	3.60 ± 0.75	0.04 ± 0.01	48.9 ± 7.2	0.42 ± 0.07
Centaurea stoebe	43.5 ± 3.4	20.1 ± 1 .7	1.31 ± 0.19	3.97 ± 0.75	0.06 ± 0.01	69.6 ± 7.3	0.38 ± 0.07
Senecio vulgaris	41.1 ± 3.1	19.6 ± 1.3	1.26 ± 0.09	4.04 ± 0.95	0.07 ± 0.01	14.0 ± 1.8	0.45 ± 0.04
Senecio inaequidens	36.8 ± 6.3	17.5 ± 3.2	1.42 ± 0.19	2.84 ± 0.45	0.09 ± 0.01	35.5 ± 4.5	0.46 ± 0.05
Artemisia vulgaris	44.0 ± 4.6	20.7 ± 2.4	1.66 ± 0.35	3.53 ± 0.34	0.08 ± 0.02	45.9 ± 3.2	0.41 ± 0.05
Artemisia biennis	37.8 ± 4.7	18.1 ± 2.2	1.21 ± 0.21	2.81 ± 0.32	$0.06 \ 0.01$	23.4 ± 6.2	0.45 ± 0.21
Tragopogon pratensis	39.0 ± 3.1	17.9 ± 1.2	1.13 ± 0.14	4.41 ± 0.97	0.06 ± 0.01	51.0 ± 6.9	0.46 ± 0.08
Tragopogon dubius	43.4 ± 2.9	19.6 ± 1.2	1.37 ± 0.18	5.04 ± 1.16	0.07 ± 0.01	65.7 ± 7.2	0.67 ± 0.05
Pots without plants	29.8 ± 3.7	14.7 ± 1.9	0.42 ± 0.06	2.78 ± 0.37	0.03 ± 0.001	8.81 ± 1.3	0.64 ± 0.07



Fig. S3.1 Total plant carbon a) and total plant biomass b). Average and SE are presented for native (grey bars) and exotic (white bars) plants. * indicates p < 0.05 for pair wise within genus comparisons



Fig. S3.2 Average and SE of concentration of potentially available P (P_{olson}) are presented for native (grey bars) and exotic (white bars) plant species. # indicates P < 0.10 for within genus comparison



Fig. S3.3 Average and SE of phospholipids fatty acids (PLFA) that are indicators of a) microbial biomass, b) actinomycetal biomass, c) bacterial biomass, and d) fungal 18:2 ω 6 PLFA biomarker / bacterial ratio. Native plant species are in grey bars and exotic plant species are in white bars for each genus. * indicates P < 0.05 and # indicates P < 0.10 for within genus comparison



Fig. S3.4 shoot and root N concentration (mean \pm SE). Native plant species are in grey bars and exotic plant species are in white bars for each genus. * indicates P < 0.05 and # indicates P < 0.10 for within genus comparison

Chapter 4

Litter feedback from exotic plant species can promote both exotic and phylogenetically related native plant species



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Abstract

Introduced exotic plant species can be a major problem when they change ecosystem processes of invaded ecosystems. A key question is whether an altered quality of litter produced by exotic plant species may contribute to their dominance by reducing growth conditions for natives. However, relatively little evidence exists to support this possibility. Here we compare litter effects of three exotic plant species with litter effects of three phylogenetically related natives that co-occur in the same riverine habitat. Under controlled conditions, we tested the hypothesis that litter from an exotic plant species enhances soil processes thereby creating a positive feedback while reducing the performance of a related native. We observed that soil with litter from exotic plant species had higher respiration rates and accumulated more often higher inorganic nitrogen concentrations than soil with litter from related natives. which was probably due to higher initial litter quality of the exotics. However, litter from exotic plant species did not inhibit germination rates and biomass of natives. Instead, when litter from an exotic plant species had a positive effect on itself, it also had a positive effect on the related native species. Therefore, we conclude that higher initial litter quality of exotic species can not only creates a positive feedback to its own performance, but can also create a positive effect to a related native from the same habitat. This suggests that changed quality of litter inputs from exotic plant species alone cannot explain the supremacy of exotics over related native species.

Introduction

Exotic plant species can be introduced into new ecosystems due to transport, tourism, trade (Hodkinson and Thompson 1997; Mack *et al.* 2000) or recent climate change (Parmesan and Yohe 2003; Tamis *et al.* 2005a; Walther *et al.* 2009). These introductions can result in biological invasions, which can have profound effects on the invaded habitats and the biodiversity therein (Vitousek *et al.* 1997; Chapin *et al.* 2000). Indeed, exotic plant species can have a large impact on ecosystem processes, because their altered quality of litter inputs can alter the cycling of nutrients (Levine *et al.* 2003; Liao *et al.* 2008a; Ehrenfeld 2010). These altered soil processes have often been hypothesised to provide a positive feedback to the exotic plant species that produce the litter (Ehrenfeld 2004; Farrer and Goldberg 2009; Liao *et al.* 2008; Raizada *et al.* 2008), but there are very few experimental tests showing that exotic plants indeed influence the legacy of the soil to their own benefit (Levine *et al.* 2003).

Litter effects of plant species on soil nutrient availability are mediated indirectly by the soil decomposer subsystem (Hobbie 1992; Aerts and Chapin 2000; Wardle et al. 2004). Differences in initial litter chemistry between exotic and native plant species are important for processes involved in litter decomposition (Wardle et al. 1998; Meier and Bowman 2008). For example, a higher lignin content can slow down the phased processes of litter breakdown (Cornwell et al. 2008), because this recalcitrant component needs specialist lignolytic fungi for degradation and can shield the more easily available components (e.g. cellulose) from decomposers in the earlier phases of litter breakdown (De Boer et al. 2005; Osono 2007). Indeed, differences in litter decomposition between exotic and native plant species strongly depend on their initial litter quality (e.g. Allison and Vitousek 2004; Godoy et al. 2010; but see Kurokawa et al. 2010). When litter of exotic plant species is of higher quality than the plant species being replaced, this may increase soil nutrient mineralization (Rothstein et al. 2004; Petsikos et al. 2007) and nutrient availability (Ehrenfeld 2003; Ashton et al. 2005). Therefore, the question is whether exotic plants may benefit more from an increased nutrient availability when their litter is of higher quality than plant species that are native in the invaded range.

Increases in soil nutrient availability and nutrient cycling can enhance plant productivity (Bardgett and Wardle 2010). For example, litter from an exotic grass has been observed to increase biomass of the exotic grass itself and of a native shrub (Wolkovich *et al.* 2009). On the other hand, growth of native plant species can be inhibited when the litter of other natives added contains defense compounds (Dorrepaal *et al.* 2007). Indeed, inhibition of native plant species is also to be expected when toxic compounds from exotic plant species are released during litter decomposition, especially if the toxic compounds are novel for the native plant species (Callaway and Ridenour 2004). For example, soils influenced by exotic forbs may inhibit the native grass, *Dactylis glomerata*, more than soil influenced by native forbs or grasses (Scharfy *et al.* 2011). Therefore, litter from exotic plant species may not only be of higher quality than litter from natives, but may also contain compounds that inhibit the performance of natives if not adapted to these compounds.

In the present study, we examine how litter from three exotic and three phylogenetically related native plant species, all co-occurring in the same habitat, reciprocally influence each other. We specifically tested the hypothesis that litter from an exotic plant species enhances soil processes thereby creating a positive feedback to its own performance while reducing the performance of a phylogenetically related native plant species. A phylogenetic controlled comparison assumes that closely related taxa are likely to share many traits thereby reducing confounding effect other than traits that explain invasiveness of the exotic plant species (Pyšek and Richardson 2007). The hypothesis was tested by three experiments in a climate chamber. In the first two experiments, we tested how soil mixed with litter from exotic plant species influenced soil respiration, soil mineralization and soil availability of nitrogen compared to soil mixed with litter from exotic and native plant species affected germination rates and plant biomass production of exotic and native plant species.

Material & methods

Plant selection

Three exotic and three phylogenetically related native plant species were selected using three criteria: (1) exotic plant species are recent invaders that increased in frequency in the second half of the 20th century (Tamis *et al.* 2005a), (2) the exotic and related native plant species had to be both present in the invaded habitat in order to make a phylogenetically controlled comparison (e.g. Agrawal *et al.* 2005; Ashton *et al.* 2005; Godoy *et al.* 2010), and (3) sufficient litter, seeds and root fragments should be available to enable the collection of plant material for the experiments. We focused on riverine habitats where recently many exotic plant species have established and could select three species pairs that fulfilled these criteria: *Artemisia biennis* and *A. vulgaris; Rorippa austriaca* and *R. sylvestris; Senecio inaequidens* and *S. jacobaea* (Table 4.1). They all occurred in the Geldersche Poort region (Dirkse *et al.* 2007), which is a region along the river Rhine delta, where the Rhine enters the Netherlands and branches of in three rivers.

Collection of plant and soil material

Soil, litter, seeds and root fragments were all collected from the Gelderse Poort

Plant name	Plant origin ¹	Time of	Litter ch	emistry	
		introduction ¹	% C	% N	lignin
					(mg C / g litter)
Artemisia biennis	North-Asia	1950-1975	44	2.5	121
Artemisia vulgaris	Native ²		46	1.7	205
Rorippa austriaca	East Europe	1900-1925	35	1.3	43
Rorippa sylvestris	Native ²		39	2.2	84
Senecio inaequidens	South-Africa	1925-1950	46	2.3	113
Senecio jacobaea ³	Native ²		44	1.8	130

Table 4.1 Names of plant species used in experiment, their origin, time of introduction and their initial litter chemistry. Nomenclature according to Van der Meijden (2005)

¹(Tamis et al. 2005b)

²Native to the Netherlands

³recently *Senecio jacobaea* has been renamed as *Jacobaea vulgaris* (Pelser et al. 2006)

region. Soil was collected from 5 locations in Millingerwaard, a nature reserve within this region (51°52'N; 5°59'E). After sampling, soil was homogenized and sieved through a 10 mm mesh to remove coarse fragments and plant material. The homogenized soil had a pH of 7.8 and a moisture content of 14.7 % (w/w).

In autumn 2008, litter was collected from the Gelderse Poort region by selecting senesced leaves from standing plants (Cornelissen *et al.* 1996). Litter was air-dried and stored in paper bags until use. Litter was chopped into 0.5×0.5 cm pieces and homogenized for subsequent use in the experiment. Initial chemical composition of litter was determined on dried (at 70°C) and ground litter (see Table 4.1). Total carbon (C) and nitrogen (N) were determined using a NC analyzer (Thermo flash EA 1112). Lignin content was determined according to Poorter and Villar (1997). Briefly, the litter material was subjected to polar, non-polar and acid extraction steps. The mass of the remaining residue was corrected for ash and the ash-adjusted C and N content of the residue was used to calculate lignin concentrations. This lignin fraction has been used successfully as litter quality index, but may contain small amounts of other recalcitrant C compounds besides lignin (Dorrepaal *et al.* 2007).

Root fragments and seeds were surface sterilised in a 0.5 % sodium hypochlorite solution to kill potential root and seed pathogens. Root fragments of *R. sylvestris* were also rinsed with 70 % ethanol, because a pilot showed higher root sprouting.

Experiment 1: litter effects on soil respiration

A series of six replicate 315 ml flasks per litter type were created to determine the effects of litter on soil respiration (four flasks for *R. austriaca* due to limited amount

of available litter). Field moist soil equivalent to 40 gram dry weight was placed in each 315 ml flask, on top of which a 29.6 gram mixture of soil and litter (71.6: 1) was placed. The amount of litter added to the pots represents the amount of litter that a unit of top soil in temperate systems receives yearly (Penuelas et al. 2007). Six flasks without litter in the top layer were included as controls. Soil was kept at 50 % water holding capacity (WHC), which equals 17.7 % w/w. Flasks were closed with a rubber septum, placed in randomized order in an incubation chamber and incubated at 10 °C, which is the yearly average temperature of the Netherlands (www.knmi. nl). At days 3, 7, 15, 22 and 29, gas samples were collected from the headspace using a gastight syringe and stored in an Exetainer® vial until analysis. After each sampling, flasks were opened to allow ventilation for an hour to prevent high CO, levels in the flasks and to adjust the moisture if needed by adding dematerialized water. CO₂-concentrations were measured against a reference line on a Thermo FOCUS GC equipped with a RT-QPLOT column from Restek (30 m long and 0.53 mm diameter). The average CO₂ concentration in control pots was subtracted from the CO₂ concentration in the pots that contained litter. Cumulative CO₂ production was calculated for each litter type.

Experiment 2: litter effects on soil nutrient parameters

We created a series of 15 replicate 0.5 L microcosms with a top-layer of soil mixed with each of the different litter types (but only 8 replicates for *R. austriaca* and 10 for *R. sylvestris* due to limited availability of litter) to determine the effects of litter on soil N availability, enzyme activities and fungal biomass. Each microcosm pot was filled with field-moist soil equivalent to 450 gram dry soil per pot and on top of this soil we added 83 gram of the same litter-soil mixture as used in experiment 1. The microcosms were incubated in a climate room at 10 °C and 83 % humidity and soil was kept at 50 % WHC (= 17.7 % w/w). Five random microcosms were harvested after 2, 9 and 18 weeks of incubation. At each harvest, subsamples were collected of the top layer and soil assays were performed.

Soil available mineral N was extracted by shaking moist soil (equivalent to 10 g dry weight) in 50 ml 1 M KCl for 2 h. N-NH₄⁺ and N-NO₃⁻ concentrations were measured on a Technicon TrAAcs 800 auto-analyzer. pH_{water} was measured in a 1: 2.5 soil to water ratio. Ergosterol, a specific fungal biomarker in the cell wall, was used to measure fungal biomass. This biomarker is not present in arbuscular mycorrhizal fungi (AMF) (Olsson *et al.* 2003). Ergosterol was extracted from soil using an alkaline-extraction method and measured on a Dionex HPLC equipped with a C 18 reverse-phase column and a UV-detector set at 282 nm (De Ridder-Duine *et al.* 2006). Lignin degrading enzyme activity (Mn-peroxidase) and cellulose degrading enzyme activity (endo-1,4- β -glucanase) were measured according to Van der Wal et

al. (2007), modified so that 6 gram of soil was extracted with 9 ml of milli-q water. Endo-1,4- β -glucanase is an indicator of cellulase activity and is therefore called cellulase throughout the remainder of the text.

Experiment 3: litter effects on seedling germination and plant biomass production Seeds or root fragments of exotic and native plant species were placed on top of the microcosms pre-incubated with litter from exotic or native species. We carried out this experiment by placing seeds of exotic and native plant species on their own soil, as well as on soil of the related native species, so that these comparisons have been made within genera. We created 10 microcosms (8 for *R. austriaca*) per litter type, which were pre-incubated for 18 weeks as in experiment 2 in order to mimic litter decomposition in winter prior to plant growth in spring. For Artemisia and Senecio species, 50 seeds of exotic or native plant species were placed on each microcosm within the genera. For *Rorippa*, 10 root fragments were placed in the soil of every microcosm. Microcosms were placed in a climate chamber for 9.5 weeks at 19°C/ 10°C (average May-September day/night temperature, www.knmi.nl) with daylight for 16 h per 24 h. Germination or sprouting rates were registered after 17 days for Senecio, after 22 days for Rorippa, and after 36 days for Artemisia, because time of germination or sprouting rates differed by genera. After germination, seedlings or cuttings were thinned so that one seedling with median length was left. All harvested plants were dried to constant weight at 70 °C and weighed.

Data analysis

The results were analyzed with Statistica version 9.0 (StatSoft, Inc. (2009), Tulsa, USA) by considering the three genera separately. Repeated measure ANOVA was performed for soil respiration with origin (litter from exotic or native plant species)

I fant ge	inci a							
Artemis	ia		Rorippa			Senecio		
d.f.	F	Р	d.f.	F	Р	d.f.	F	Р
ject								
1	2.77	0.13	1	0.96	0.36	1	14	0.004
10			8			10		
ct								
1.4	461	< 0.001	1.2	1141	< 0.001	1.6	635	< 0.001
1.4	5.5	0.027	1.2	4.5	0.054	1.6	14	< 0.001
14			9.9			16		
	<i>Artemis</i> d.f. iect 1 10 ct 1.4 1.4 1.4	Artemisia d.f. F ject 1 2.77 10 0 ct 1.4 461 1.4 5.5 14	Artemisia Artemisia d.f. F P ject 1 2.77 0.13 10 0 0 ct 1.4 461 < 0.001	Artemisia Rorippa d.f. F P d.f. iect 1 2.77 0.13 1 10 8 8 8 ct 1.4 461 < 0.001	Artemisia Rorippa d.f. F P d.f. F iect 1 2.77 0.13 1 0.96 10 8 8 0.96 0 ct 1.4 461 < 0.001	Artemisia Rorippa d.f. F P d.f. F P iect 1 2.77 0.13 1 0.96 0.36 10 8 8 0.36 0 0.36 ct 1.4 461 < 0.001	ArtemisiaRorippaSeneciod.f.FPd.f.FPd.f.iect12.770.1310.960.36110810ct	Artemisia Rorippa Senecio d.f. F P d.f. F P d.f. F iect 1 2.77 0.13 1 0.96 0.36 1 14 10 8 10 10 10 10 10 ct 1.4 461 < 0.001

 Table 4.2 Repeated-measure ANOVA of soil respiration as affected by litter from exotic versus native plant species (named Origin) of three genera (*Artemisia, Rorippa and Senecio*)

 Factors
 Plant genera



Fig. 4.1 Mean cumulative soil respiration (\pm SE) in flasks with litter from exotic (filled circles) and native plant species (open circles) for *Artemisia* (a), *Rorippa* (b), and *Senecio* (c)

as the between-subject factor. As the sphericity assumption was violated for all species-pairs, Greenhouse-Geisser adjusted P values and degrees of freedom were calculated (Table 4.2). An ANOVA was performed for the effects of litter on soil with origin (litter from exotic or native plant species) and time (2, 9 and 18 weeks of incubation) as fixed factors. Cellulase was log-transformed to meet assumptions of ANOVA. Inorganic N concentration was log-transformed for the genera *Artemisia* and *Rorippa* and fourth-root transformed for *Senecio* to meet assumptions of ANOVA. Effects of litter origin on germination rates and plant biomass production were analyzed by ANOVA with litter (litter from exotic or native plant species) as fixed factors. Germination rates were arcsine transformed and biomass was log transformed to meet assumptions of ANOVA.

Results

Experiment 1: Soil respiration

The Time by Origin interactions were significant or (in the case of *Rorippa*) close to significant (Table 4.2). This interaction indicated that litter from all three exotic plant species increased cumulative soil respiration more over time than litter from related native plant species (Fig. 4.1).

Experiment 2: Litter effects on soil nutrient parameters

Soil with litter from exotic *Artemisia* and *Senecio* accumulated more inorganic N than soil with litter from their related natives (Fig. 4.2a and 4.2c) as indicated by the origin by time interaction (Table 4.3). However, the origin by time interaction for *Rorippa* (Table 4.3) indicated that soil with litter from exotic *Rorippa* had lower soil N concentrations than soil with litter from the native after 2 weeks of incubation and N concentrations became similar over time (Fig. 4.2b). These differences in

inorganic N accumulation between soils with litter from exotic and native plant species corresponds with the initial litter N concentrations (Table 4.1). Soil with litter from exotic plant species had less fungal biomass than soil with litter from native plant species in the case of *Rorippa* and *Senecio*, but not in the case of *Artemisia* (Table 4.3, Fig. 4.2d-f). The highest activity of cellulase was observed after 9 weeks of incubation (Fig. 4.2g-i, Table 4.3). Significant differences at peak activity were observed in the case of *Artemisia* (Table 4.3), where litter from exotics induced the



Fig. 4.2 Soil available inorganic nitrogen (N) (a, b, c), fungal biomass (d, e, f) and cellulase activity (g, h, i) in soil mixed with litter from exotic plant species (filled circles) and litter from native plant species (open circles). Means (\pm SE) are presented for *Artemisia* (a, d, g), *Rorippa* (b, e, h) and *Senecio* (c, f, i)

Factors	Plant gener	·a				
	Artemisia ¹		<i>Rorippa¹</i>		Senecio ¹	
	F	Р	F	Р	F	Р
Soil Inorganic N						
Origin (O)	52	< 0.001	12	0.005	19	< 0.001
Time (T)	55	< 0.001	6.8	0.01	35	< 0.001
OxT	13	< 0.001	11	0.002	5.9	0.008
Fungal biomass						
Origin (O)	0.8	0.38	5.1	0.043	7.6	0.01
Time (T)	1.0	0.38	0.49	0.63	2.7	0.087
OxT	0.2	0.82	0.54	0.59	0.85	0.44
Cellulase activity						
Origin (O)	28	< 0.001	0.02	0.89	0.05	0.83
Time (T)	17	< 0.001	17	< 0.001	31	< 0.001
OxT	2.97	0.07	5.8	0.018	3.03	0.07
Mn-peroxidase activi	tv					
Origin (O)	0.89	0.35	0 4 4	0.42	0.18	0.67
Time (T)	14	< 0.001	0.36	0.67	63	0.006
OxT	0.57	0.57	0.44	0.34	0.29	0.75
nH						
Origin (O)	44	0.046	12	0.005	4 0	0.057
Time (T)	4 4	< 0.01	24	< 0.000	37	< 0.007
OxT	1.9	0.17	0.78	0.78	4.3	0.026

Table 4.3 ANOVA for effects of litter from exotic versus native plant species (Origin) of three plant genera (*Artemisia, Rorippa* and *Senecio*) at three destructive sampling points (Time) on soil properties

¹Numerator d.f. is 2 for time, 1 for origin and 2 for Time X Origin. Denominator d.f. is 24 for *Artemisia* and *Senecio* and 12 for *Rorippa* pair

highest cellulase activity (Fig. 4.2g). Mn-peroxidase activity in soil with litter was relatively low and did not show significant differences between soil with litter from exotics and natives (Table 4.3, See Fig. S4.1a-c). Soil pH showed some significant differences. However, they were minor (Table 4.3, See Fig. S4.2d-f).

Experiment 3: Litter effects on seedling germination and plant biomass production Seed germination and root sprouting of natives were not inhibited by litter from related exotics. There was even a trend (Table 4.4) that litter from the exotic *Rorippa* species increased the rate of sprouting for both exotic and native plants (Fig. 4.3). The rates of germination (and sprouting) of exotic plant species were lower than of natives for both *Artemisia* and *Rorippa*, whereas the reverse was observed for *Senecio* (Fig. 4.3a-c, Table 4.4). Biomass production of native plant species was not reduced by litter from exotics. Instead, litter from the exotic *Artemisia* species increased biomass production of both exotic and native *Artemisia* (Table 4.4, Fig. 4.3d). There was a similar trend for *Senecio* (Table 4.4, Fig. 4.3f). The exotic *Rorippa* species produced more biomass than the related native, whereas biomass production was not different between exotic and native species for *Artemisia* and *Senecio* (Table 4.4, Fig. 4.3e).

Discussion

Our results supported the first part of our hypothesis in that litter from exotic plant species increases microbial activity and soil processes more than litter from native plant species. This may have been due to differences in initial litter quality, because litter from exotics contained less lignin and lower lignin:N ratios than the natives within the three genera (Table 4.1). The higher quality litter of exotic species may have increased respiration rates and cellulose breakdown, because the degradable carbon pool in litter from exotics may have been better accessible by decomposers than inlitter from natives (Berg and McClaugherty 2008). However, cellulose was more available only in litter from the exotic *A. biennis*.

Soil available N concentrations reflected initial litter N concentrations, which were highest in litter from exotic *Artemisia* and *Senecio* species, but not in

Factors	Plant genera					
	Artemisia ¹		<i>Rorippa</i> ¹		Senecio ¹	
	F	Р	F	Р	F	Р
Germinatio	on/sprouting					
Litter (L)	1.78	0.20	4.1	0.06	1.86	0.19
Plant (P)	24	< 0.001	18	< 0.001	14	0.002
LxP	0.02	0.88	0.06	0.81	0.79	0.39
Plant bioma	ass					
Litter (L)	9.5	0.007	1.23	0.29	3.60	0.078
Plant (P)	1.04	0.32	7.5	0.016	0.03	0.87
LxP	0.02	0.89	1.52	0.24	0.86	0.37

Table 4.4 ANOVA for effects of litter from exotic versus native plant species (Litter) on germination or (in the case of Rorippa) sprouting rates and plant biomass production as well as the differences between exotic and native plant species (Plant) within three genera (*Artemisia, Rorippa*, and *Senecio*)

 $^1\!Numerator$ d.f. is 1 for all factors. Denominator d.f. is 16, except for Rorippa-pair where denominator d.f. is 14



Fig. 4.3 Mean germination or (in *Rorippa*) sprouting rate (a, b, c) and plant biomass (d, e, f) production $(\pm SE)$ of exotic and native plant species in litter from exotic (grey bars) or native plant species (white bars) belonging to three genera. Exotic plant species are: *A. biennis, R. austriaca* and *S. inaequidens* and native plant species are *A. vulgaris, R. sylvestris* and *S. jacobaea*

litter from the exotic *Rorippa*. Our results may therefore support the priming theory, which predicts that a higher quality input of litter increases microbial activity and the flux of nutrients released from soil organic matter (Kuzyakov *et al.* 2000). This priming-induced increase of soil organic matter mineralization has also been proposed to be an important consequence of exotic grass invasion into hardwood forest (Strickland *et al.* 2010). Yet, fungal biomass was more often lower in soil with litter from exotics than litter from natives, which is likely due to the lower initial lignin concentration of exotics as lignin is mainly broken down by lignolytic fungi (Cadisch and Giller 1997; Osono 2007).

Our results did not support the second part of our hypothesis in that litter from exotic plant species inhibits the germination rates or biomass production of native plant species. Instead, we observed that if litter from an exotic plant species increased the biomass production or germination rate of itself, this litter also promoted the related native plant species. This finding is in line with increased biomass production of native plant species by living and senesced *S. jacobaea*, which was introduced in New Zealand (Wardle *et al.* 1995). Similarly, litter of an exotic grass favored not only its own biomass production, but also the biomass production of a native shrub (Wolkovich *et al.* 2009). These studies and our results suggest that not only exotic plant species, but also native plant species may benefit from increased decomposition of litter from exotic plant species.

If litter from exotic plant species does not necessarily provide exclusive benefit to exotic plant species themselves, it seems more likely that litter effects on soil processes may contribute to exotic plant invasions only in interaction with other mechanisms (Blumenthal *et al.* 2009; Catford *et al.* 2009; Eppinga *et al.* 2011). For example, an increasing amount of exotic plant species appears to be released from enemies when introduced in a new range (e.g. Reinhart *et al.* 2003; Callaway *et al.* 2004; Van Grunsven *et al.* 2010a). Indeed, two of the exotics used in our study, *A. biennis* and *S. inaequidens*, experienced a less negative effect from their soil biota than related native plant species when grown in soils from the same sampling area as we have used (Engelkes *et al.* 2008). Therefore, an interaction between enemy release and increased nutrient availability by litter inputs might favor these two exotic plant species over natives.

We conclude that in our study the positive effect of litter from exotic plant species on soil nutrients is likely related to higher initial litter quality. This increase in soil nutrients could promote the performance of both exotic and phylogenetically related native species. As exotic and natives co-occur in the same habitat, exotic plants may increase their own performance via litter inputs only when this interacts with other factors. If not, litter feedback of exotic may not necessarily result in increased dominance of the exotic as related natives may share that same benefit.

Acknowledgements

We thank Staatsbosbeheer regio Oost for giving us permission to collect plant and soil material in the Gelderse Poort region. We thank Ciska Raaijmakers, Wiecher Smant, Gera Hol, Henk Duyts, Paulien Klein Gunnewiek and Richard van Logtestijn for their help and advice during the experiment; Heike Schmitt for advice on respiration measurements; Daan Blok for help with setting up the experiment; Fernando Monroy Martinez, Remy Hillekens, Mirka Macel and Tim Engelkes for discussion about the experiment; Koen Verhoeven, Martijn Bezemer and Arjen Biere for discussions about statistics.


Supplement

Fig. S4.1 Mn-peroxidase activity (a, b, c) and pH (d, e, f) in soil incubated with litter from exotic plant species (filled circles) or with litter from native plant species (open circles). Means (\pm SE) are presented for *Artemisia* (a, d), *Rorippa* (b, e) and *Senecio* (c, f)

Chapter 5

Soil legacy effects of climate change-induced stresses are critical for the dominance of exotic plant species



Annelein Meisner, Wietse de Boer, Gerlinde B. de Deyn, Wim H. van der Putten

Abstract

Climate change is expected to increase abiotic stresses on plant communities, because of increased periods of drought and heavy rainfall in future. These stresses can affect plant communities. However, little is known about the effects of water stresses on the dominance of exotics in plant communities. Here we test the hypothesis that water stresses before or during the growing season will influence the dominance of exotic plant species in plant communities. Water stresses before the growing season are expected to affect the dominance of exotic plant species via soil legacies, whereas water stresses during the growing season are expected to affect the dominance of exotic plant species directly. In a full-factorial experiment, soils were pre-exposed to drought followed by flooding. Then, a plant community of exotics and related natives was planted in these soils and exposed to drought during plant growth. In a second experiment, we tested if stress-induced changes in soil biota affected the exotic and native plant species.

Here we present novel evidence that the timing of water stresses can influence the dominance of exotic plant species in plant communities via soil legacies. A single drought stress before or during the growing season increased the dominance of exotic plant species, whereas the exotics remained as dominant as in control soils when the mesocosms were exposed to both drought stresses. The second experiment showed that soil biota benefitted exotics but not natives in soils pre-exposed to water stresses. However, the opposite was seen for the control soils. In the first experiment, altered soil processes during drought before the growing season may have resulted in higher accumulation of inorganic N. The net effects of these soil legacies may influence the dominance of exotics and interact with their dominance when the community is exposed to a second drought stress. Therefore, the dominance of exotics in plant communities can be affected by the legacy of stress in the soil, but the order of occurrence of these stresses will be of key-importance.

Introduction:

Climate change is expected to increase future abiotic stresses on ecosystems. These stresses include prolonged periods with drought and short periods with extremely high amounts of precipitation, but the frequencies of these stresses are unpredictable (IPCC 2007; KNMI 2009). These water stresses can affect the productivity of plant communities (Wu *et al.* 2011), especially when the abiotic stress occurs in the middle of the growing season (De Boeck *et al.* 2011). Exotic plant species are expected to be less affected by climate-related stresses than native species, because they can have traits that help them to deal with various climate change stresses (Dukes & Mooney 1999). However, little is known about the effect of timing of abiotic stresses on the dominance of exotic plant species in the plant community. Here we test the hypothesis that abiotic stresses before or during the growing season will influence the dominance of exotic plant species in the plant community.

When abiotic stresses occur before the growing season, they will likely affect the abiotic soil conditions as is often observed in drying and rewetting experiments of soils (Schimel *et al.* 2007; Borken & Matzner 2009). Furthermore, they may also affect the soil biota, because microbial communities can differ in composition after drying and rewetting (Degens *et al.* 2001; Fierer *et al.* 2003; Butterly *et al.* 2009). These altered abiotic and biotic soil conditions may persist as legacy effects to plant species, thereby influencing the priorities of plant species to become more dominant in the community (Grman & Suding 2010). For example, some species might be promoted by these legacies while other species might be reduced. These changes can be subtle, but they may have strong effects when enforced by plant community interactions (e.g. Kardol *et al.* 2007; Petermann *et al.* 2008). However it is unknown if changes in soil legacies due to abiotic climate change stresses will prioritize exotic plant species to be more dominant in plant communities (Van der Heijden *et al.* 2008).

When abiotic stresses occur during the growing season, they can directly affect the performance of individual plant species in the plant community. For example, drought has been shown to increase the proportional biomass of subdominant plant species in a plant community when the dominant plant species are more sensitive to this stress than the subdominants (Kardol *et al.* 2010). As exotic plant species have overcome many abiotic and biotic filters to establish in new environments (Theoharides & Dukes 2007), it is expected that they will be less affected by abiotic stresses than native plant species (Dukes & Mooney 1999). Indeed, warming has been shown to decrease the biomass of individual native plant species whereas individual exotic plant species were resistant to warming (Verlinden & Nijs 2010). However, it is unknown if this gives the exotics a priority over natives in gaining dominance in the plant community.

We are especially interested in determining how drought and flood before or/and during the growing season can affect the dominance of recent-established exotic plant species in plant communities in the Netherlands. Most of these exotic plant species originate from warmer climate regions (Tamis et al. 2005a) and the recent climate warming is expected to provide more favourable conditions for these plant species to become established (Bakkenes et al. 2002; Walther et al. 2002; Parmesan & Yohe 2003). In addition, many exotics are introduced along flood-plains of Dutch rivers, because the European river system of canals and rivers form an excellent migration route for many propagules (Galil et al. 2007; Jacquemyn et al. 2010). Plant communities in riverine floodplains are exposed to irregular water levels due to precipitation and upstream snow melting, as well as human management of water levels downstream (Nienhuis 2008). These irregular water levels are expected to be more frequent in future, because of climate warming induced changes in precipitation in Europe (KNMI 2009). Therefore, exotics may increase in dominance in future plant communities, which can be further facilitated by neutral instead of negative feedbacks (Van Grunsven et al. 2010). Thus, exotics might take advantage from stress more easily than natives (Alpert et al. 2000).

Here we study if abiotic stresses before or during plant growth affect the dominance of exotic plant species in the plant community. We tested the hypothesis that exotic plant species become more dominant when the community is exposed to drought or flooding stresses before or during the growing period. In order to test this hypothesis, we performed a full factorial experiment where we applied drought and/ or flooding stresses before as well as drought stress during plant growth in order to test if timing of stresses played a role. Before the plant communities were planted in the soils, we measured if microbial processes were affected by the stresses before the growing season. In a second experiment we determined if soil biotic legacies may influence the capacity of exotics to become more dominant in plant communities. In that experiment, we exposed plant communities to living and sterilized soil inocula of soil pre-exposed to drought and/ or flood.

Material and methods

Soil and plant material

Soil was collected from nature reserve Millingerwaard that is located along the Rhine river system in the Netherlands (51°52'N; 6°00'E). After sampling, soil was homogenized and sieved through a 10 mm mesh to remove coarse fragments and plant material. Part of this soil was sterilized via γ -irradiation for sterile bulk soil in the soil biota legacy effect experiment (see later).

Exotic and native plant species were selected based on the following criteria: exotic plant species were first recorded in the Netherlands in the 20th century, i.e.

Plant family	Plant species	Time of introduction ¹	Origin ¹
Asteraceae	Artemisia biennis	1975-2000	North Asia
	Artemisia vulgaris		Native ²
	Centaurea stoebe	1975-2000	Mid-Europe
	Centaurea jacea		Native
	Tragopogon dubius	1925-1950	Mid-Europe
	Tragopogon pratensis subsp pratensis		Native
Brassicaceae	Rorippa austriaca	1900-1925	East-Europe
	Rorippa sylvestris		Native
Fabaceae	Vicia lutea	1975-2000	Mid-Europe
	Vicia sativa subsp nigra		Native
Fabaceae	Vicia lutea Vicia sativa subsp nigra	1975-2000	Mid-Europe Native

 Table 5.1 Plant species used in experiment, their origin, and the time of introduction for the nonnative plant species. Nomenclature according to Van der Meijden (2005)

¹ (Tamis et al. 2005b)

² Native in the Netherlands

which may have benefited from recent climate warming in the Netherlands (Tamis *et al.* 2005a); exotic plant species had to originate from the Eurasian continent in order to include species that have expanded their range spontaneousely, especially along the European network of canals and rivers; plant species had to be present in the riverine ecosystem; a native plant species had to be present within the same genus, i.e. be a congener (Dirkse *et al.* 2007). The selected plant species were from three different families (Table 5.1).

Seeds were collected in the field or bought from small seed suppliers. All seeds were surface sterilized with a 0.5% hypochlorite solution and germinated on glass beads, moistened with demineralized water and placed in a germination cabinet. Seeds of *Vicia*-species were exposed to sulfuric acid for 45 minutes to overcome seed dormancy. Because not all seeds germinated at the same moment, seedlings were placed in a 4°C climate chamber with 11 h daylight until the start of the experiment. The experiment was carried out in a greenhouse at 21 °C (\pm 2 °C) day temperature and 16 °C (\pm 2 °C) night temperature. There was 16 h daylight supplemented up to 225 µmol m⁻² s⁻¹ if light levels dropped below that level.

Soil exposure to drought and flooding stress

The experiment included 64 mesocosms filled with unsterilized soil that were preexposed to one of four water treatments in a full factorial design (Fig. 5.1): no stress, only drought stress, only flooding or drought followed by flooding. Briefly, wet soil (equivalent to 6000 gram dry soil) was put in 64 mesocosms of 7 L and adjusted to a moisture level of 20 % w/w (= 50 % WHC). Then, 32 pots were air-dried for 28 days until 7.5 % w/w and 32 mesocosms were maintained at a control moisture content of 20% w/w. At 28 days of incubation, all dried pots received water again.



Fig. 5.1 Schematic overview of study design. Soils were exposed to drought and flood in a full-factorial design (a). At start of incubation, water content in mesocosms was adjusted to control humidity of 20 % w/w, which equaled 50 % water holding capacity (WHC). Then, mesocosms did not receive water (drought) or remained at control humidity for 28 days of incubation. At 28 days of incubation, mesocosms received water up to 40 % w/w (flood) or 20 % w/w (control) and were left to recover for 20 days until 48 days of incubation. When turning below 20 % w/w, mesocosms received water again. Soil samples were collected at 25, 31 and 46 days of incubation (arrows). At 48 days of incubation a community of seedlings of 10 different plant species (Table 5.1) was planted in every mesocosms that differed in soil pre-treatment (b). Three weeks after planting, half of the mesocosms of each pre-treatment were exposed to a drought period of 2 weeks. The plants were grown for 69 days.

Sixteen mesocosms per treatment received a water flush to a level of 40 % w/w (= 100 % WHC) and 16 mesocosms per treatment received water to a level of 20 % w/w. Then the soil was left to recover for 3 weeks. The flooded soils were at 20 % w/w after 13 days and were kept at that soil moisture level. Soil was sampled from half the mesocosms per pre-treatment (32 mesocosms in total) at 25, 31 and 46 days of incubation to determine if soil functions and nutrients were affected by these stresses. After 46 days of incubation, soil inocula were taken for soil biota legacy experiment (see later).

Experiment 1: Soil legacies and post-drought stress to plant species

At day 48 of the experiment, every mesocosm was planted with five seedlings of exotics and five seedlings of natives (Table 5.1) in a random order with 5 cm distance

between seedlings. At day 69 of the experiment half of the mesocosms were exposed to a 14 day drought period until the soil moisture was 3.6 % w/w, after which soils were rewetted up to 20 % w/w. At day 117 all plant biomass was harvested and sorted to species (Fig. 5.1). Seven mesocosms were excluded from further analyses, because they did not contain the intended plant species.

Experiment 2: Biotic soil legacy effects to plant species

In this experiment, we established the same plant communities as in experiment 1 in 56 mesocosms with sterile bulk soil. To half of the mesocosms, living soil inoculums was added and the remaining mesocosms received sterilized inoculums. The inocula originated from seven replicates of each of the four soils with the four different legacies (Fig 5.1). Briefly, at day 46 of the abiotic and biotic legacy effect experiment, 28 wet soil samples (equivalent to 650 gram dry soil) were collected from seven replicas of every pre-treatment and divided in two portions of 325 gram dry soil. One of the two portions was sterilized twice at 120 °C for one hour in an autoclave with an 48 h time interval to kill all soil biota present in the inoculum (Van der Stoel *et al.* 2002). Then, one part of sterilized or living inoculum was mixed with the sterilized bulk soil resulting in a 1:8 mixture of inoculum and bulk soil. The soil-mixture was put in three liter mesocosms. A plant community similar as in experiment 1 was planted in these soils.

Soil analysis and plant biomass measurements

Soil mineral N was extracted by shaking wet soil (equivalent to 10 g dry weight) in 50 ml 1 M KCl for 2 h. Arginine ammonification was measured according to Alef and Kleiner (1987) with minor modifications. For each mesocosm, 2 replicate samples of fresh soil (equivalent to 1 gram dry weight) were spiked with 0.25 ml arginine solution (10 mg L-arginine ml⁻¹ milli-q water) and 1 control was left unspiked. After 3 h of incubation at 25 °C, ammonium was extracted with a 2 M KCl solution and arginine solution was added to control for background color. Samples were centrifuged at 6000 rpm for 10 minutes and dilutions (1:1 with water) were stored at – 20 °C until analyses. N-NH₄⁺ and N-NO₃⁻ concentrations were measured on a Technison TrAAcs 800 auto analyzer. Basal respiration was measured according to Orwin and Wardle (2005). C-CO₂ was measured against a reference line on a Thermo Focus GC equipped with a RT-QPLOT column (Restek) (30 m long and 0.53 mm diameter). Plants were harvested individually and roots and shoots were separated, dried to constant mass at 70 °C and weighed.

Data analysis

Data was analyzed with Statistica 9.0 (StatSoft, Inc. (2009), Tulsa, USA). The evenness of the biomass among the plant species was calculated as $J' = H'/ \ln (S)$. H' is the Shannon diversity index and is calculated as $H'= -\Sigma P_i \ln(p_i)$. P_i is the proportional biomass of the ith species and S the total number of species in a mesocosm. To estimate if exotic plant species became more dominant in the community, the total biomass of exotics in a mesocosm was divided by the total biomass of natives in that mesocosm and is referred to as the dominance of exotic plant species. Dominance of exotic plant species, total plant biomass, total exotic biomass, total native biomass and the biomass evenness were analyzed with factors pre-drought, pre-flooding and post plant drought to determine if plant community was affected by soil legacy and drought during plant growth. The biomass data were square-root transformed to meet the assumptions for the ANOVA.

The soil biotic effect on total biomass, total biomass of exotic plants species and total biomass of native plant species was calculated by taking the natural log of the biomass in the living soil divided by the biomass in the sterile soil of the original mesocosms (Brinkman *et al.* 2010). The soil effect was analyzed with predrought and pre-flood as fixed factors. Shannon evenness was calculated as above and analyzed with a mixed model procedure (REML) in SPSS 17 (IBM corporation (2008), Somers, NY, USA) with fixed factors pre-drought, pre-flood and inoculum and with random factor the mesocosm where the inoculum originated from.

Repeated measure ANOVA was performed for the soil measurements with drought and flooding as fixed factors. The Mauchly's sphericity test indicated that the sphericity assumption was violated for N concentration and basal respiration in soil, because the variances at each time point were not similar to each other. Therefore, Greenhouse-Geisser adjusted P values and degrees of freedom are presented in Table 5.2. Arginine ammonification was square-root transformed to meet assumptions for ANOVA.

The effect of treatments on square-root transformed species was tested with CANOCO 2.55 (Ter Braak and Šmilauer 1997-2006, biometris, Wageningen, the Netherlands) by a Principal Component Analysis (PCA) and Redundancy Analysis (RDA) with 999 permutations (Lepš & Šmilauer 2003). The effect of initial inorganic N concentrations on the total productivity of exotics and natives was tested with RDA on averaged data per treatment, because soil N concentrations were not measured in all mesocosms.

Results

Experiment 1: soil legacies and post-drought stress to plant species Drought stress before or during plant growth affected the dominance of exotics in the plant community (Fig. 5.2). However, there was a pre-drought by post plant drought interaction ($F_{1,49} = 8.24$, P = 0.006), indicating that the effects of drought during the growing season dependent on the soil legacy of drought before the growing season. The natives were more dominant in mesocosms unexposed to drought before or during the growing season (Fig. 5.2). However, drought stress prior to plant growth increased the dominance of the exotic plant species in comparison with soils unexposed to drought (paired t-test P = 0.024). Drought during plant growth decreased the performance of the total plant community by on average 4.7 g from 24.0 g ± 0.6 to 19.3 g ± 0.5 ($F_{1,49} = 28.05$, P < 0.001). In soil without a legacy of drought, exotic plant species also tended to be more dominant when they were exposed to drought during plant growth than in control mesocosms unexposed to drought (paired t-test P = 0.059), but this increase in exotic dominance was due to a decrease in the biomass of native species (Fig. 5.2). In mesocosms exposed to drought before and during the growing season, native plant species remained as dominant as in mesocosms unexposed to drought (paired t-test P > 0.1).



Fig. 5.2 Total biomass of natives versus exotic plant species (mean \pm SE). The plant community was grown in soil without a legacy of drought (called no drought); in soil with a legacy of drought (pre-drought); in soil without a legacy of drought, but exposed to plant drought during the growing period (post-drought); or in soil with a legacy of drought and exposed to drought during the growing period (pre- and post drought). * indicates that P < 0.05 and # indicates that P < 0.1 in comparison to mesocosms unexposed to drought (paired t-test)



Fig. 5.3 Soil biotic effect to native versus exotic plant species (mean \pm SE). Plants were grown in a mixed community in sterilized soil mixed with a sterilized or living inoculum that originated from control soil (filled triangle), soil pre-exposed to drought (filled diamond), soil pre-exposed to flood (open triangle) or soil pre-exposed to drought and flood (open diamond) (Fig. 1). The soil biotic effect has been calculated for exotic and native plant species seperately. The soil biotic effect to exotic and native plant species on soils pre-exposed to the stresses were different from soil not exposed to stresses (t-test, P < 0.05)

Soils pre-exposed to flooding did not influence the dominance of exotic plant species in the plant community (P > 0.1). However, there was less total plant community biomass in soils pre-exposed to flooding than in control soils (20.1 g \pm 0.6 versus 23.5 g \pm 0.9 respectively; $F_{1,49} = 13.3$, P = 0.007). Total biomass of natives in the plant community decreased on average from 12.3 g \pm 0.6 in soils without a legacy of flood to 10.7 g \pm 0.4 in soils with a legacy of flood ($F_{1,49} = 4.16$, P = 0.047). Similarly, the total biomass of exotics also tended to decrease, namely from 11.2 g \pm 0.7 in soils without a legacy of flood to 9.4 g \pm 0.7 in soils with a legacy of flood ($F_{1,49} = 3.50$, P = 0.067).

The evenness of plant community biomass was not influenced by drought and/ or flooding stress (P > 0.1). However, the response of individual plant species was variable among treatments (See Figs. S5.1 and S5.2). Therefore, the altered dominance of exotic plant species in soils pre-exposed to drought or when the plant community was exposed to drought during the growing period could be a consequence of an increase in dominance of some exotics and/or a decrease in dominance of some natives.

Experiment 2: soil biotic legacy effects to plant species

In our second experiment we observed that exotic and native species in the plant community responded differently to soil biota in soil with a legacy of drought and/or flood. Indeed, the soil biota pre-exposed to drought and/or flooding had a positive effect on exotic plant species and a negative effect on native plant species in comparison with biota from control soils (Fig. 5.3). This is indicated by the interaction between pre-flood and pre-drought for exotics ($F_{1, 24}$ 4.21 P 0.05) and for native species ($F_{1, 24}$ 10.07, P 0.004). Total plant biomass was not affected by soil inocula or soil pre-treatment (P > 0.1). However, the contribution of plant species to total biomass was less even in soils mixed with a living inoculum than in soil mixed with a sterilized inoculum ($F_{1, 24}$ 14.80, P 0.001).

Legacies of soil microbial processes

Drought increased arginine ammonification, but tended to decrease soil respiration. Flooding decreased arginine ammonification, but increased basal soil respiration (Table 5.2, Fig. 5.4). Arginine ammonification and basal respiration had almost completely recovered by the time the plant communities were established in the soils. However, at the date the seedlings were planted, the accumulation of inorganic N differed in the soil with the four pre-treatments. Soils only exposed to drought

Table 5.2	Repeated	measure	ANOVA	for	microbial	biomass,	arginine	ammonification	and N
concentrati	ion in soil.	Half of th	e mesoco	sms	were treat	ed with dr	ought for	the first 28 days	(named
drought), a	fter which	a water fl	ux named	(flo	od) was giv	ven to half	of mesoc	osms. Bold indic	ate that
P < 0.05									

Factor	Arginin ammon	e ification		Basal re	espiration	n	N conce	ntration	in soil
	d.f.	F	Р	d.f.	F	Р	d.f.	F	Р
Between subjects									
Drought (D)	1	0.5	0.470	1	3.0	0.110	1	62.0	< 0.001
Flood (F)	1	16.0	<0.001	1	5.5	0.035	1	78.0	< 0.001
D x F	1	0.1	0.721	1	0.8	0.400	1	0.9	0.360
Error	28			13			28		
Within subjects									
Time (T)	2	178	<0.001	1.41	15.7	<0.001	1.63	55.0	< 0.001
T x D	2	19	<0.001	1.41	5.2	0.025	1.63	0.9	0.411
ТхF	2	21	< 0.001	1.41	1.7	0.222	1.63	33.0	< 0.001
T x D x F	2	0.35	0.700	1.41	0.7	0.460	1.63	0.4	0.375
Error	56			18.39			45.7		



Fig.5.4 Arginine ammonification (a), basal respiration (b), soil N concentration (c) over time. Mean and SEM are presented for control soil without stress (filled triangles), soil that was exposed to drought stress (filled diamonds), soil that was exposed to flooding (open triangles) and soil that was exposed to drought and flooding (open diamonds). The first sampling points was during the drought stress, the second sampling point was three days after the flooding and the third sampling point was after 3 weeks of recovery (see Fig. 5.1)

had highest and soil only exposed to flooding had lowest amounts of inorganic N by the time the plant communities were planted in the soils (Table 5.2, Fig. 5.4c). Both native and exotic plant species benefitted from a higher N concentration if they were not exposed to drought during the growing season (Fig. S5.3), also coinciding with an increase in total plant community biomass (r = 0.98, P = 0.023, n = 4).

Discussion

Our results were in support of the hypothesis that altered water regimes due to climate change related abiotic stress can influence the dominance of exotic plant species in plant communities. However, whether or not exotic plant species increased their dominance depended upon the type and the timing of the water stress. Natives were more dominant under control situations. Exotics, on the other hand, were more dominant when the plant community was exposed to a single drought stress. Interestingly, a second stress could reverse a pre-planting stress effect. Indeed, natives were again more dominant when soils were exposed to two drought stress in a row, one before and one during the growing season. Such effect of stress on stress has been observed in ecotoxicology (e.g.Griffiths *et al.* 2000; Højer *et al.* 2001), but our results provide the first evidence that serial stress effects as potentially caused by climate change may influence the dominance of exotic plant species via soil legacies.

The stress-induced changes in soils may have operated to plant species via priority effects. Priority effect are effects of a first species on the performance of a second species (Fukami 2010; Grman & Suding 2010) and can operate via changed soil biotic conditions. These changes in soil conditions can affect the productivity or identity of a subsequently establishing species (Facelli & Facelli 1993; Grman & Suding 2010; Hausmann & Hawkes 2010; Van de Voorde *et al.* 2011). Our results show that drought before the growing season ('spring droughts') may remain in the soil as a legacy effect. These spring droughts are not unimaginable. For example, in 2011, parts of North-Western Europe are facing the severest drought in early season since one century (www.knmi.nl). Our results suggest that such droughts may influence exotic dominance in invaded plant communities, provided that there is no summer drought to reset the balance between exotic and native plant species.

The legacy of drought may have been due to altered abiotic and/or biotic soil conditions. Indeed, soil processes were directly affected by drought, but were almost recovered before plant growth. The legacies of these altered soil processes may have remained in the soils as a higher availability of inorganic N to the benefit of especially exotic plant species, which would support the fluctuations in resource hypothesis (Davis *et al.* 2000; Gonzalez *et al.* 2010). However, the natives also benefitted from the increased nutrient availability and not only the exotics, which has also been observed for increased nutrient availability after litter break down

(Chapter 4). In addition, the net effect of plant symbionts and pathogens on plant species was tested (Bever 2003) in our second experiment. This experiment revealed that in soils with a legacy of water stresses the soil biotic conditions had a positive effect on the exotics and a negative effect on natives, but not in soil without a legacy of water stress. The biotic effect may be caused by an altered composition of soil biota, which has often been observed after drying and rewetting (Degens *et al.* 2001; Fierer *et al.* 2003; Butterly *et al.* 2009). Therefore, the net effect of legacies in soil processes and biota promoted the dominance of exotic plant species in future plant communities of intermixed native and exotic species in soils pre-exposed to drought stress when there is no second drought during the growing season.

Exotic plant species increased in dominance when exposed to drought stress during plant growth. Plant species differ in their tolerance to environmental stresses as has been observed for flooding (Van Eck *et al.* 2004; Banach *et al.* 2009). Therefore, exotic plant species may have tolerated drought stress better than natives as has been observed for warming (Verlinden & Nijs 2010). However, in soil with a legacy of drought and where plant communities were exposed to drought during plant growth, native plant species were as dominant as in control microcosms. One possible explanation could be that the natives remained more dominant, because the symbionts may have selectively promoted the natives, but not the exotics under drought stress (Compant *et al.* 2010). Thus, soils with a legacy of drought may have benefitted natives and not exotics when the community was exposed to drought stress during plant growth.

Not only climate change stresses will affect future plant communities, but also altered species distributions when plant species expand their range to the poles with their climate optimum (Walther et al. 2002; Parmesan & Yohe 2003). Therefore, future plant communities in temperate regions will likely exist of a mixture of exotic plant species from the Southern region and native plant species (Walther et al. 2009). These new plant communities may already be occurring in the Dutch plant communities (Tamis et al. 2005a). Moreover, exotics in these communities are suggested to benefit from climate change stresses (Dukes & Mooney 1999; Vilà et al. 2007). However, our results show that the type, timing and the number of stresses can influence the potential of exotic plant species to dominate the plant community. Moreover, soil legacies may be important for the dominance of exotics, because a single drought stress before or during the growing season could increase the dominance of exotic plant species in our study. However, an additional drought stress during the growing season may reverse this effect. Therefore, two climate change stresses in a row had different outcomes than one single climate change stress. In climate change scenario's, abiotic changes are being presented as changes

in temperature and water availability (KNMI 2009; IPCC 2007). Our results show that these changes as well as their order of occurrence can have effects on the dominance of exotic plant species in plant communities.

Acknowledgement

We thank staatsbosbeheer regio Oost for permission to collect soil and plant material. We thank Freddy ten Hooven, Wiecher Smant, Ciska Raaijmakers, Roel Wagenaar, Slavica Milanovic-Ivanovic, Maria Hundscheid and Gregor Disveld for their advice and assistance during the experiment. We thank Tanja Scheublin for advice on the germination of both *Vicia* species.

Supplement



Fig. S5.1 Proportional biomass of each species in the plant communities grown in soils with different legacies (pre-drought and/ or pre-flood) and exposed to drought during the growing period (post-drought) (Fig. 5.1). Grey bars are mean (\pm SE) of the proportional biomass of the five exotic plant species and white bars are mean (\pm SE) of the proportional biomass of the five native plant species. A is *A. biennis*, B is *A. vulgaris*, C is *T. dubius*, D is *T. pratensis*, E is *C. stoebe*, F is *C. jaceae*, G is *R. austriaca*, H is *R. sylvestris*, I is *V. luteae* and J is *V. sativa*



Fig. S5.2 principal component analysis (PCA). First axis explains 28.6 % of variation and second axis explains 21.2% of variation. Redundancy analysis (RDA) revealed that the effect of drought on plant species during the growing season (post-plant drought in graph) tended to dependent on the legacy of soil to drought (pre-drought in graph) (permutation test, F = 1.54, P = 0.051, 8.1 % of variance explained) (see Table 5.1 for origin of species)



Fig. S5.3 Redundancy analysis (RDA) plot of the total biomass of exotic and native plant species in relation to the N concentration in the soil at the start of the plant growing phase. Both exotic and native plant species had more biomass when there was more inorganic N available at planting date and when they were not exposed to drought during plant growth (permutation test for first axis, F = 5.97, P = 0.004, 54.4 % of variance explained). However, when they were exposed to drought during plant growth, the relationship between inorganic N and plant biomass disappeared

Chapter 6

Synthesis and discussion



Synthesis and discussion

I studied how exotic plant species interact with soil and climate change-induced abiotic stresses in this thesis. Currently an increasing number of exotic plant species is establishing in new habitats where they may have an impact on soil ecosystem processes (Ehrenfeld 2003). These altered soil processes are often hypothesized to create a positive feedback to the exotic plant species (e.g. Levine *et al.* 2003; Raizada *et al.* 2008). However, it has rarely been studied if this is really the case. Therefore, I studied if exotic plant species can consistently alter nutrient cycling, thereby creating a positive feedback to their own productivity (Chapter 2-4) and if they could inhibit related native species by litter inputs (Chapter 4). I further studied if recent established exotic plant species in the Netherlands could dominate in plant communities when exposed to abiotic climate stresses (Chapter 5), because many of these exotic plant species originate from warmer climate regions (Tamis *et al.* 2005a). In the current chapter, I synthesize my results.

Effects of exotic plant invasions on ecosystem processes

Exotic invasive plant species can affect ecosystem processes (Ehrenfeld 2003; Levine et al. 2003), by having different plant traits than native plant species (Ehrenfeld 2004). However, exotic invasive plant species do not seem to consistently increase or decrease N cycling (Chapter 2). Moreover, many effects of exotics on soil processes are context dependent, because they depend on species comparisons (e.g. Kourtev et al. 2003) and on conditions of the invaded sites (e.g. Dassonville et al. 2008). Therefore, there is no evidence for a general pattern of effects of invasive exotic plant species on ecosystem processes. This supports the conclusions of two recent review papers that there is not one general mechanism that explains the impact for all invaders on soil processes (Ehrenfeld 2010; Vilà et al. 2011). The recently established exotic plant species in the Netherlands did not consistently differ from native congeners with respect to their effects on rhizosphere nutrient mineralization (Chapter 3). Yet, there may be a pattern. When I compared the litter effects of exotics with related natives, increases of soil processes and N availability were apparent when the litter quality of exotics was higher than that of natives (Chapter 4). Therefore, litter inputs rather than inputs of root exudates may have created a higher nutrient availability in soils.

Recently established exotics in the Netherlands did not consistently increase their nutrient uptake in comparison with native congeners. I measured a range of soil processes in the rhizosphere of these plants to identify interspecific differences in nutrient uptake (Chapter 3). Plant presence increased microbial biomass and N mineralization, which can make nutrients available for plant growth (Kuzyakov *et al.* 2000; Dijkstra *et al.* 2009). However, the measured rhizosphere processes did not have a positive relationship with plant N and P uptake. Therefore, rhizosphere processes alone could not explain nutrient uptake of the plant species. Plant species may have used additional strategies for nutrient uptake than the ones measured in chapter 3. Nutrients may be released from soil microbes when they were grazed by soil fauna as protozoa and nematodes (Clarholm 1985; Ingham *et al.* 1985; Bardgett and Chan 1999; Bonkowski 2004). Indeed, the number of bacterial- and fungal-feeding nematodes varied in species-specific ways between similar plant species as I used in Chapter 3 (Morriën *et al.* 2011b; Viketoft and Van der Putten ms in submission).

Although N acquisition strategies did not consistently differ between exotic and native plant species, shoot N concentrations were more often higher for exotic than for native plant species (Fig. S3.4). Increased shoot N concentrations have been observed for many exotic plant species in their new range (e.g. Agrawal et al. 2005; Dassonville et al. 2008; Kurokawa et al. 2010). It is still unknown what causes these higher shoot N concentrations. I proposed in chapter 2 that a higher N availability in soil in the new range may increase shoot N concentrations (Fig. 2.1b), because increased N availability has indeed been observed to increase the number of exotic plant species in plant communities (e.g. Huenneke et al. 1990; Weiss 1999; Vinton and Goergen 2006) and can increase leaf N concentrations (Lowe et al. 2002). However, higher shoot N concentrations for exotic plant species than for native plant species has been observed in soil with similar starting conditions (Chapter 3 and Agrawal et al. 2005). Recent established exotic plant species in the Netherlands as well as exotic plant species in Canada had less negative effect from their rhizosphere biota (Agrawal et al. 2005; Van Grunsven et al. 2007; Engelkes et al. 2008), but did not consistently experience less aboveground herbivore damage than natives (Agrawal et al. 2005; Engelkes 2010). Therefore, the less negative effect of soil biota might have selected those exotics plant species that invest more of their resources in shoot N concentrations instead of belowground defense.

The increased shoot N concentration suggests that the litter inputs of exotic plant species may be of higher quality and could be decomposed faster (Cornwell *et al.* 2008). I collected litter from three exotic plant species and from three related native species in the field. Litter from exotic species was of higher quality. This higher quality litter did indeed increase cumulative microbial respiration (Chapter 4). Furthermore, soil N availability was also higher after litter decomposition if litter of the exotic plant species had higher N content than litter of the native species (Chapter 4). Exotics with a higher litter quality than native species have been observed with higher decomposition rates than the natives (Allison and Vitousek 2004; Ashton *et al.* 2005), which can increase soil nutrient mineralization and nutrient availability (Rothstein *et al.* 2004; Ashton *et al.* 2005; Petsikos *et al.* 2007). However, exotic plant

species can slow down litter decomposition when their litter is of lower quality than the native species (Drenovsky and Batten 2007; Godoy *et al.* 2010). Therefore, initial litter chemistry may indeed be very important for predicting the outcome of litter on soil processes (Wardle *et al.* 1998; Meier and Bowman 2008). These differences in initial litter quality between native and exotic plant species may explain differences in litter decomposition and carbon mineralization between invaded and uninvaded plots in Belgium grasslands (Koutika *et al.* 2007). Yet, litter of exotic plant species in New Zealand had higher quality litter than related native species, but did not result in increased litter decomposition. This was probably due to phosphorus limitation and not nitrogen limitation in that region (Kurokawa *et al.* 2010). Furthermore, site differences, but not litter quality determined the litter decomposition rates for the exotic invasive *Falcataria moluccana* and native *Metrosideros polymorpha* species (Hughes and Uowolo 2006). Therefore, species-specific differences between plant species as well as regional differences between sites can likely affect the impact of exotic plant species on soil processes involved in nutrient cycling.

Consequences of exotic plant invasions on native plant species

One of the consequences of established exotic plant species is that they are expected to affect native plant species via altered soil processes in the invaded range (Chapin *et al.* 2000). Indeed, a modeling study showed that an exotic wetland plant has likely evolved a mechanism to produce litter of lower quality that takes longer to decompose, which reduces light and nutrient availability. This reduced the dominant native plant species, because the exotic plant species was a better competitor for light than the native plant species when the litter remained at the water surface (Eppinga *et al.* 2011). I observed that increased nutrient availability due to faster break down of higher quality litter of exotic plant species promoted both native and exotic plant species (Chapter 4). This confirms the suggestion in Chapter 2 that not only exotic plant species, but also native plant species has also been observed to benefit native plant species in California (Wolkovich *et al.* 2009) and New Zealand (Wardle *et al.* 1995) in different study designs. Therefore, exotic plant species may not necessarily inhibit native plant species when they alter soil processes with litter inputs.

The litter inputs of native and exotic plant species created a priority effect to the following establishing exotic or native plant species (Chapter 4). Priority effects are defined as the effect of the first species on the (negative or positive) performance of a second species (Fukami 2010; Grman and Suding 2010). Such priority effects via litter have been observed to be important for the community composition of native plant species in the next growing seasons, because some plant species will be promoted and others inhibited by the litter legacy of one species (Facelli and Facelli



Fig. 6.1 The exotic *Artemisia biennis* and the native *A. vulgaris* were grown in intraspecific competition (focal plant surrounded by itself) or intrageneric competition (focal plant surrounded by congener). The experiment was performed in field soil in a common garden. The biomass production of the focal plant is presented in the figure. Asterisk indicates difference for the focal plant biomass surrounded by itself or its congener at P < 0.05

1993; Berendse 1994; Hofland-Zijlstra and Berendse 2010). The question remains if these altered priority effects could favor the outcomes of competition to the benefit for the exotic species. An exotic grass is not always prioritized by the long-term legacy it has left in soil when it is grown in pair-wise competition with one of the two dominant native forbs it displaced (Yelenik and Levine 2011). Furthermore, an invasive forb can have a negative feedback from the legacy of its own soil when it is grown in competition with native plant community (Scharfy et al. 2010). In the Netherlands, recent established exotic Artemisia biennis may also be outcompeted by its native congener, because the germination rate of the native species was higher than the germination rate of the exotic (Chapter 4). Furthermore, the exotic A. biennis produced less biomass and native A. vulgaris produced more biomass when the species were surrounded by their congeners than when they were surrounded by themselves in soil uninfluenced by Artemisia-species (Fig 6.1). Moreover, native and exotic Artemisa-species produced more biomass when grown in litter of the exotic species than in litter of the native species (Chapter 4). This suggests that the native A. vulgaris may out-compete exotic A. biennis when grown in competition with each other. Therefore, exotics may not always be promoted by litter feedbacks when grown in competition with native plant species.

Trait differences between exotic and native plant species

I compared exotic plant species with related natives to ensure that I could identify traits associated with the invasive status of exotic plant species (Pyšek and Richardson

2007; Tecco *et al.* 2010). Moreover, this comparison is very relevant for my study system, as both related species co-occur in that habitat. However, effects of exotic plant species may have a greater impact in the new range when the exotic plant species are less related to the native plant species (Strauss *et al.* 2006). Therefore, trait differences between exotic and native plant species may matter more for local soil processes and may inhibit unrelated natives adapted to pre-invasion conditions. However, unrelated exotic species may not necessarily have a stronger effect on soil properties when invading plant communities with different than similar traits (Scharfy *et al.* 2010). Therefore, the impact of exotic plant species on soil properties may be dependent on the traits of the invader.

The invasion of a N-fixing species may disproportionally increase the N-status in the invaded environment (Intermezzo, Chapter 2). For example, invasive N-fixing *Myrica faya* in Hawaii, which was a novel functional group, increased the N status of these ecosystems. This higher N status is suggested to have decreased the abundance of native species that were adapted to nutrient poor conditions (Vitousek et al. 1987; Vitousek and Walker 1989). Moreover, N-fixing invasive species have greater effect on soil processes than non-N-fixing species (Liao et al. 2008a). However, the N inputs of N-fixing invasive plant species in their invaded environment have only been examined in few studies. Moreover, the most detailed one was *M. fava* invasion in Hawaii (Levine et al. 2003; Scherer-Lorenzen et al. 2007). Furthermore, the effects of N fixing plant species on soil N cycling can be site- and comparison-specific (Stock et al. 1995; Yelenik et al. 2007). Other traits besides high litter N concentrations of N-fixing invaders may matter for effects on N cycling, because an invasive Acacia had recalcitrant litter inputs, which may have made the N less accessible for soil decomposers (Yelenik et al. 2007). Therefore, not all N-fixing invasive plant species may increase soil N-status in the invaded environment (Ehrenfeld 2010). Instead, it depends on the environment and the traits of the N-fixing species.

Future plant communities and climate change stresses

Future plant communities are expected to be composed of a mixture of exotic and native plant species (Walther *et al.* 2009), because climate change is expected to create novel species assemblages (Parmesan and Yohe 2003). These new plant communities may already be present in the Netherlands, because the number of exotic species from warmer climate regions has increased (Tamis *et al.* 2005a). They are expected to be exposed to irregular frequencies of abiotic climate change stresses as drought and flood in the future (IPCC 2007). These water stresses can affect the performance of plant communities (Wu *et al.* 2011), as well as soil biota and soil functions (Schimel *et al.* 2007; Borken and Matzner 2009). We expected



Fig. 6.2 microbial and fungal biomass in soils exposed to drought for 28 days and then one water flood. The last soil sampling was after 3 weeks of recovery and just before the plant species were planted in the soils (See Fig. 5.1 for study design). (a) Total microbial biomass was measured with the fumigation extraction method (Vance *et al.* 1987) at the last sampling point (so drought and flood refers to the soil legacy). Soils with a legacy of flood slightly increased the total microbial biomass ($F_{1,28}$ = 13, P = 0.0011). (b) Fungal biomass was measured as the concentration ergosterol (see Chapter 3 for details of method). The first sampling point was during drought stress, the second three days after flood and the third was just before seedlings were planted in the soils. Soil water stresses did not affect fungal biomass (P > 0.05), but fungal biomass decreased over time ($F_{2,56}$ = 19; P < 0.001)

that altered soil conditions may remain in soils as a legacy effect to plant species. Therefore, I tested how the dominance of exotic plant species may respond to these drought and/ or flooding stresses before or during the growing season (Chapter 5).

Exotic plant species are expected to benefit from abiotic stresses (Dukes and Mooney 1999) and have been observed to be resistant to warming whereas individual natives were not (Verlinden and Nijs 2010). However, I showed that effects of water stresses on the dominance of exotic plant species depend on timing and on type of stress (Chapter 5). Moreover, single drought stresses before and during the growing season increased the dominance of exotic species, but natives remained dominant with two drought stresses in a row. Therefore, I conclude that the type, timing, as well as the repetitiveness of abiotic stress are crucial determinants for exotics to

become dominant in invaded plant communities under climate change.

Drought or flood before the growing season could remain in the soil as a legacy to the plant community. It may be possible that soil biota contributed to this legacy effect. Therefore, an additional experiment was performed to estimate if soil biota affected the dominance in soils with a legacy of water-stresses (Chapter 5). The soil biotic legacies were positive to exotic plant species and negative to natives in soil pre-exposed to stresses, but were the opposite in control soil unexposed to stresses. Our measurements do indicate that the total microbial biomass was slightly higher in soils with a legacy of flood (Fig. 6.2a). In addition, fungi were tolerant to drought and flood stresses (Fig. 6.2b), which follows the common expectation (Schimel et al. 2007). However, it remains a black box which soil organisms contributed to the soil biotic effect to plant species. Changes in soil microbial diversity is often observed after water stresses (Degens et al. 2001; Fierer et al. 2003; Butterly et al. 2009). Such changes may affect individual plant species in the plant community. Although exotics experienced a more positive effect from soil biota in soils pre-exposed to water stresses in the additional experiment, they only became more dominant in soils pre-exposed to drought in the main experiment (Chapter 5). Possible, other soil conditions differed between the microcosms with water stresses. Indeed, the two measured soil processes, ammonifiation and respiration, were directly affected by the climate stresses, but were almost recovered before plant growth. Moreover, inorganic N concentrations were highest in soils pre-exposed to drought and lowest in soil pre-exposed to flood. As increased soil N concentrations benefitted both native and exotic plant species, this may not necessarily explain exotic dominance (Chapter 5). Therefore, the net effect of the abiotic and biotic soil legacies may have been important for determining the dominance of exotic plant species in these soils.

Exotic plants in NL: consequences for ecosystem functioning

Species within Europe have expanded their ranges during natural climate fluctuations in the past. For example, fossils of Arctic plant species has been observed in the Netherlands (Van Geel *et al.* 2010) and fossils of temperate trees in Arctic regions (Francis 1988, Taylor *et al.* 1992). However, habitat fragmentation in Europe may limit range-expansion of species with limited long-distance dispersal capacities to occur in future. Therefore, it is expected that a number of plant species cannot adapt to a warmer climate and go extinct (Thomas *et al.* 2004a; Thomas *et al.* 2004b) especially for species with no long-distance dispersal capacities. Therefore, connected ecosystems within Europe and the Netherlands could insure that plant species can expand their range and insure ecosystem functioning in the future (Walther *et al.* 2009). Creating connected ecosystems has recently been proposed as one of the mechanisms to increase the biodiversity of species in Dutch fragmented



Fig 6.3 Schematic overview of effects of recent established exotics in the Netherlands. Grey boxes indicate that the measurement were done for individual plant species and compared with related natives or the native range. Black circles indicate interactions between exotics and natives, because they were grow after each other on similar soils or in a plant comunity with native plant species. See text for detailed explanations. ¹ (Van Grunsven et al. 2007; Engelkes et al. 2008; Van Grunsven et al. 2010a; Van Grunsven et al. 2010b; ².³ Chapter 3; ³Chapter 4; ⁴(Engelkes 2010); ⁵Chapter 5

ecosystems, which could contribute to the adaptation of ecosystem functions to future stresses (Vonk *et al.* 2010). However, warmth-loving exotics have already established in the second half of the previous century in the Netherlands (Tamis *et al.* 2005a). They originate from intra- and inter-continental origin, can use vectors as wind and rivers for long-distant dispersal and may be important for future ecosystem functioning (Morriën *et al.* 2010).

Recent studies suggest (see Fig. 6.3 for schematic overview) that these plant species may be beneficial or detrimental in future ecosystems. These recent established exotics may become very dominant over a short time-period just after establishment, because they experience a less negative effect from above- and belowground biota than native congeners (Van Grunsven *et al.* 2007; Engelkes *et al.* 2008) under current and future temperatures (Van Grunsven *et al.* 2010b). In addition, the exotic *T. dubius* experienced a less negative effect from its soil biota in the new range compared with the native range in Europe (Van Grunsven *et al.* 2010a). This net effect from soil biota may have been caused by a release from soil-borne enemies, because AMF did not contribute to increased nutrient uptake and therefore might not contribute to the less negative plant-soil feedback (Chapter 3) and did not differ between exotic and native plant species (Van Grunsven 2008).

Thermophilic exotics may become more exposed to pathogens over time. Connected ecosystems may create pathways for plant enemies to migrate along with the plant species. Indeed, especially aboveground herbivores are expected to migrate with these exotic plant species (Berg *et al.* 2010), whereas for belowground enemies it is more difficult to keep up with their host plants (Morriën *et al.* 2010). In addition the effects of aboveground herbivores and belowground soil biota are additive to both native and exotic plant species (Morriën *et al.* 2011), so that exotics may be more controlled by above- and belowground enemies over time. Moreover, as enemies may adapt to the exotic plant species (Verhoeven *et al.* 2009), the effect of these biota to exotic plants may reduce the dominance of exotics over time (Diez *et al.* 2010). Therefore, enemies may reduce the dominance of these exotic plant species over time, which also occurs in early stages of primary (Van der Putten *et al.* 1993) and secondary succession (De Deyn *et al.* 2003; Kardol *et al.* 2006).

Other studies suggest that recent established exotics may not become a problem on the short term, because they do not have a generally better nutrient acquisition strategy than natives (Chapter 3). Furthermore, exotic plant species did not dominate in mixtures of native and exotic plant species that were exposed to aboveground herbivory (Engelkes 2010) and did not always dominate when exposed to climate change-related water stresses (Chapter 5). Moreover, exotics do not show an increased biomass production when exposed to warming (Verlinden and Nijs 2010). In addition, litter legacy effects can increase the performance of both related native and exotic plant species and not only the exotic plant species (Chapter 4). However, it is unclear what the consequences of these newcomers will be for Dutch ecosystems on the longer-term. The combined effect of an altered dominance of individual plant species due to climate change and an altered litter input from exotic plant species may affect native and exotic plant species in the next growing season. Therefore, the next step in studying the effects of these thermophilic exotic species should be to assess the long-term effects of these species introductions into heavily managed ecosystems, as the relatively short-term experiments discussed above suggest that plant community responses can develop in different ways. Moreover, connected ecosystems may also increase the range-expansion of species

with short-distance dispersal strategies, which may introduce other plant species into the Netherlands. Thus, in order to enhance accuracy of predictions on future community composition and ecosystem functioning requires more precise and longterm estimates made under realistic scenarios of climate change. This may help managers to anticipate on species-gain and loss processes that will be a very likely consequence of ongoing climate change.

Suggestions for future research

There are still many topics important for future research and here, I highlight research areas that need priority in future studies.

Future plant communities and ecosystem processes.

I think that it is really important to predict if exotic plant species are welcome passengers needed for ecosystem stability in future or invasive pests that can dominate whole ecosystems (see discussion above). Therefore, long-term experiments are needed that answers the question if a change in ecosystem processes by exotic plant species will make future ecosystems less stable or more resistant to climate changeinduced stresses.

Linking invasion ecology with community ecology

In line with recent suggestions (Davis 2009 and Wardle *et al.* 2011), I suggest that the ecology of invasive exotic plant species should be more integrated with community ecology of plant species. It is very likely that the dominance of exotic plant species in communities is based on similar mechanisms as native species. For example, dominant native and exotic plant species can have similar population dynamics under succession (Meiners 2007). Furthermore both native and exotic plant species can allocate more N to their leaves when they are grown in soils with added N (Lowe *et al.* 2002). Most invasive exotic plant species under study are very dominant in their new ecosystem, but only 1 out of 100 introduced species is suggested to become invasive in the new habitat (Williamson and Fitter 1996). Therefore, a starting point to integrate invasion ecology with community ecology could be to study why so many native and exotic plant species remain rare or subordinate and why only few species become dominant in plant communities.

Litter feedbacks and competition for resources

Exotic plant species are often released from enemies in the new range (Mitchell and Power 2003; Reinhart *et al.* 2003; Van Grunsven *et al.* 2010a). In addition, fluctuation in resource availability has been suggested to promote the establishment of exotic plant species (Davis *et al.* 2000). Moreover, an interaction between enemy release

and increased nutrient availability has been suggested to increase the establishment of fast-growing species that originate and invaded nutrient rich habitats (Blumenthal 2005), because enemy release may favor these exotics to take more advantage over an increase in nutrient availability than natives (Blumenthal *et al.* 2009). These increases in nutrient availability may result in increased litter quality (Lowe *et al.* 2002), which may contribute to the increased nutrient cycling at these sites and create priority effect to plant species in the next growing season. I observed that the higher quality litter of exotic plant species could promote the performance of both exotic and native plant species and not exclusively the exotic plant species after litter breakdown (Chapter 4). Therefore, a series of experiments may be set up to test if increased soil nutrient availability increases the quality of litter of exotics more than that of natives and promote their establishment and local dominance via litter feedbacks when the plants are grown in competition with native plant species. Moreover, it should be estimated if a release from soil enemies could also contribute to these increased dominance as I suggested in chapter 2 (Fig 2.3).

Litter inputs of exotic species

Litter decomposition rates have been observed to be higher in soil that shared a history with the foliar litter (Ayres *et al.* 2009a; Ayres *et al.* 2009b; Strickland *et al.* 2009b). This so-called 'home-field advantage' is suggested to be caused by adaptation of soil microbes and soil biota to decompose the litter from the plant above them (Ayres *et al.* 2009b). After establishment, exotic plants may create this home-field advantage for their litter as well, because soil decomposers have to adapt to changed chemical compounds in root exudates and litter. However, litter of both native and exotic plants has been observed to decompose faster under exotic plants than under native plants (e.g. Ashton *et al.* 2005). This phenomenon should be considered together with other factors that promote the dominance of invasive exotic plant species.

Conclusion

I conclude that exotic plant species can affect soil nutrient cycling related processes, but that the direction of change depends on the invaded exotic plant species and local conditions in the invaded habitat. Litter inputs may be more important to affect soil processes than rhizosphere inputs. However, altered soil processes did not give recently established exotics in the Netherlands the advantage to increase their productivity more than natives, because nutrient uptake strategies did not consistently differ between exotic and related native plant species. In addition, litter inputs from recent established exotic plant species did not inhibit native plant species. Therefore, multiple mechanisms must be involved in the increase in dominance of exotic plant species in plant communities (Catford *et al.* 2009). Future climate change induced stresses may increase the dominance of exotics, but these effects depend on the timing and the type of stress. My results suggest that water-stress induced changes in soil biota and microbial functions can remain in the soil as a legacy that may have been important for the dominance of exotics in the plant community. Therefore, the results in my thesis indicate that indirect interactions between plants and soil processes do not necessarily increase the productivity of exotic species more than that of natives. Moreover, soil legacy effects developed as a response to water stresses are very important for the dominance of exotic plant species in future ecosystems under climate warming.

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Nederlandse samenvatting (Dutch summary)

Mensen dragen bij aan de verspreiding van vele plantensoorten over de hele wereld. Deze soorten worden bijvoorbeeld geïntroduceerd in nieuwe gebieden doordat mensen onbewust zaden meebrengen na een verre reis die aan kleren zijn blijven plakken. Uitheemse plantensoorten worden ook bewust in een nieuw ecosysteem geïntroduceerd door bijvoorbeeld het aanplanten van soorten in tuinen. Vele van de recent gevestigde uitheemse plantensoorten in Nederland komen uit warmere klimaatzones. Een deel van deze soorten is via natuurlijke verspreiding naar Nederland gekomen, waarna het warmer wordende klimaat ervoor zorgt dat ze zich in Nederland kunnen vestigen en handhaven.

Uitheemse plantensoorten kunnen zeer dominant worden in hun nieuwe leefomgeving. Een van de redenen hiervoor is dat de uitheemse soorten natuurlijke vijanden achterlaten in hun oorspronkelijke leefomgeving en geen vijanden in hun nieuwe omgeving ontmoeten. Maar ze kunnen ook de groei van de inheemse planten remmen als ze andere eigenschappen hebben dan de inheemse planten. Bijvoorbeeld als de uitheemse planten de bodem zodanig beïnvloeden dat de voedingstofkringlopen ten voordele van zichzelf veranderen. De voedingstofkringlopen zijn belangrijk voor het beschikbaar maken van voedingstoffen voor plantengroei. Stikstof is bijvoorbeeld zeer belangrijk voor de plantengroei, maar ondanks dat lucht uit zo'n 80 % stikstof bestaat, moeten de meeste planten stikstof opnemen via hun wortels. De stikstof in de bodem wordt echter niet allemaal in de juiste vorm aangeboden voor opname via de wortels. Het bodemleven speelt een belangrijke rol in het omzetten van stikstof in beschikbare vormen. Echter, inheemse plantensoorten kunnen een nadeel ondervinden als uitheemse planten de voedingstofkringlopen veranderen, bijvoorbeeld doordat ze zijn aangepast aan de huidige, maar niet aan de door de uitheemse plant veranderde bodemcondities. In mijn proefschrift onderzoek ik of uitheemse planten de bodemprocessen die belangrijk zijn voor de kringlopen van voedingstoffen in hun eigen voordeel kunnen veranderen. Omdat veel uitheemse soorten in Nederland uit warmere klimaatzones komen, onderzoek ik eveneens of de toekomstig te verwachten extremen in regenval en droogte een voordeel kunnen zijn voor de uitheemse planten.

Er bestaan in de literatuur verschillende opvattingen over de vraag of uitheemse planten de stikstofkringloop in hun eigen voordeel kunnen versnellen. In een literatuuronderzoek vond ik echter geen algemeen patroon dat deze opvatting bevestigt. De effecten van uitheemse planten op de stikstof kringloop hangen zeer waarschijnlijk af van vele andere factoren, zoals de identiteit van de bestudeerde plant, de vergelijking met inheemse soorten en de verschillen tussen de bemonsterde velden. De meeste gepubliceerde studies maken bovendien alleen gebruik van veldwaarnemingen om de conclusies te onderbouwen. Dit heeft als nadeel dat er bijvoorbeeld onderliggende verborgen verschillen kunnen zijn tussen de velden waar de uitheemse planten wel en niet voorkomen. Het is bovendien zelden bestudeerd of uitheemse planten een voordeel hebben ten opzichte van de inheemse soorten als ze de stikstof kringloop veranderen. Mijn conclusie in het literatuuronderzoek is dat er meer experimentele studies nodig zijn om te kunnen beoordelen of uitheemse planten de voedingstofkringlopen op een consistente manier veranderen en daarmee een positieve terugkoppeling creëren in hun eigen voordeel zodanig dat ze daardoor dominant kunnen worden. Daarom heb ik een aantal experimenten uitgevoerd om te bestuderen hoe recent gevestigde uitheemse plantensoorten in Nederland de bodemprocessen kunnen veranderen en of ze hierbij een groter voordeel ondervinden dan verwante inheemse plantensoorten.

In de Nederlandse uiterwaarden komen veel uitheemse plantensoorten voor, mogelijk omdat deze gebieden goed met andere Europese en Nederlandse streken verbonden zijn via rivieren. Recent gevestigde uitheemse planten in de uiterwaarden kunnen mogelijk hun nutriëntenopname vergroten via versnelde omzetting van voedingstoffen in de wortelzone. Deze verwachting heb ik getoetst door middel van een kasexperiment waarbij ik de hoeveelheid nutriëntenopname in planten, de voedingstofkringlopen in de buurt van de wortels en verschillen tussen microbiële biomassa van uitheemse soorten vergeleek met die van inheemse soorten. Uitheemse planten verschilden niet consistent in de opname van stikstof en fosfaat met nauwverwante inheemse plantensoorten. Ook verschilden de effecten van uitheemse soorten op bodemprocessen en microbiële biomassa niet consistent met die van verwante inheemse soorten. Uitheemse soorten hadden echter wel een hogere stikstofconcentratie in hun scheuten dan de inheemse soorten.

De hogere stikstofconcentratie in scheuten van uitheemse plantensoorten suggereert dat ze een hogere kwaliteit strooisel produceren dan inheemse plantensoorten. Dit strooisel zou sneller kunnen worden afgebroken en daarmee mogelijk omzettingsprocessen in de bodem versnellen. Als dit gebeurt, kan het zo zijn dat de uitheemse soorten daar meer voordeel van ondervinden dan inheemse soorten. Om het effect van strooisel op omzettingsprocessen en plantengroei te onderzoeken, verzamelde ik plantenmateriaal van drie paren van een uitheemse en verwante inheemse soort in het veld en deed een experiment onder gecontroleerde omstandigheden in een klimaatkamer. Het strooisel van de uitheemse planten versnelde inderdaad vaker de bodemprocessen in vergelijking met het strooisel van inheemse plantensoorten. Maar als dit strooisel de uitheemse plantengroei stimuleerde, dan verhoogde het strooisel ook de groei van de inheemse plantensoort. Daarom zullen uitheemse soorten met een hogere strooiselkwaliteit niet alleen een positieve terugkoppeling naar zichzelf creëren, maar ook naar inheemse plantensoorten. Deze resultaten suggereren dat een positieve terugkoppeling van de bodem op uitheemse planten alleen kan optreden in interactie met andere mechanismen die de groei van uitheemse plant meer stimuleren dan de groei van de inheemse plant.

Ecosystemen kunnen niet alleen worden verstoord door uitheemse plantensoorten, maar ook door de gevolgen van klimaatsveranderingen. Klimaatsverandering zorgt er niet alleen voor dat de temperatuur verandert, maar er wordt ook voorspeld dat frequenter extreme weersituaties zullen ontstaan zoals hevige regenval en langdurige droogte. De vraag is of uitheemse plantensoorten zouden kunnen gaan domineren in ecosystemen als gevolg van zulke stress, bijvoorbeeld doordat ze uit gebieden afkomstig zijn waar dergelijke stressvolle omstandigheden al frequenter voorkomen. Extreme weersomstandigheden doen zich echter niet alleen voor tijdens de plantengroei, maar ook in de periode voorafgaand aan het groeiseizoen. Als de extreme weersomstandigheden zich voordoen in de periode voor de plantengroei, dan kan dit een effect hebben op de vegetatie via de bodem en het bodemleven. Een verandering in de bodem of het bodemleven kan zodoende zijn weerslag hebben op de plantengroei tijdens het groeiseizoen en daarmee een indirect effect hebben op de abundantie van uitheemse soorten in plantengemeenschappen. Als extreme weersomstandigheden zich voordoen tijdens de plantengroei, zal dit een direct effect kunnen hebben op de abundantie van uitheemse soorten. Deze verwachtingen heb ik getest in een experiment door bodems bloot te stellen aan droogte, gevolgd door extreme neerslag voordat deze werden beplant. Daarna werd een plantengemeenschap geplant die werd blootgesteld aan droogte tijdens de groeiperiode. De resultaten wezen uit dat de uitheemse soorten echter niet altijd meer dominant waren in de plantengemeenschap ongeacht de stress die werd opgelegd. Dit hing af van het type stress en de combinatie van stressen of uitheemse soorten meer dominant werden. Een droogtebehandeling voor of tijdens het groeiseizoen verhoogde de dominantie van uitheemse planten, terwijl de inheemse plantensoorten meer dominant bleven als zich een combinatie voordeed van droogte voorafgaand aan en tijdens het groeiseizoen.

Een bodemblootstelling aan droogtestress voor beplanting was dus heel belangrijk voor het domineren van uitheemse plantensoorten in de plantengemeenschap. In een tweede experiment bestudeerde ik of een verandering in het bodemleven belangrijk was voor de dominantie van uitheemse plantensoorten. Bodemorganismen hadden een positief effect op de groei van uitheemse planten en een negatief effect op de groei van inheemse planten als de bodem was blootgesteld aan droogte en/of een extreme hoeveelheid neerslag voorafgaand aan de plantengroei. Maar het tegenovergestelde vond plaats in bodems die niet waren blootgesteld aan deze stressen. In het eerste experiment waren bodemprocessen ook veranderd in de bodems tijdens de blootstelling aan de stressen en zijn daarom mogelijk ook verklarend voor de gevonden resultaten. Er bleef namelijk een verandering in stikstof concentratie achter in de bodems die waren blootgesteld aan de stressen. In bodems die waren blootgesteld aan droogte voorafgaand aan het groeiseizoen werd de hoogste stikstofconcentratie gemeten en in bodems die waren blootgesteld aan een extreme hoeveelheid water werd de laagste. Een hogere stikstof concentratie had echter zowel een voordeel voor de inheemse als voor de uitheemse soorten. Daarom is waarschijnlijk het netto-effect van veranderingen in het bodemleven en veranderingen in bodemprocessen belangrijk voor een verandering in de dominantie van uitheemse soorten in de plantengemeenschap.

Conclusies

Uitheemse plantensoorten kunnen bodemprocessen in hun nieuwe leefgebied veranderen in vergelijking met verwante inheemse plantensoorten, maar de richting van de verandering hangt af van de plantensoort en de lokale condities in het nieuwe leefgebied van de uitheemse plantensoort. Strooisel heeft waarschijnlijk een grotere impact op bodemprocessen dan de directe wortelinvloeden. Veranderingen in bodemprocessen geven recent gevestigde uitheemse soorten echter geen voordeel ten opzichte van inheemse plantensoorten, omdat uitheemse soorten niet een consistent hogere of lagere voedingsstofopname hadden dan inheemse soorten. Verder stimuleerde het strooisel van de uitheemse planten de groei van zowel de inheemse als uitheemse planten. Daarom zullen ook andere mechanismen dan veranderde voedingsstofprocessen een rol spelen om de dominantie van uitheemse planten in nieuwe ecosystemen te verklaren. Toekomstige klimaat gerelateerde stresseffecten kunnen een effect hebben op de dominantie van uitheemse soorten. Ik vond echter dat de richting van stresseffecten op dominantie van soorten afhing van het type stress en de combinatie van stressen. Mijn resultaten suggereren dat droogtestress voor de plantengroei tot een verandering in bodemorganismen en bodemfuncties kan leiden die in de bodem achterblijft en in een later stadium de groei van planten kan veranderen. Deze veranderingen kunnen belangrijk zijn voor de dominantie van uitheemse planten in hun nieuwe ecosysteem, maar het hangt af van de combinatie van stresseffecten of dit de dominantie van uitheemse planten zal verhogen. De conclusie van mijn proefschrift is dat veranderde interacties tussen uitheemse soorten en bodemprocessen er niet voor zorgen dat uitheemse soorten per definitie een voordeel hebben ten opzichte van nauw verwante inheemse plantensoorten. Daarnaast hangt de dominantie van uitheemse soorten in toekomstige plantengemeenschappen eveneens af van de historie van de bodem, die bijvoorbeeld kan worden veranderd door droogtestress die voorafgaat aan het groeiseizoen.

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Dankwoord (Acknowledgement)

Dit boekje was er nooit gekomen zonder de bijdrage van velen. Er zijn namelijk een hoop mensen die direct of indirect een bijdrage hebben geleverd aan de totstandkoming van mijn proefschrift.

Allereerst wil ik mijn begeleiders Wim en Wietse bedanken. Ik vond het heel erg fijn dat jullie deur altijd voor me openstond als het nodig was. Jullie kennis en commentaar vulde elkaar altijd heel goed aan. Wim, allereerst bedankt dat je zo'n 4 ½ jaar geleden het vertrouwen in mij had om mij aan te nemen als promovendus. Er was een plan van ongeveer een half A4tje waar we eigenlijk zeer weinig van hebben uitgevoerd. In plaats daarvan liet je me mijn eigen experimenten bedenken en uitvoeren. Ik vind het heel leuk dat je mij de kans hebt gegeven om mijn eigen weg te gaan en wil je daarvoor dan ook heel erg bedanken. Verder wil ik je ook bedanken voor al het kritische meedenken over de richting van de experimenten en de kritische commentaren op manuscripten. Wietse, ook jou wil ik heel erg bedanken. Ik vond het heel leuk samen te brainstormen over experimenten. Bedankt voor je commentaren op mijn experiment-voorstellen en manuscripten. Dankzij je zeer kritische, maar enthousiaste reacties op mijn voorstellen en manuscripten, wist je me te wijzen op bepaalde punten die ik toch nog even goed moest overdenken.

Tess, ik vond het heel leuk om samen een werkkamer te delen de afgelopen jaren. Ik wil je bedanken voor het beantwoorden van mijn simpele praktische vragen over van alles en het meedenken met study design of statistiek problemen. Ook vind ik het heel leuk dat je me enthousiast hebt gemaakt voor leuke fietstochtjes met de racefiets en hoop dat we nog vaak samen gaan fietsen. Ik vind het daarom heel leuk dat je mijn paranimf wilt zijn. Sabrina, I really enjoyed sharing the room with you during the last years of my PhD. Thank you for the time you took to discuss issues about exotic plants, study designs and introducing me to Portuguese coffee. Good luck with finishing your PhD-thesis. I am sure it will work out fine. Bao li and Prisca, thank you for your company when you visited Nicole. Paul, bedankt om mij gedurende de eerste 4 maanden van mijn promotieonderzoek alle ins en outs over promoveren uit te leggen. Pella, jij hebt mijn laatste loodjes meegemaakt. Bedankt voor al de leuke gesprekken op onze kamer.

Dit proefschrift was praktisch onmogelijk geweest zonder de hulp van vele handen tijdens mijn experimenten. Wiecher, bedankt voor alle hulp bij de vele bodemmetingen die er uitgevoerd waren. Roel, bedankt voor al je hulp in de kas, mijn bestellingen en de vele metingen zoals die vele buisjes met lucht van het respiratie experiment op de GC. Ciska, bedankt voor al je hulp die je hebt geboden tijdens mijn experimenten. Ik vond het gezellig dat je me een weekje hebt geholpen bij de PLFA extracties in Yerseke. Zonder jouw hulp was het trouwens nooit gelukt om in 3 weken al die monsters te extraheren. Freddy, ik wil je heel erg bedanken voor al je hulp bij het laatste experiment. Leuk dat je nu weer terug bent op het NIOO en veel succes. Slavica en Maria, bedankt voor al jullie hulp bij de monstername. Paulien en Henk, bedankt voor al jullie hulp bij het opzetten van de CO_2 metingen op de GC. Tanja, Henk en Saskia bedankt voor al jullie hulp bij praktische zaken binnen het lab. Gregor bedankt voor alle hulp in het fytotron en bij het opslaan van al mijn bodemmonsters. André bedankt voor je hulp bij het verzamelen van bladstrooisel. Ab en Gilles bedankt voor jullie hulp bij het verzamelen van bodem. Ook wil ik hier Staatsbosbeheer en speciaal Harry Woesthuis en Jaap Rouwenhorst bedanken voor de mogelijkheid om planten en bodem-materiaal te verzamelen.

Er zijn een hoop collega's die ik wil bedanken voor hun inbreng en suggesties over experimenten, statistiek en praktische zaken. Elly en Tim, jullie waren al een tijdje bezig toen ik ook op het VICI-project mocht gaan werken. Bedankt, voor de tijd die jullie hebben genomen om veel uit te leggen over het regelen van de vergunningen, locaties waar de planten groeien en hoe ik ze kon identificeren. Ook wil ik jullie bedanken voor al onze discussies over exoten. Elly, bedankt voor de introductie in Wageningen toen ik hier net woonde. Mirka, bedankt voor alle hulp en kritische commentaren op mijn onderzoek. Wil, bedankt voor de hulp bij het selecteren van planten. Roy, bedankt voor de informatie over jouw ervaringen met morgenster en discussies over exoten. Taia, thank you for your company when we went to Millingerwaard and for discussions about exotics.

Jennifer and Gera, I enjoyed working with you on the review paper. Jennifer, thank you for your enthusiasm and positive input in the review paper. Gera, bedankt voor al je kritische, maar zeer nuttige commentaren. Koen, bedankt voor alle tijd die je nam om mij alle moeilijke statistiek uit te leggen die vooral te maken hadden met die random en fixed factors. Eric, bedankt voor al je hulp en uitleg bij de PLFA metingen in Yerseke. Marco, bedankt voor al je hulp bij de PLFA-metingen. Hans, bedankt voor je enthousiaste inbreng in mijn litter paper en je aanbod om de lignine metingen bij de VU uit te voeren. Richard, bedankt voor alle hulp bij de lignine metingen en al je tijd om mijn vragen te beantwoorden. Gerlinde, ik vond het heel erg leuk dat je betrokken bent geweest bij het vijfde hoofdstuk van dit boekje. Ik wil je daarom bedanken voor al je hulp en kritische suggesties bij het bedenken, uitvoeren en opschrijven van het experiment. Arjen en Martijn bedankt voor alle antwoorden op mijn statistiek vragen en jullie kritische suggesties op mijn experimenten. Maarten en Martine bedankt voor jullie waardevolle inbreng in de experimenten tijdens jullie stage. George, bedankt voor de discussies over mijn project in het begin van mijn promotieonderzoek. Jeff, thank you for all our discussions as well as your advice about strange insects in the fytotron. Remy, bedankt voor al onze discussies over litter-bodem interacties. Paolina, bedankt voor je gastvrijheid toen we met het VICI-team naar Bulgarije toekwamen. I further thank Ciska V., Henk, Olga, Patrick,

Tanja Sp., Emilia, Marjolein, Maria V., Agaat, Fernando, Luc, Sonja, Marcel, Louis, Roeland, Tibor, Saschie, Eico, Tanja Sc., Mitja, Kathrin, Michiel, Maaike, Annemieke, Max, Yani, Marlies, Almudena, Christiaan, Mary-lou, Alexandra, Sarah, Sabine, Sarash, Gerda, Gerrie, Elly and all other NIOO-people for the nice atmosphere at NIOO. I really enjoyed your company during coffee breaks, lunch breaks, borrels, uitjes, courses and conference visits. You all made NIOO a really nice place to work.

Tijdens mijn eerste onderzoeksstage ben ik geïntroduceerd in de leuke wereld van bodemonderzoek. Heike en Gerdit, ik wil jullie bedanken voor jullie leuke begeleiding tijdens de stage. Ik vond ons een heel leuk team en vond het een hele leuke stage. Dankzij jullie enthousiasme heb ik besloten om een promotieonderzoek te gaan doen.

Buiten het NIOO hebben een heleboel mensen indirect bijgedragen aan mijn proefschrift. Stephanie, Doris, Mark, Marieke S., Marieke O., Bas, Eva, Serap, Christo, Martijn, Wouter, Marloes en Thijs heel erg bedankt voor alle leuke etentjes, goede gesprekken, weekendjes weg en spelletjes avonden. Jullie hebben er misschien geen idee van, maar het is altijd heel fijn om er even uit te zijn. Marieke O., ik wil jou speciaal bedanken voor de tijd die je nam om Indesign uit te leggen toen je zelf ook heel druk was. Marloes, heel erg bedankt voor je enthousiasme om mijn cover te ontwerpen. Ik wil je ook bedanken voor alles wat we gedeeld hebben sinds de tijd dat we bij elkaar in de klas kwamen op de middelbare school. Ik vind het daarom heel erg leuk dat je mijn paranimf wilt zijn.

Ook wil ik mijn ouders en mijn broertje Marten bedanken voor hun steun bij de keuzes die ik nam. Joke, heel erg bedankt dat je me altijd gestimuleerd hebt mijn eigen weg te gaan. Gerda en Willem, bedankt voor de leuke vakantie in Oostenrijk.

Daan, ik vind het heel spannend dat we nu op dezelfde dag onze boekjes verdedigen. Ik wil je heel erg bedanken voor je onvoorwaardelijke steun die je me altijd geeft. Tijdens mijn promotieonderzoek waren dit de fietstochtjes in het weekend om toch nog even planten water te geven en het bespreken van manuscripten. Vooral wil ik je bedanken omdat je me gemotiveerd hebt alles goed af te ronden. Gelukkig waren we niet altijd met werk bezig. Ik wil je daarom ook bedanken voor alles buiten ons werk en hoop dat we daar nu meer tijd voor gaan krijgen.



Curriculum vitae

Annelein Meisner was born 22nd July 1979 in Amsterdam. In 1999 she started the study veterinary sciences at Utrecht University. During her study, she particularly liked the more research-oriented courses. Therefore, after obtaining her master-degree, she decided to start the research master environmental toxicology and chemistry at Utrecht University. During her major research-project, she studied the effect of veterinary antibiotics on soil functions with Dr. Heike Schmitt at IRAS, Utrecht University and RIVM. Her minor research project was about the diversity of IncP-1 plasmids in sewage systems together with Dr. Martin Ian Bahl and Prof. Dr. Søren Sørensen at Copenhagen University. During her major research project, she became fascinated by the topic of soil functioning in relation with environmental changes. Therefore, she decided to apply for a PhD position in soil ecology at NIOO. During her PhD-project, supervised by Dr. Wietse de Boer and Prof. Dr. Wim van der Putten, she studied how exotic plant species interacted with soil processes and how these plant-soil interactions are influenced by climate change stresses, which resulted in this thesis.

List of publications

A. Meisner, W. de Boer, K.J.F. Verhoeven, H.T.S. Boschker, W.H. van der Putten. 2011, Comparison of nutrient acquisition in exotic plant species and congeneric natives, Journal of ecology, early view: doi: 10.1111/j.1365-2745.2011.01858.x

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

Review of literature (6 ECTS)

- Effects of exotic invasive plants on soil nutrient cycling

Post-graduate courses (6.8 ECTS)

- Soil ecology; PE&RC (2007)
- Biodiversity and ecosystem services in a sustainable world; PE&RC (2008)
- Advanced statistics; PE&RC (2008)
- Multivariate analysis of ecological data; University of South Bohemia, Czech Republic (2009)

Laboratory training and working visits (0.6 ECTS)

- Lignin measurements; VU (2010)

Deficiency, refresh, brush-up courses (2 ECTS)

- An introduction in statistics using R; Imperial College London (2009)

Competence strengthening / skills courses (4 ECTS)

- Competence assessment; WGS, WUR (2007)
- Presentation skills course; Centa, WUR (2007)
- Promotie in eigen regie; FOM en KNAW (2008)
- Writing and presenting a scientific paper; WGS, WUR (2009)
- NWO talent class: moving on in your career; NWO (2010)

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- Symposium: current themes in ecology: polar biodiversity, past, present and future (2007)
- Symposium: current themes in ecology: plants-insects-microbes, a current theme for three (2008)

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- Microbial ecology symposium of NVVM (2007)
- PhD Discussion group NIOO, TE (2007-2011)
- NIOO-Seminars (2007-2011)
- NIOO Days with poster presentation (2009)
- Netherlands annual ecology meeting with oral presentation (2010)
- Netherlands annual ecology meeting with poster presentation (2011)
- WEES Mater class by Hans Cornelissen (2011)

International symposia, workshops and conferences (12.8 ECTS)

- Rhizosphere 2; poster presentation; Montpellier, France (2007)
- Biorhiz workshop Integrating direct and indirect interactions in the rhizosphere (2007)
- Ecological society of Germany, Austria and Switzerland (GFOE); oral presentation; Leipzig, Germany (2008)
- Workshop Global Change & Plant Microevolution; University of Bern (2009)
- Section Plant Population Biology of the Ecological of Germany, Austria and Switzerland; annual meeting; oral presentation Nijmegen, the Netherlands (2010)
- International society of microbial (ISME), oral presentation and session chair; bi-annual meeting, Seattle, USA (2010)
- Ecological society of America (ESA), oral presentation, annual meeting, Pittsburgh, USA (2010)





The research presented in this thesis was conducted at the Department of Terrestrial Ecology of the Netherlands Institute of Ecology (NIOO-KNAW) in Wageningen. This is NIOO thesis 90. The research described in this thesis was funded by the Dutch Organization for Scientific Research (NWO).



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Photo information Page 11 + 135 river dune Millingerwaard Page 21 Senecio inaequidens Page 37 Solidago gigantea Page 91 Artemisia biennis Photo on page 91 taken by Tim Engelkes; Photos on pages 130 and 135 taken by Daan Blok. All other photos taken by Annelein Meisner.

Cover-design by Marloes Moraal Printed by Ponsen & Looijen, Ede, The Netherlands

