

# **Living apart together**

Interactions between aboveground grazers,  
plants and soil biota



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 groningen



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RIJKSUNIVERSITEIT GRONINGEN

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A black and white photograph of a grassy field. The foreground is filled with tall, dense grass and some small plants. In the background, there is a line of trees and shrubs under a bright sky.

# *Chapter one*

# 1

## Introduction

## Aboveground-belowground interactions

Terrestrial ecosystems consist of aboveground and belowground subsystems that differ vastly in physical structure. The aboveground world is open and contains mainly air, while the belowground one consists of a matrix of soil particles and pores filled with water or air. This has major consequences for the mobility of organisms living aboveground and belowground. Aboveground organisms may explore areas of several kilometers within a day, whereas most soil organisms are constrained to areas of millimeters to centimeters in their whole lifetime and mostly stay in the vicinity of an individual plant (Mortimer et al. 1999, van der Putten et al. 2009).

Despite these large differences in scale, medium and mobility, aboveground and belowground organisms interact because they are connected by plants that position their functionally linked organs in both aboveground and belowground worlds and mediate flows of carbon, nutrients and energy between them (Bardgett and Wardle 2003, Wardle et al. 2004). Plants provide organic carbon for decomposer organisms, as well as resources for root and shoot herbivores, pathogens and symbiotic mutualists. In turn, these aboveground and belowground organisms influence plant performance and plant community composition via direct removal of plant material, by enhancing access to limiting resources, and by altering soil nutrient cycling. As a result, both aboveground and belowground organisms can be important drivers of plant community composition, structure and diversity (e.g. Grime et al. 1987, Crawley 1989, Huntly 1991, Milchunas and Lauenroth 1993, Olff and Ritchie 1998, van der Putten and van der Stoep 1998, van der Heijden et al. 1998b, Klironomos 2002, Wardle 2002, De Deyn et al. 2003, Bardgett and Wardle 2010). Moreover, by affecting plants aboveground and belowground organisms influence each others performance as well (e.g. Gange and Brown 1989, Masters and Brown 1992, Masters et al. 1993, Masters et al. 2001, Bezemer et al. 2003, Soler et al. 2005, Soler et al. 2007). As a result, interactions between aboveground and belowground subsystems will have major consequences for community structure and ecosystem functioning in terrestrial systems (Wardle et al. 2004, van der Putten et al. 2009).

Only few studies have investigated how aboveground-belowground interactions affected mixed plant communities (Brown and Gange 1989, Brown and Gange 1992, van Ruijven et al. 2005, Ilmarinen and Mikola 2009). These studies mainly focused on effects of invertebrate aboveground and belowground organisms. How vertebrate aboveground grazers and soil organisms interact to drive plant community structure and ecosystem processes is still poorly understood. Therefore, in this thesis I focus on consequences of interactions between aboveground vertebrate herbivores and soil organisms for plant community structure and composition.



## Aboveground vertebrate herbivores

Aboveground vertebrate grazers are identified as key determinants of community composition and functioning of grassland systems worldwide (Olff and Ritchie 1998, Knapp et al. 1999). Aboveground vertebrate herbivores affect plant community composition and ecosystem functioning via different pathways. Firstly they remove aboveground biomass, which alters competitive interactions between plant species (Olff et al. 1997, Knapp et al. 1999). Secondly they alter the quality and quantity of nutrient input into the soil by changing root exudation patterns, litter quantity and quality, and the return of excreta (Bardgett and Wardle 2003). Finally they affect the plant community and soil biotic and abiotic properties physically, for example, via trampling (Hobbs 1996, Knapp et al. 1999).

Depending on the scale of observation, herbivore size and ecosystem productivity, aboveground vertebrate herbivores have contrasting effects on plant community structure and diversity. In productive grasslands, vegetation patches created by vertebrate herbivores harbor a higher plant community diversity than ungrazed patches, because the removal of foliar biomass by herbivores will often relax the competition for light between plant species (Bengtsson et al. 1994, Olff et al. 1997). This promotes subdominants to become more abundant which generally results in an increase in local plant community diversity (Collins et al. 1998, Knapp et al. 1999, Bos et al. 2002, Bakker et al. 2003, Veen et al. 2008). Moreover, vertebrates increase colonization possibilities by transporting seeds and disturbing soil (Olff and Ritchie 1998, Bakker and Olff 2003, Ozinga et al. 2009). In contrast, in unproductive habitats, plants not always recover from grazing which leads to lower diversity (Proulx and Mazumder 1998). This can lead to a decrease in the local species pool and may reduce colonization possibilities for plants (Milchunas and Lauenroth 1993, Olff and Ritchie 1998).

The body size of herbivores is also an important determinant of herbivore effects on the plant community. Digestive capabilities of herbivores increase with body size. Therefore, small herbivores need to select for high quality food while large vertebrate herbivores are dependent on high amounts of food, but can process relatively low quality food (Demment and Van Soest 1985, Hopcraft et al. 2010). Consequently, larger vertebrate herbivores make foraging decisions on a coarser grain (Ritchie and Olff 1999, Cromsigt and Olff 2006, Laca et al. 2010) and therefore tend to create more coarse-grained spatiotemporal patterns in the vegetation as well (Bakker 2003, Cromsigt and Olff 2008). Still, large herbivores are able to create small-scale plant community patches (Veen et al. 2008), for example through the selective removal of plant species, revisitation of grazed patches with enhanced food quality (Bakker et al. 1983), and patchy distribution of dung and urine (Steinauer and Collins 1995).

## Plant-soil feedback

Effects of soil organisms on plants and plant communities can be understood by the concept of plant-soil feedback. This is a two-step process where plant species modify their soil biotic and abiotic environment (Bever et al. 1997, Yeates 1999), which in turn influences subsequent plant growth and the competitive ability of plant species (van der Putten and Peters 1997, Bever 2003, Wardle et al. 2004, Ehrenfeld et al. 2005, Manning et al. 2008). The biotic component of plant-soil feedback operates through direct, as well as indirect effects of soil organisms on plant growth (Wardle et al. 2004). Root herbivores, pathogens and symbiotic mutualists use and provide resources from plant tissues thereby directly affecting plants. Decomposer organisms drive soil nutrient cycling by converting organic material into inorganic nutrients, thereby regulating soil nutrient supply and, indirectly, growth of plants.

Plant-soil feedback effects can be highly species specific, particularly as a result of specific associations between plant species and soil organisms (van der Putten et al. 1993). It may range from positive to negative, depending on the balance between beneficial and parasitic interactions. On the one hand, symbiotic mutualists, such as some mycorrhizal fungi and rhizosphere bacteria, enhance the access of plants to limiting resources (van der Heijden et al. 2006). On the other hand, root herbivores and pathogens, such as plant-feeding nematodes, directly remove nutrients from plant tissues and reduce the capability of plants to take up soil nutrients, thereby reducing plant growth and competitive ability (van der Putten and Peters 1997).

Plant-soil feedback may enhance or reduce plant community diversity. Positive interactions between plants and symbiotic mutualists can promote seedling establishment (van der Heijden 2004). In general however, plant species that experience strong positive plant-soil feedback have been shown to outcompete other plant species (Klironomos 2002, Callaway et al. 2004), which reduces plant community diversity (Hartnett and Wilson 1999, Bever 2003) and enhances stability (Revilla 2009). In contrast, negative interactions reduce competitive exclusion (van der Putten and Peters 1997). A reduction in the competitive abilities of plants can stimulate replacement of plant species in space and time, which may result in increased coexistence, diversity and dynamics in the plant community (Olf et al. 2000, van der Putten 2003, Bever 2003, Kulmatiski et al. 2008, Revilla 2009).

## Aboveground and belowground scale of operation

Effects of the various aboveground vertebrate herbivores and soil organisms take place at different spatial and temporal scales due to limited mobility belowground and less restrictions aboveground. This will have an important effect on the conse-

quences of interactions between vertebrate herbivores and soil organisms for plant community structure and composition (van der Putten et al. 2009). Previous work showed that aboveground and belowground herbivores that use the same individual plant can influence each others performance (e.g. Gange and Brown 1989, Masters and Brown 1992, Masters et al. 1993, Masters et al. 2001, Bezemer et al. 2003, Soler et al. 2005, Soler et al. 2007). However, aboveground vertebrate herbivores that operate across much larger scales than many soil organisms may not be able to respond to changes at the level of an individual plant, for example resulting from interactions with soil organisms. In that situation aboveground vertebrate herbivores may be expected to overrule or modify belowground effects on the plant community.

## **Aim of this thesis**

The main objective of this thesis is to explore how aboveground vertebrate herbivores and soil organisms interact to drive plant community composition, structure and diversity. I aim to understand the potential of soil organisms to drive plant community dynamics and ecosystem processes in grazed grasslands. In particular I address (1) via which pathways aboveground vertebrate herbivores modify soil community composition, (2) how soil organisms in turn can drive plant community composition and dynamics, (3) how soil organisms drive plant community structure and dynamics in the presence and absence of vertebrate herbivores, (4) how differences in scale of operation of aboveground herbivores affect the outcome of aboveground-belowground interactions and (5) how large vertebrate herbivores affect soil nutrient cycling.

## **Thesis outline**

I start with examining the effects of cattle (*Bos taurus*) on soil community composition (chapter 2). I use a long-term exclosure experiment to investigate if cattle grazing changes species composition, functional group composition and diversity in the nematode community of a river floodplain grassland. In a structural equation model I combine data on nematode community composition, plant community composition and soil abiotic properties to explore via which pathway cattle modify the soil community.

In turn, changes in soil community composition and structure may feed back to change plant community composition and dynamics (chapter 3). I use a modeling approach to study how plant-soil interactions drive plant community dynamics and structure. I use an existing model describing plant-soil feedback effects (Bever

2003) and perform a detailed analysis on all possible interactions between plants and soil organisms. Results from the analysis are used to interpret how soil organisms drive plant community dynamics and to discuss their possibility to enhance or reduce plant community diversity.

In chapters 4, 5 and 6 I study how the interactions between vertebrate herbivores and soil organisms influence plant community patterns in time and space. I explore the potential of soil organisms to drive spatiotemporal plant community dynamics in the absence and presence of cattle (chapter 4). Cattle operate on a landscape scale, whereas soil organisms act on the scale of an individual plant. Consequently, cattle may determine large-scale plant community patterns, thereby altering the possibilities for soil organisms to affect plant community dynamics. I use a combination of a greenhouse and a field exclosure experiment to determine the potential of soil organisms to affect plant community dynamics and to investigate plant community dynamics in different grazing regimes respectively. In chapter 5 I study the interaction between aboveground grazers and soil organisms in more detail. In a full factorial greenhouse experiment I investigate how simulated grazing, presence of mounds of yellow meadow ants (*Lasius flavus*), and the actual presence of yellow meadow ants themselves affect plant community structure and composition. In this greenhouse experiment aboveground clipping was non-selective to imitate cattle grazing. However, in the field different-sized aboveground vertebrate herbivores that are more or less selective co-occur. The degree of selectivity, i.e. the scale of operation, of aboveground herbivores is expected to affect the outcome of aboveground-belowground interactions. Therefore, to follow up on the greenhouse experiment I study interactions between yellow meadow ants and different-sized aboveground vertebrate herbivores in the field (chapter 6). In a field experiment on the salt marsh of Schiermonnikoog I investigate plant community patches on mounds of subterranean yellow meadow ants (*Lasius flavus*) in the presence of European brown hares (*Lepus europaeus*; small aboveground herbivores) and cattle (large aboveground herbivores).

Aboveground and belowground organisms interact via direct interactions with plant species (e.g. through consumption of living biomass) and via indirect pathways (e.g. by affecting the soil decomposer food web and hence soil nutrient cycling). Soil nutrient cycling has been indicated as an important pathway via which large vertebrate herbivores and soil organisms interact (Bardgett and Wardle 2003). Large herbivores have been shown to enhance or slow down nutrient cycling by affecting the quality and quantity of resources (e.g. litter, herbivore excreta) entering the soil food web. However, contrasting effects of vertebrate herbivores on soil nutrient cycling are not always satisfactorily explained by changes in resource input into the soil. In chapter 7 I use a literature review combined with empirical examples which shows that incorporating effects of vertebrate herbivores on soil physical conditions into existing theories on nutrient cycling in grasslands will help

us to better understand when herbivores speed up or slow down soil nutrient cycling.

In chapter 8 I synthesize the findings of this thesis. I first discuss direct and indirect pathways via which aboveground and belowground organisms interact. Then, I look at the consequences of aboveground-belowground interactions for plant community structure and composition, thereby particularly regarding effects of differences in the scale of operation between aboveground and belowground organisms. Finally, I indicate future challenges in aboveground-belowground research.

# *Chapter two*



# 2

## Vertebrate herbivores influence soil nematodes by modifying plant communities

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## Abstract

Abiotic soil properties, plant community composition and herbivory all have been reported as important factors influencing the composition of soil communities. However, most studies thus far considered these factors in isolation, whereas they strongly interact in the field. Here, we study how grazing by vertebrate herbivores influences the soil nematode community composition of a floodplain grassland while accounting for effects of grazing on plant community composition and abiotic soil properties. Nematodes are the most ubiquitous invertebrates in the soil. They include a variety of feeding types ranging from microbial feeders to herbivores and carnivores and they perform key functions in soil food webs.

Our hypothesis was that grazing affects nematode community structure and composition through altering plant community structure and composition. Alternatively, we tested whether the effects of grazing may, directly or indirectly, run via changes in soil abiotic properties. We used a long-term field experiment containing plots with and without vertebrate grazers (cattle and rabbits). We compared plant and nematode community structure and composition, as well as a number of key soil abiotic properties and applied structural equation modeling to investigate four possible pathways via which grazing may change nematode community composition.

Aboveground grazing increased plant species richness and reduced both plant and nematode community heterogeneity. There was a positive relationship between plant and nematode diversity indices. Grazing decreased the number of bacterial feeding nematodes, indicating that in these grasslands top-down control of plant production by grazing leads to bottom-up control in the basal part of the bacterial channel of the soil food web.

According to the structural equation model, grazing had a strong effect on soil abiotic properties and plant community composition, whereas plant community composition was the main determinant of nematode community composition. Other pathways, which assumed that grazing influenced nematode community composition by inducing changes in soil abiotic properties, did not explain variation in nematode community composition significantly. We conclude that grazing-induced changes in nematode community composition mainly operated via changes in plant community composition. Influences of vertebrate grazers on soil nematodes through modification of abiotic soil properties were of less importance.





## Introduction

Grazing by aboveground vertebrate herbivores, abiotic soil properties and plant community composition all have been reported to determine soil community composition, function and diversity (see e.g. Stanton 1988, Yeates 1999, Wardle 2002, Bardgett 2005). Most studies have considered the effects of herbivores (e.g. Wall-Freckman and Huang 1998, Guitian and Bardgett 2000, Mikola et al. 2001), plant species (e.g. Salamon et al. 2004, De Deyn et al. 2004b, Viketoft et al. 2005), and soil abiotic properties (e.g. Sarathchandra et al. 2001, Housman et al. 2007) on soil biota independently, while their interactions have been rarely examined. One of the major complications in unraveling the individual effects and their interactions is that effects of grazing, soil abiotic properties and plant community composition are not independent. Therefore, it is still poorly understood whether effects of grazing on soil communities operate via plant community changes (e.g. Bardgett et al. 1998, Wardle et al. 2001, Bardgett and Wardle 2003) or altered soil abiotic properties (e.g. Bazot et al. 2005). Previous studies on determinants of soil community composition were carried out under controlled experimental conditions, i.e. common garden or pot experiments (e.g. Wardle et al. 1999, De Deyn et al. 2004b, Viketoft 2008), while little is known about field situations (Casper and Castelli 2007).

The aim of our study was to examine if grazing effects on soil biota operate through modification of soil abiotic properties, through altered plant community composition or through more complicated routes, for example when grazing causes plant-induced changes in soil abiotic properties. We collected plant and soil data from a long-term grazing exclusion experiment in the field and assessed the relative importance of alternative causal pathways by structural equation modeling (SEM). We used SEM because it can test the fit of data to a priori causal hypotheses when a particular organization among variables is assumed (Shipley 2000, Grace 2006). It allows testing of multivariate hypotheses in which some variables can act as predictor and response variables at the same time.

To characterize the soil community, we focused on nematode community composition, because this taxonomic group includes a wide variety of feeding types, including bacterial feeders, fungal feeders, plant feeders, omnivores and carnivores (Yeates et al. 1993). Consequently, the nematode community serves as a proxy for community composition of other groups of soil organisms at different trophic levels. Considering the variety of nematode feeding types in the soil, the possible limiting resources will also be variable, including plant roots, bacteria, fungi, mosses, other nematodes and insects. In general, lower trophic level organisms in soil food webs are supposed to be controlled by top-down forces, whereas higher trophic level organisms are controlled by bottom-up forces (de Ruiter et al. 1995). However, resource selectivity probably plays a more important role in soil food

webs than previously supposed (Ruess et al. 2000, De Deyn et al. 2004b). Therefore, we considered that a myriad of bottom-up, top-down and competitive control factors, as well as resource specificity may be involved in determining the nematode community composition. We analyzed main pathways that could hypothetically orchestrate grazing effects on this myriad of nematode control factors.

In order to test the hypothetical pathways that may explain grazing effects on nematode community composition, we constructed four competing conceptual models (Fig 2.1) and determined the closest fit with the best model resulting from the SEM analysis. The hypothetical pathways of grazer effects on nematode community composition was based on literature information: (1) Soil abiotic conditions can affect nematode community composition (Yeates and Bongers 1999) and can be changed by herbivore activities. For example, grazers change soil bulk density as a result of trampling and they locally alter soil pH, nutrient availability and N mineralization rates by dung and urine deposition (e.g. Ruess and McNaughton 1987, Steinauer and Collins 1995, Hobbs 1996, McNaughton et al. 1997a, Frank et al. 2000, Bakker et al. 2004). (2) Grazing-induced nematode community changes can be mediated by changes in plant community composition. As a result of selective removal of plants, herbivores alter the extinction-colonization balance and competitive interactions between plants (e.g. Milchunas and Lauenroth 1993, Olff and Ritchie 1998). Altered plant community composition may lead to a different nematode community composition, because nematodes are able to respond differently to specific plants (van der Putten and van der Stoel 1998, De Deyn et al. 2007). Consequently, plant community composition, rather than plant diversity determines community composition of soil nematodes (De Deyn et al. 2004b, Viketoft et al. 2005). (3) Grazing can also affect nematode community composition by altering soil abiotic properties indirectly via plant responses and changes in plant community composition. This, in turn, influences the amount and quality of resources entering the soil via plant nutrient allocation and root exudation (Holland and Detling 1990, Bardgett et al. 1998, Guitian and Bardgett 2000). Moreover, altered plant community composition often results in a different structural and chemical composition of plant litter returned to the soil, and in different depletion patterns of soil resources like water and nutrients (Yeates 1999, Wardle 2002). (4) Finally, the nematode community composition can be altered because grazers change soil abiotic properties, which change plant community composition, leading to changes nematode community composition.

To analyze the relative importance of the different pathways (Fig 2.1), we excluded aboveground vertebrate grazers (cattle and rabbits) from a species-rich floodplain grassland and compared plant and nematode community composition, as well as various soil abiotic properties between the grazed and ungrazed plots. We expected the effects of grazing by large aboveground vertebrate herbivores, soil abiotic properties and plant community composition on nematode community

composition to be hierarchically structured according to the size of organisms involved (grazers>plants>soil biota) (Bradford et al. 2002, Ettema and Wardle 2002, Bardgett et al. 2005). Therefore, the four alternative pathways proposed include unidirectional effects only. Although there may be feedback effects of the soil community on the plant and herbivore community (e.g. Blomqvist et al. 2000, Bardgett and Wardle 2003, De Deyn et al. 2004a), we did not include these effects in our analysis.

## Methods

### Site description

The study was conducted in the Junner Koeland area, a 100-ha nature reserve along the river Overijsselse Vecht in the Northeast of the Netherlands (52°32'N, 6°36'E). The area includes a 50-ha floodplain, where our experiment was carried out. This site used to be flooded regularly until canalization of the river Vecht (around 1910) and regulation of the water level. The Junner Koeland was common grazing land, used by farmers from the village Junne, and has been grazed by livestock for centuries. Nowadays, it is managed as a nature reserve by the National Forestry Service (Staatsbosbeheer), and is grazed by cattle (*Bos taurus*) from April till November at stocking rates of about 0.3 animals/ha. The most abundant natural vertebrate herbivores in the area are European rabbits (*Oryctolagus caniculus*) and common voles (*Microtus arvalis*). Dominant plant species in the grasslands are the graminoids *Agrostis capillaris*, *Festuca rubra*, *Holcus lanatus*, and *Luzula campestris*. Frequently occurring dicots are *Rumex acetosa* and *Trifolium repens*. Also, the grasslands locally host several endangered plant species, such as *Dianthus deltoids*, *Succisa pratensis*, *Thymus serpyllum* and *Mentha pulegium* (nomenclature follows van der Meijden 2005).

### Experimental design

In 1994, a long-term enclosure experiment was set up consisting of 5 randomly established blocks, each containing a fenced enclosure plot of  $12 \times 12\text{m}^2$  from which cattle and rabbits were excluded (Ungrazed) and at 2 m distance an adjacent plot of  $12 \times 12\text{m}^2$  to which cattle and rabbits had free access (Grazed). Within each plot 4 permanent subplots of  $2 \times 2\text{m}^2$  were established to record long-term vegetation and abiotic responses to cattle and rabbit grazing. All 40 subplots were positioned at least 1 m from the fences to avoid edge effects.

### Measurements

In each of the 40 subplots of  $2 \times 2\text{m}^2$ , we recorded plant species composition in July 2006 at peak standing biomass. We estimated cover of each plant species visually

according to the decimal Londo scale (Londo 1976). In the first week of November 2006 we randomly selected three locations per subplot from each of which we collected 2 kg of soil from the top 15 cm using a hand shovel. After gentle homogenization and removing of stones, we collected 2 subsamples of 100 g each. We used one sample to isolate, identify and count nematodes and the other to measure soil organic matter content (SOM) and soil texture. We extracted nematodes using Oostenbrink elutriators (Oostenbrink 1960). A subsample of 10% of the extraction was preserved, the nematodes were heat-killed and fixed (35% formaldehyde diluted to 4%). In each sample we counted total number of nematodes and a minimum of 150 nematodes were identified to family, genus or species level according to Bongers (1988). Nematodes were classified into feeding types according to Yeates et al. (1993): plant feeders, fungal feeders, bacterial feeders, carnivores and omnivores and distributed into c-p (colonizer-persister) classes to calculate a maturity index, MI (Bongers 1990).

We determined soil organic matter content (SOM) of dry soil samples (achieved by drying for 24 hrs at 105°C) by loss-on-ignition at 550°C for 3 hrs. SOM was expressed as the relative weight difference before and after ignition. We determined soil texture in freeze-dried soil as volume % particle size classes. The freeze-dried soil was first dispersed by ultrasonification and subsequently analyzed with a coulter counter LS230 (fluid module; Beckman Coulter Inc., USA).

Additionally, per subplot we collected two PVC cylinders filled with soil (diameter 4.3 cm, length 16 cm) to measure potential nitrogen (N) mineralization rates. We homogenized soil from half of all tubes within 24 h after collection to measure soil moisture content (%), pH, and extractable concentrations of inorganic nitrogen ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ). The remaining tubes were kept at 21°C and constant soil moisture levels for 6 weeks. After 6 weeks the soil was homogenized and extractable levels of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  were measured again. The differences between the first and second analysis yield potential mineralization rates per subplot (Olff et al. 1994). To determine  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations 25g of soil was dissolved in 60 ml 1 mol/L KCl. Concentrations were determined colorimetrically with a Flow Solution Autoanalyzer (Skalar Analytical, Breda, The Netherlands). We determined soil moisture content as the weight loss after drying soil at 105°C for 24 hrs. To measure pH we dissolved 15g of soil in 20 ml demineralized water and measured the samples with a Sentron pH meter (Sentron, Roden, The Netherlands).

## Data analysis

To characterize plant and nematode community structure in each subplot ( $n = 40$ ) we calculated plant species and nematode taxon richness ( $S$ , the total number of plant species or nematode taxa per subplot), Shannon's diversity index ( $H'$ ,  $H' = -\sum p_i \cdot \ln(p_i)$  where  $p_i$  is the relative cover or abundance of species  $i$  (Magurran 2003) and evenness ( $E$ ,  $E = H'/\ln(S)$ ). We calculated plant and nematode

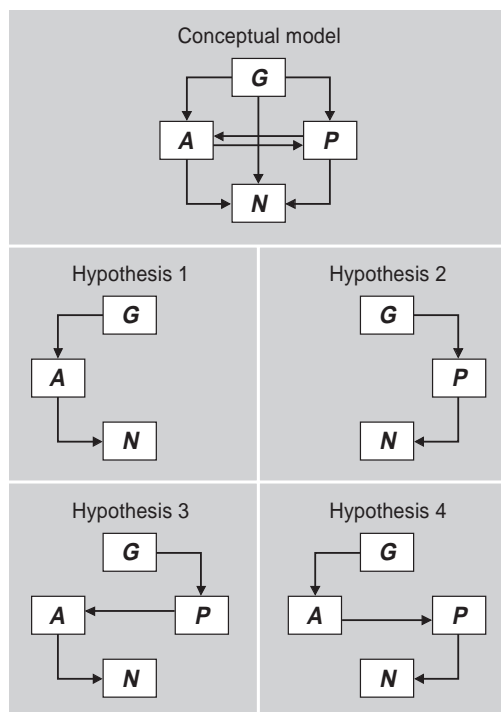
community dissimilarity from Euclidian Distance (*ED*) using the percentage cover of each plant species or the abundance of each nematode taxon, respectively, according to the following formula:

$$ED = \sqrt{\sum_{i=1}^S (xi_1 - xi_2)^2},$$

where *S* is species/taxon richness, *xi<sub>1</sub>* is the percentage cover or abundance of species *i* in plot 1 and *xi<sub>2</sub>* is the percentage cover or abundance of species 1 in plot 2. We calculated *ED* by comparing all 4 subplots within one plot. Thus, in total we had 5 replicated dissimilarity values for grazed and 5 for ungrazed areas. We tested community structure and soil abiotics data for normality (Kolmogorov-Smirnov test) and homogeneity of variances (Levene's test). When necessary data were log(*x*+1)-transformed.

We tested grazing effects on plant and nematode community structure (*S*, *H'*, *E*, *ED*, and *MI*) and soil abiotic properties using mixed model ANOVA's with the metrics of community structure or soil abiotics as response variables. Grazing was used as a fixed factor and block as a random factor. We tested the relationship between nematode and plant community structure (*S*, *H'*, *E*, *ED*) using ANCOVA with metrics for nematode community structure as response variables, grazing as a fixed factor, and metrics for plant community structure as continuous predictor variables.

To analyze via which of the four hypothetical pathways grazing was affecting nematode community composition (i.e., taxon and feeding type composition) we used structural equation modeling. Prior to the SEM procedure we reduced the number of variables for abiotic soil properties, plant community composition and soil nematode community composition through principle component analyses (PCA) on each group of variables separately. For each group the first principle component (PC) was used in the subsequent SEM analysis to represent soil abiotic properties (PC1 explained ~70% of variation), plant community composition (PC1 explained ~30% of variation) and soil nematode community composition (PC1 explained ~25% of variation), respectively. All variables used in the SEM analysis were observed variables. We started the SEM procedure with the specification of a conceptual model of relationships in our system (Fig 2.1), based on a priori and theoretical knowledge. In this model we hypothesized that grazing may alter soil abiotic properties and plant community composition, which in turn may affect nematode community composition. Soil abiotic properties and plant community composition could influence each other as well. The conceptual model provided a framework for the actual SEM analysis and test of our four alternative pathways. The subplots in the grazing experiment were grouped into blocks (see experimental design) to account for unmeasured variation due to the position of the plots on an environmental gradient which was determined by the distance to the river. In the SEM analysis we were not able to include the factor "block". To account for poten-



**Figure 2.1** The top panel shows the conceptual model of pathways via which grazing may affect nematode community composition and diversity. The four bottom panels show the models for the four alternative hypotheses outlining how aboveground grazing may affect nematode community composition. Variables are: *G*, grazing treatment, (grazed or ungrazed), *A*, soil abiotic properties, *P*, plant community composition, *N*, nematode community composition. The four hypothesis are (1) grazing alters soil abiotic properties, which directly affect nematode community composition; (2) grazing changes plant community composition, which alters nematode community composition; (3) grazing changes plant community composition, which affects nematode community composition indirectly by changing soil abiotic properties; and (4) grazing changes soil abiotic properties, which affect nematode community composition indirectly by changing plant community composition.

tial unmeasured effects of the block structure we added the variable “distance” (DIST; Fig 2.3) to the model. This variable describes the distance of each subplot to the river, therefore serving as an indicator for subplot position on the gradient, which corresponds to the blocking structure in the experiment. As minimum sample size in SEM, Bentler and Chou (1987) recommend to use 15 cases per measured variable, or even fewer when there are no missing values. In our study we just met these recommendations as we measured three variables and our sample size was 40 without missing values.

In the SEM analysis we compared the model-implied variance-covariance matrix against the observed variance-covariance matrix. Data were fitted to the models using the maximum likelihood estimation method. The  $\chi^2$  goodness-of-fit

statistic and its associated  $P$  value were used to judge the model fit to the data. A large  $P$  value associated with the  $\chi^2$  value indicates that the covariance structure of the data does not differ significantly from expected, based on the model. By step-wise removal of non-significant paths from the initial model (i.e., the model closest to our conceptual model), we selected the model that fitted our data best. We compared this SEM model to the conceptual models describing our hypothetical pathways (Fig 2.1). This procedure enabled us to determine which proposed pathways explained grazing effects on nematode community composition best. We also tested the models with direct effects of grazing on nematode community composition included to see whether this effect would explain significant amounts of variation in nematode community composition.

All univariate statistical analyses were performed using STATISTICA (release 7.1, Statsoft, Inc., Tulsa, Oklahoma USA). Multivariate statistical analyses (PCA) were performed using CANOCO for windows (version 4.5, ter Braak and Šmilauer, Wageningen-UR, The Netherlands). SEM analyses were performed using AMOS 5.0.1 student version (Amos Development, Spring House, PA, USA).

## Results

### Grazing effects on plant community composition, soil abiotic properties and nematodes

In grazed plots we found a significantly higher pH, whereas potential  $\text{NH}_4^+$  mineralization rates and soil organic matter content tended to be increased. However, potential  $\text{NO}_3^-$  mineralization rates were significantly lower in grazed plots (Table 2.1). Grazing decreased heterogeneity ( $ED$ ) in nematode communities and caused a close to significant reduction of heterogeneity in plant communities. In the grazed plots, the vegetation was lower and plant species richness was higher than in the plots where grazing was excluded. The grazing regime did not influence taxon richness in the nematode community. Grazing neither influenced evenness ( $E$ ) and diversity ( $H'$ ) in both plant and nematode communities. The maturity index ( $MI$ ) for the nematode community tended to be lowest in the grazed plots (Table 2.1). The grazed plots had significantly fewer numbers of bacterial feeding nematodes (Fig 2.2,  $F_{1,30} = 10.26$ ,  $P = 0.033$ ), whereas other nematode feeding groups showed the same tendency, although not significant ( $P > 0.05$ ).

Nematode community diversity ( $H'$ ) was positively related to plant community diversity ( $P = 0.006$ ,  $F_{1,37} = 8.54$ ), and this relationship was not different between grazed and ungrazed plots ( $P = 0.307$ ,  $F_{1,37} = 1.07$ ). However, there was neither a relationship between plant species richness and nematode taxon richness ( $P = 0.159$ ,  $F_{1,37} = 2.07$ ), nor between indices of plant and nematode evenness ( $E$ ) ( $P = 0.129$ ;  $F_{1,37} = 2.40$ ) and heterogeneity ( $ED$ ;  $P = 0.181$ ,  $F_{1,7} = 2.20$ ).



### Effects of grazers on nematodes: alternative pathways

The SEM model finally selected and used for interpretation explained 52% of the variation in nematode community composition by grazers, plant community composition, soil abiotic factors and distance to the river ( $\chi^2 = 0.568$ ,  $P = 0.989$ ,  $df = 5$ ; Fig 2.3). Grazing had strong pathways to soil abiotic properties ( $P < 0.001$ ) and plant community composition ( $P < 0.001$ ). However, plant community composition was the only factor that was associated significantly with nematode community composition ( $P < 0.001$ ). The non-recursive (i.e., reciprocal) paths between soil abiotic properties and plant community composition, as well as the path from soil abiotic properties to nematode community composition were not significant ( $P > 0.05$ ). Therefore, we found the strongest evidence for hypothetical pathway 2,

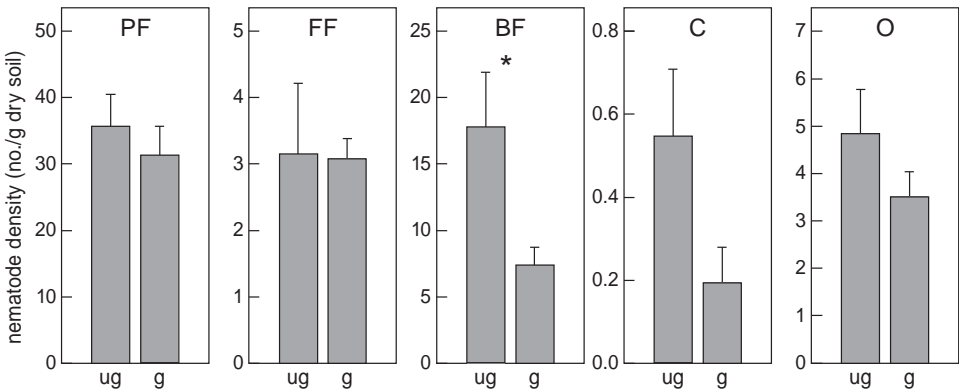
**Table 2.1** Overview of effects of grazing treatment (ungrazed or grazed) on soil abiotic properties, plant community structure and nematode community structure. Results are presented as averages  $\pm$  1 standard error of mean.

	Ungrazed	Grazed	F <sub>1,30</sub> *	P
<b>Soil abiotic properties</b>				
pH	4.48 $\pm$ 0.15	4.85 $\pm$ 0.10	24.20	<b>0.008</b>
Soil organic matter (g 100g <sup>-1</sup> )	7.58 $\pm$ 0.85	8.65 $\pm$ 1.07	5.38	0.081
Soil moisture (g 100g <sup>-1</sup> )	25.3 $\pm$ 1.21	23.4 $\pm$ 1.41	2.29	0.205
Potential NO <sub>3</sub> <sup>-</sup> mineralization rate (mg kg <sup>-1</sup> )	42.7 $\pm$ 4.00	14.7 $\pm$ 3.55	23.00	<b>0.009</b>
Potential NH <sub>4</sub> <sup>+</sup> mineralization rate (mg kg <sup>-1</sup> )	0.83 $\pm$ 1.24	8.09 $\pm$ 2.86	6.63	0.062
% clay	4.32 $\pm$ 0.28	3.95 $\pm$ 0.52	0.44	0.546
% silt	25.6 $\pm$ 1.86	24.9 $\pm$ 2.27	0.05	0.831
% sand	70.0 $\pm$ 2.12	71.0 $\pm$ 2.79	0.09	0.778
<b>Plant community structure</b>				
Species richness ( <i>S</i> )	8.00 $\pm$ 1.06	13.0 $\pm$ 0.73	11.30	<b>0.028</b>
Evenness ( <i>E</i> )	0.66 $\pm$ 0.05	0.55 $\pm$ 0.03	3.45	0.137
Diversity ( <i>H'</i> )	1.33 $\pm$ 0.06	1.41 $\pm$ 0.08	0.80	0.422
Heterogeneity ( <i>ED</i> )	53.7 $\pm$ 10.01	21.7 $\pm$ 3.94	7.02	0.057
Vegetation height (cm)	24.01 $\pm$ 2.68	5.61 $\pm$ 0.60	48.60	<b>0.002</b>
<b>Nematode community structure</b>				
Species richness ( <i>S</i> )	25.8 $\pm$ 1.81	24.2 $\pm$ 2.01	1.96	0.234
Evenness ( <i>E</i> )	0.76 $\pm$ 0.02	0.75 $\pm$ 0.04	0.07	0.805
Diversity ( <i>H'</i> )	2.45 $\pm$ 0.12	2.38 $\pm$ 0.17	0.69	0.454
Heterogeneity ( <i>ED</i> )	19.1 $\pm$ 2.06	12.2 $\pm$ 1.58	13.70	<b>0.021</b>
Maturity Index (MI)	2.06 $\pm$ 0.30	1.51 $\pm$ 0.13	4.93	0.091

\* Degrees of freedom where 1 and 30, except for ED for which they were 1 and 4

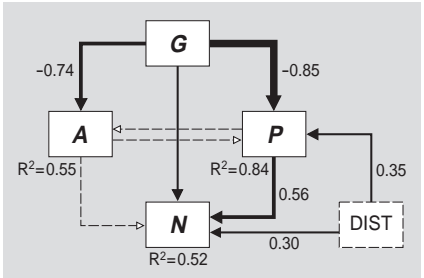


that grazing changes plant community composition, which in turn alters nematode community composition (Fig 2.1). The other pathways, which assumed that grazing influenced nematode community composition by inducing direct and indirect changes in soil abiotic properties, were less important in explaining variation in nematode community composition. Within the context of our SEM analysis we did not find a direct effect of aboveground grazing on nematode community composition ( $P = 0.926$ ). Distance to the river (DIST) significantly affected both nematode and plant community composition (Fig. 2.3), and therefore was kept in the model.



**Figure 2.2** Density of nematodes (mean  $\pm$  SE) per feeding type in each grazing treatment. Grazing treatments are plotted on the x-axis (ug, ungrazed; g, grazed). The different graphs represent different nematode feeding types (PF, plant-feeding nematodes; FF, fungal-feeding nematodes; BF, bacterial-feeding nematodes; C, carnivorous nematodes; and O, omnivorous nematodes). The Asterisk indicates a significant difference at  $P < 0.05$ .

**Figure 2.3** Final model results of SEM analysis ( $\chi^2 = 0.57$ ,  $P = 0.989$ ,  $df = 5$ ). Square boxes display variables included in the model: *G*, grazing treatment (grazed or ungrazed); *A*, soil abiotic properties (PC1); *P*, plant community composition (PC1); *N* = nematode community composition (PC1); and DIST, distance to river, used as an indicator for position on the environmental gradient. Solid arrows indicate significant effects ( $P < 0.05$ ); dashed arrows represent effects that were not significant. Arrow width corresponds directly to the standardized path coefficient.  $R^2$ -values associated with response variables indicate proportion of explained variation by relationships with other variables. Values associated with solid arrows represent standardized path coefficients.



## Discussion

Twelve years of excluding cattle and rabbits from an extensively managed grassland changed soil abiotic properties, plant and nematode community structure. Aboveground grazing decreased the abundance of nematodes, specifically bacterial feeders. Grazing reduced spatial heterogeneity (*ED*) in both plant and nematode communities. Diversity indices in both plant and nematode communities were positively related, indicating structural linkages between plants and nematodes. SEM revealed that grazing by cows and rabbits most likely influenced nematode community composition by altering plant community composition, instead of by altering soil abiotic properties. Therefore, our SEM approach supports conclusions from plant addition experiments (De Deyn et al. 2004b, Viketoft et al. 2005) and plant removal studies (Wardle et al. 1999) that nematode community composition depends on plant community composition. One previous study that also tried to tease apart effects of plant community composition and soil abiotic properties on soil microbial community composition and structure (Broughton and Gross 2000) was constrained by plant community composition changing with soil fertility. This will be a usual limitation when performing field sampling along environmental gradients. Our SEM approach based on a long-term grazing exclusion experiment, therefore, is the first to our knowledge to tease apart effects of grazing, plant communities and soil abiotic properties in influencing soil community composition.

The effects of plant community composition on nematode community composition may result from effects directly related to living plant roots and indirect effects as a result of root exudates and inputs of detritus and leaf litter into the soil food web (Wardle et al. 2004). Direct effects will work mostly through root-feeding nematodes. Since root-feeding nematodes may be host-specific (van der Putten and van der Stoep 1998) a change in plant species composition will directly alter nematode composition in this feeding group (Yeates and Bongers 1999). De Deyn et al. (2004b) showed that particular plant species enhanced the abundances of specific plant-feeding nematode types. Other nematode feeding types, such as bacterial and fungal feeders, are affected indirectly by plant community change through altered inputs of root exudates and plant litter, which first influence the soil microbial community and then the bacterial and fungal feeding nematodes (Stanton 1988, Wardle et al. 2004).

We found higher numbers of bacterial feeding nematodes in ungrazed plots, which could result from an altered microbial community composition due to changed plant species composition. Plant species may discharge different root exudates, resulting in plant-specific microbial communities (Grayston et al. 1998, Johnson et al. 2003, Marschner et al. 2004). Relatively little is known about feeding selectivity of bacterial and fungal feeding nematodes, but considering the variety in mouth parts, it is possible that these nematodes have some degree of resource specialization or preference (Ruess et al. 2000). Therefore, altered bacterial commu-

nity composition can be expected to result in a change in bacterial feeding nematode composition. Indeed, preliminary analysis of the microbial community in our study system revealed that aboveground vertebrate grazing reduced bacterial activity, which may explain lower numbers of bacterial feeding nematodes in grazed plots. Moreover, bacterial activity tended to be positively related to the number of bacterial feeding nematodes in a plot (G.F. Veen, unpublished data). Previous studies on the consequences of aboveground vertebrate grazing for microbial biomass or activity reported both positive (e.g. Bardgett et al. 1998, 2001, Wang et al. 2006) and negative effects (e.g. Stark and Grellmann 2002, Sankaran and Augustine 2004). A reduction in microbial activity by aboveground vertebrate grazing can be explained as a result from lower plant litter quality due to grazing-induced dominance of unpalatable species (Bardgett and Wardle 2003) or from lower plant litter quantity due to decreased litter production under cattle grazing (Bakker 2003).

The quality and quantity of plant litter input to the soil is affected by plant community composition. High litter quality and inputs from herbivore droppings will promote the bacterial decomposition chain of the soil food web, whereas lower quality litter and the absence of droppings may enhance the fungal decomposition chain (Wardle et al. 2004). Interestingly, in our case grazing reduced bacterivorous nematodes, suggesting that the grazing pressure was too high to promote the bacterial food chain. Omnivores may be influenced similarly to the herbivores, bacterivorous and fungal feeding nematodes, whereas carnivores will be affected more indirectly, i.e. via other nematode feeding groups, mites, collembolans, or altered insect communities in the soil (Yeates et al. 1993). As carnivores tended to be reduced as well in the grazed plots, grazing also appeared to influence higher trophic level organisms in the soil food web.

## Conclusion

We conclude that effects of grazing by aboveground vertebrate herbivores on soil nematode community diversity, heterogeneity and composition mainly operate via altering plant community structure and composition. Abiotic soil properties were of less influence on nematode community composition. Grazing effects showed up at various positions in the soil food web, as it reduced the numbers of bacterivorous and carnivorous nematodes. Grazing effects on the soil community as a result of altered plant community composition may be mediated by altered quality, quantity and distribution of resources into the soil.

## Acknowledgements

Thanks to Nelly Eck, Saskia de Vries and Rineke Gieles for their assistance with analysis of soil abiotic properties. Thanks to James B. Grace and T. Michael Anderson for their help on structural equation modeling. We thank two reviewers for their useful comments on previous versions of the manuscript. We are grateful to the National Forest Service (Staatsbosbeheer) for permission to perform our field work in the "Junner Koeland nature reserve".



# *Chapter three*

# 3

## Plant-soil feedbacks and the coexistence of competing plants

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\*These authors contributed equally to this paper

## Abstract

Plant-soil feedbacks can have important implications for the interactions among plants. Quantification of these effects is a major challenge since it is inherently difficult to measure and manipulate highly diverse soil communities. There is a need for mathematical models to single out the parameters that are crucial for a better understanding of plant-soil interactions. Here we consider a pioneering plant-soil feedback model developed by Bever and colleagues (1997, 1999, 2003). We analyze this model by means of a new graphical method that provides a complete set of scenarios describing how plant competition may be altered through soil community effects. Due to the graphical character of the method, the results are still relatively easy to obtain and understand.

We show that plant diversity can be explained by the interactions of two key parameters which can be interpreted as measures of the direction and strength of soil feedback and intensity of plant competition, respectively. We present a detailed explanation of how negative soil feedback enhances the range of plant coexistence by means of competitive oscillations. Our analysis provides a formal underpinning of earlier claims that plant-soil feedbacks, especially when they are negative, may enhance the diversity of plant communities.

The complete set of possible scenarios presented in this study can be used to formulate hypotheses about the way soil community effects drive observed plant competition dynamics. However, we have to be careful to translate the model findings to natural systems because the representation of soil community dynamics is highly simplified, i.e., the soil community is assumed to be saturated always. Therefore, a combination between more mechanistic plant-soil feedback models and plant-soil feedback experiments will provide a promising approach for future empirical research.





### 3.1 Introduction

Ecologists have long recognized that interactions between plants are mediated by many biotic (e.g. grazing, plant competition and facilitation) and abiotic factors (e.g. soil texture, nutrient availability, topography) (e.g. Harper 1977, Tilman 1988). More recent research has stressed the influence of the soil community on interactions between plants (Klironomos 2002, Callaway et al. 2004), by exerting positive or negative effects on the growth of specific plants (e.g. van der Putten et al. 1993, Gange et al. 1993, van der Putten and van der Stoep 1998, van der Heijden et al. 1998a, Olf et al. 2000, De Deyn et al. 2003, Klironomos 2003, van der Heijden et al. 2003). These effects have been shown to influence species at higher trophic levels (e.g. plant herbivores and their predators) as well (Soler et al. 2005). Therefore, plant-soil interactions can potentially be of crucial importance for species composition of plant communities and, more generally, the diversity of terrestrial ecosystems (van der Putten et al. 2001, Wardle et al. 2004).

The interaction between a plant and the soil community, referred to as plant-soil feedback, is a two-step process: the presence of a specific plant changes the composition of the soil community, which in turn alters the growth rate of that specific plant (Bever 2003, Reynolds et al. 2003). Quantifying the effect of soil organisms on plant growth and vice versa is difficult due to the vast below-ground diversity, and the technical problems inherent to measuring and manipulating soil communities (Bever 2003, van der Putten et al. 2009). Therefore, there is a need for mathematical models to single out the parameters that are crucial for a better understanding of plant-soil interactions (van der Putten et al. 2009).

Pioneering mathematical models of the effect of plant-soil feedback on plant diversity were developed by James Bever and colleagues (Bever et al. 1997, Bever 1999, 2003). These models are appealing to empirical plant-soil ecologists because they do not require specific knowledge on the diversity of soil communities, or the effects of individual soil-borne species on plant growth. As a result, model predictions can be tested with relatively straightforward experiments (Bever 1994, Bever et al. 1997). Bever (2003) proposed a framework that introduces plant-soil feedback into the classical Lotka-Volterra competition model, which has motivated several subsequent model studies on similar topics (Bonanomi et al. 2005, Umbanhowar and McCann 2005, Eppinga et al. 2006, Eppstein et al. 2006, Eppstein and Molofsky 2007).

Using this model framework, Bever (2003) highlighted two particular cases where plant-soil feedback affected the coexistence of two competing plant species: (1) negative plant-soil feedback facilitating coexistence between plants, and (2) negative plant-soil feedback driving oscillations in plant abundances (Bever 2003). Bever only performed a partial analysis of his model. Here we will provide a more systematic and complete analysis of this model (henceforth called the “Bever

model"). Our analysis reveals that there are more ways in which plant-soil feedbacks affect coexistence and dynamics of competing plant species. Moreover, it shows that we need to redefine some of Bevers conclusions (e.g. the definition of his feedback parameter  $I_s$  and the range in which plant-soil feedback drives oscillations in plant abundances) to be able to explain all outcomes of the model. In this paper we first introduce the Bever model (section 3.2), which we analyze with a graphical method (section 3.3). In section 3.4 we describe the implications of our results for plant species coexistence and in section 3.5 we present a general discussion.

### 3.2. The Bever model

The "Bever model" (Bever 2003) studies the effect of two soil communities with densities  $S_A$  and  $S_B$  on the growth of two competing plant species with densities  $N_A$  and  $N_B$ . Figure 3.1 shows a schematic representation of potential interactions between the two plant species and the two soil communities. Each soil community in the model is specifically associated with one of the plant species (Yeates 1999, Wardle et al. 2004). The growth of a soil community is positively affected by the presence of its associated plant species, while it is negatively affected by the presence of the other plant species. On their turn, specific soil communities can have both positive and negative effects on the growth of both plant species (e.g. van der Putten and van der Stoep 1998, De Deyn et al. 2003, Klironomos 2003). The dynamics of the system consisting of two plant populations and two soil communities is described by the following system of four differential equations:

$$\frac{dN_A}{dt} = r_A N_A (1 + \alpha_A S_A + \beta_A S_B - \frac{N_A + c_B N_B}{K_A}) \quad (1a)$$

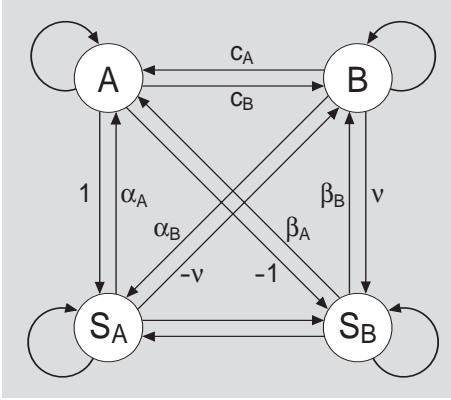
$$\frac{dN_B}{dt} = r_B N_B (1 + \alpha_B S_A + \beta_B S_B - \frac{N_B + c_A N_A}{K_B}) \quad (1b)$$

$$\frac{dS_A}{dt} = S_A S_B \frac{N_A - v N_B}{N_A + N_B} \quad (1c)$$

$$\frac{dS_B}{dt} = S_A S_B \frac{v N_B - N_A}{N_A + N_B} \quad (1d)$$

In the absence of the soil communities, the interaction of the two plant populations is described by a Lotka-Volterra competition model (e.g. Case 2000);  $r_A$  and  $r_B$  denote the intrinsic per capita growth rates of the plant species,  $K_A$  and  $K_B$  are the carrying capacities of the plant species when growing in isolation, and  $c_A$  and  $c_B$  are the competition coefficients which are expressed as the per capita effects of each species on the growth rate of the competitor species (interspecific competition), relative to the per capita effect on the growth rate of its own population (intraspecific competition).  $S_A$  and  $S_B$  are the densities of the two soil communities,





**Figure 3.1** Schematic representation of potential interactions between two plants and their associated soil communities in the Bever model.

where  $S_A$  is specifically associated with plant species A and  $S_B$  with plant species B. The per capita growth rate of each plant species is affected by the presence of the two soil communities. The effect of the soil communities on plant growth is characterized by the parameters  $\alpha_A$ ,  $\alpha_B$ ,  $\beta_A$  and  $\beta_B$  respectively, which can be either positive, negative or zero.

The Bever model represents soil community growth in a highly simplified way. The soil community is positively affected by the relative abundance of its associated plant species and negatively affected by the relative abundance of the other plant species. The parameter  $v$  is a scaling factor that quantifies the relative strength of the positive and negative effects of plant on soil community growth.

Notice that  $\frac{dS_A}{dt} + \frac{dS_B}{dt} = 0$ .

This implies that the total abundances of both soil communities are constant at all times. Without loss of generality we can assume that the total size of the soil community is scaled to 1:  $S_A + S_B = 1$ . This allows us to express  $S_B$  in terms of  $S_A$  (i.e.,  $S_B = 1 - S_A$ ) and to reduce the system (1) to a system of three differential equations, which can be written in the form:

$$\frac{dN_A}{dt} = \rho_A N_A \left( 1 - \frac{N_A + c_B N_B}{\kappa_A} \right) \quad (2a)$$

$$\frac{dN_B}{dt} = \rho_B N_B \left( 1 - \frac{N_B + c_A N_A}{\kappa_B} \right) \quad (2b)$$

$$\frac{dS_A}{dt} = S_A(1 - S_A) \left( \frac{N_A - v N_B}{N_A + N_B} \right) \quad (1c)$$

The intrinsic growth rates  $\rho_A$  and  $\rho_B$  and the carrying capacities  $\kappa_A$  and  $\kappa_B$  of the

two plant species depend on the densities of the soil communities  $S_A$  and  $S_B = 1 - S_A$ , and they are given by:

$$\rho_A = \rho_A(S_A) = r_A(1 + \alpha_A S_A + \beta_A(1 - S_A)) \quad (3a)$$

$$\rho_B = \rho_B(S_A) = r_B(1 + \alpha_B S_A + \beta_B(1 - S_A)) \quad (3b)$$

$$\kappa_A = \kappa_A(S_A) = K_A(1 + \alpha_A S_A + \beta_A(1 - S_A)) \quad (3c)$$

$$\kappa_B = \kappa_B(S_A) = K_B(1 + \alpha_B S_A + \beta_B(1 - S_A)) \quad (3d)$$

The species-specific effects of the soil communities on their plants are  $\alpha_A$  and  $\alpha_B$ , for plant A and B respectively and the non-specific effects are  $\alpha_B$  and  $\beta_A$ , for plant A and B respectively. These effects can take any sign, in correspondence with mutualistic or pathogenic relationships. In mutualistic relationships plant growth may be favored by the presence of their symbionts, e.g. arbuscular mycorrhizal fungi (AMF), which enhance plant access to limiting resources. On the other hand, soil pathogens and root herbivores, e.g. root feeding nematodes, can negatively affect plant growth by direct removal of nutrients from root tissue and by reduction of soil nutrient uptake (Bever et al. 1997).

### 3.3. Graphical analysis

Our analysis of the Bever model will focus on equilibria and their stability. It will be useful to perform the analysis in four steps. First, we discuss the effects of the soil dynamics on plants growing in monoculture, which leads us to the concept of *soil stability*. Second, we consider the effects of a fixed soil composition on plant competition, which helps us to define the concept of *competitive stability*. Third, we take into account the net effects of the plant-soil *feedbacks*, and how to distinguish whether they are positive or negative. Fourth, we integrate our criteria for soil stability, competitive stability, and feedbacks in a graphical method for the analysis of the complete system (2).

#### 3.3.1 Plant monocultures

Let us start by considering the absence of plant B, i.e.  $N_B = 0$ . This means that plant A is a monoculture that grows according to the logistic equation (2a). In the absence of plant B, the soil dynamics (2c) simplifies to  $dS_A/dt = S_A(1 - S_A)$ . The right-hand side is positive for  $0 < S_A < 1$ , which implies that  $S_A$  converges to its maximal value  $S_A = 1$ , while  $S_B$  converges to zero. Hence in a monoculture of plant A the soil community associated with plant A completely eliminates the soil community associated with plant B. As a consequence, in the long term plant A attains an equilibrium density that is given by the carrying capacity  $N_A = \kappa_A(1) = K_A(1 + \alpha_A)$  that is characteristic for a soil where plant A's soil biota is dominant.

There is another monoculture equilibrium of plant A, corresponding to the situation that plant A's soil biota is absent ( $S_A = 0$ ), which is given by  $N_A = \kappa_A(0) = K_A(1 + \beta_A)$ . This equilibrium is unstable, since the smallest deviation of  $S_A$  from zero will induce convergence to  $S_A = 1$ . Accordingly, we call the two monoculture equilibria of plant A *soil-stable* and *soil-unstable*, respectively. Obviously, the same arguments apply, *mutatis mutandis*, to monocultures of plant species B. It will turn out that the concept of *soil stability* is also useful for judging the stability of community equilibria that happen to be associated with an 'extreme' soil fauna (i.e.,  $S_A = 0$  or  $S_A = 1$ ). For later reference, we give the four monoculture equilibria a name and summarize our findings as follows:

$$\mathbf{A_A}: S_A = 1, N_A = K_A(1 + \alpha_A) \quad (\text{soil-stable monoculture A}) \quad (4a)$$

$$\mathbf{A_B}: S_A = 0, N_A = K_A(1 + \beta_A) \quad (\text{soil-unstable monoculture A}) \quad (4b)$$

$$\mathbf{B_A}: S_A = 1, N_B = K_B(1 + \alpha_B) \quad (\text{soil-unstable monoculture B}) \quad (4c)$$

$$\mathbf{B_B}: S_A = 0, N_B = K_B(1 + \beta_B) \quad (\text{soil-stable monoculture B}) \quad (4d)$$

For the rest of this paper we assume  $\alpha_A, \beta_A, \alpha_B, \beta_B > -1$ , since the four monoculture equilibria only exist if this condition is satisfied.

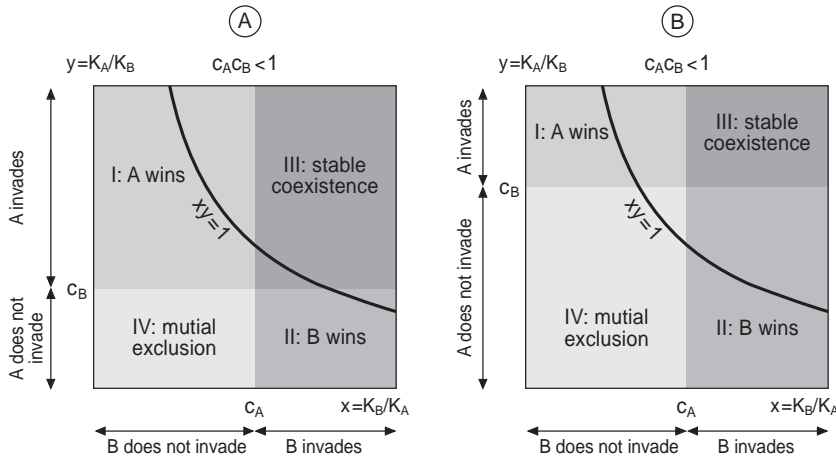
### 3.3.2 Effect of a static soil community on plant competition

Assume for the moment that the density of soil organisms remains at a constant level  $S_A$ . Then the dynamics of the plant species is fully described by the Lotka-Volterra competition model (2a, 2b), where the carrying capacities  $\kappa_A$  and  $\kappa_B$  depend on the static level  $S_A$ . It is a standard result from Lotka-Volterra theory (e.g. Case 2000) that there are four different scenarios for the dynamics and outcome of competition. These scenarios can be characterized on the basis of the stability properties of the two monoculture equilibria ( $N_A = \kappa_A, N_B = 0$ ) and ( $N_A = 0, N_B = \kappa_B$ ). The stability of such an equilibrium depends on whether or not the absent plant species can grow when rare. Take, for example, the monoculture of plant species A. In view of eqn (2b), plant species B has a positive growth rate whenever  $N_B + c_A N_A < \kappa_B$ . Inserting the monoculture densities  $N_A = \kappa_A$  and  $N_B = 0$  into this condition, we see that species B can invade the monoculture of species A if  $c_A \kappa_A < \kappa_B$ . Similarly, plant species A can invade a monoculture of plant species B, if  $c_B \kappa_B < \kappa_A$ . Equivalently, these stability conditions for monocultures can be phrased as:

$$c_A < \kappa_B / \kappa_A \quad \text{species B can invade monoculture of species A} \quad (5a)$$

$$c_B < \kappa_A / \kappa_B \quad \text{species A can invade monoculture of species B} \quad (5b)$$

Based on (5a) and (5b), the dynamics of competition can be classified as follows (see Figure 3.2):



**Figure 3.2** Outcomes of the Lotka-Volterra system (1a,1b). The plane is divided into four invasibility zones I, II, III, IV, such that B invades if  $x > c_A$  and A invades if  $y > c_B$ , where  $x \equiv \kappa_B/\kappa_A, y \equiv \kappa_A/\kappa_B$  are the carrying capacity ratios in (5). (a) If  $c_A c_B < 1$  stable equilibria exist at the intersection of the hyperbola  $xy = 1$  with zone III, in which A and B invade. (b) If  $c_A c_B > 1$  unstable equilibria exist at the intersection of  $xy = 1$  with zone IV, where neither A or B can invade, and depending on the initial conditions A or B wins the competition. Intersection at I or II do not result in equilibrium, instead A or B always wins, respectively.

- I.  $c_A > \kappa_B/\kappa_A$  and  $c_B < \kappa_A/\kappa_B$ : species A can invade species B, but B cannot invade A; A is competitively dominant over B and will in the long term completely displace B irrespective of the initial conditions.
- II.  $c_A < \kappa_B/\kappa_A$  and  $c_B > \kappa_A/\kappa_B$ : B can invade A, but A cannot invade B; B is dominant over A and will displace A irrespective of initial conditions.
- III.  $c_A < \kappa_B/\kappa_A$  and  $c_B < \kappa_A/\kappa_B$ : both monocultures can be invaded by the other species and are hence unstable; there exists a unique “interior” equilibrium (also called “coexistence equilibrium” or “community equilibrium”) that is globally stable; in the long run, both species will stably coexist at equilibrium.
- IV.  $c_A > \kappa_B/\kappa_A$  and  $c_A > \kappa_A/\kappa_B$ : both monoculture equilibria cannot be invaded by the other species and are therefore stable; there exists a unique interior equilibrium, but this equilibrium is unstable; in the long run, one plant species will displace the other, but the winner of the competition completely depends on the initial conditions.

Hence a community equilibrium does exist whenever  $c_A - \kappa_B/\kappa_A$  and  $c_B - \kappa_A/\kappa_B$  have the same sign. By multiplying the left- and right-hand sides of the inequalities defining scenarios III and IV, it is evident that a community equilibrium, if it exists, is stable whenever  $c_A c_B < 1$ , a condition that is often interpreted as “interspecific competition is on average weaker than intraspecific competition.” Summarizing:

$$\begin{aligned} \text{existence of community equilibrium: } (c_A - \kappa_B / \kappa_A)(c_B - \kappa_A / \kappa_B) &> 0 & (6a) \\ \text{stability of community equilibrium: } c_A c_B &< 1 & (6b) \end{aligned}$$

Figure 3.2 illustrates the conditions for equilibrium and stability in a plot where the co-ordinate axes correspond to the ratios of (soil dependent) carrying capacities:  $x = \kappa_B / \kappa_A$  and  $y = \kappa_A / \kappa_B$ . In view of (5), the relation between  $x$  and  $c_A$  determines whether B can invade the monoculture of A, while the relation between  $y$  and  $c_B$  determines whether A can invade the monoculture of B. The parameters  $x$  and  $y$  are not independent but constrained by  $xy = (\kappa_B / \kappa_A)(\kappa_A / \kappa_B) = 1$ . In other words, all parameter combinations describing a competitive plant system actually lie on the hyperbola  $xy = 1$  in the  $xy$  plane. If  $c_A c_B < 1$  (Fig 3.2A), this hyperbola intersects the coexistence region III (doubly hatched area), which means that stable coexistence is possible for certain values of  $\kappa_B / \kappa_A$ . But in case of  $c_A c_B > 1$  (Fig 3.2B), the hyperbola intersects the mutual exclusion region IV and stable coexistence is not possible at all.

To characterize stability with respect to the competition among the plant species A and B, we will henceforth use the term *competitive stability*, which we define as follows: a monoculture is competitively stable if it cannot be invaded by the other plant species and competitively unstable otherwise; a community equilibrium is competitively stable if  $c_A c_B < 1$  and competitively unstable if  $c_A c_B > 1$ .

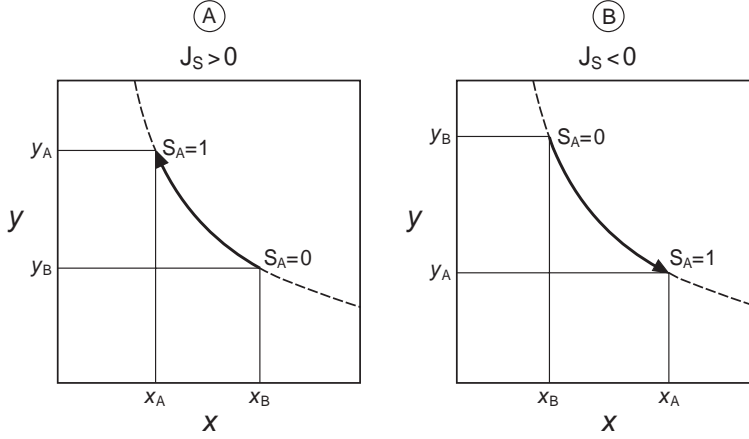
### 3.3.3 Positive and negative plant-soil feedback

For any fixed value of  $S_A$ , the corresponding competitive system corresponds to a single point on the hyperbola  $xy = 1$  in Figure 3.2. If we vary  $S_A$  from 0 to 1, we obtain all feasible competitive systems. Graphically, these systems correspond to a segment of the hyperbola  $xy = 1$ . This *feasibility arc* is illustrated in Figure 3.3, where also the direction of increasing  $S_A$  is indicated by an arrow. The two endpoints of the feasibility arc have the coordinates:

$$S_A = 1: x = x_A = \frac{\kappa_B(1)}{\kappa_A(1)} = \frac{K_B(1 + \alpha_A)}{K_A(1 + \alpha_B)}, \quad y = y_A = \frac{1}{x_A} = \frac{K_A(1 + \alpha_A)}{K_B(1 + \alpha_B)} \quad (7a)$$

$$S_A = 0: x = x_B = \frac{\kappa_B(0)}{\kappa_A(0)} = \frac{K_B(1 + \beta_B)}{K_A(1 + \beta_A)}, \quad y = y_B = \frac{1}{x_B} = \frac{K_A(1 + \beta_A)}{K_B(1 + \beta_B)} \quad (7b)$$

As indicated in Figure 3.3, when increasing from  $S_A = 0$  to  $S_A = 1$ , the system can move along the feasibility arc either upward and left (Fig 3.3A) or downward and right (Fig 3.3B). In the first case, large values of  $S_A$  correspond to that part of the feasibility arc that is closest to parameter regime I where plant species A is competitively dominant, while large values of  $S_B$  (i.e., small values of  $S_A$ ) shift the system to regime II where B is dominant. In other words, there is a *positive plant-soil feedback* in the sense that dominance of one of the two soil communities favors the



**Figure 3.3** Effects of soil feedbacks on the feasibility of equilibria. Since soil composition  $S_A$  is bounded by  $[0,1]$  the set of feasible Lotka-Volterra systems belong to a *feasibility arc* of the unit hyperbola  $xy = 1$ . (a) If  $\partial y / \partial S_A > 0$  (the arc points up) the net feedback is positive  $J_S > 0$ : plants attain maximum relative densities when their associated soil biota are dominant, i.e.  $\kappa_A / \kappa_B$  is maximum at  $S_A = 1$ , and  $\kappa_B / \kappa_A$  is maximum at  $S_A = 1$ . (B) If  $\partial y / \partial S_A < 0$  (the arc points down) net feedback is negative  $J_S < 0$ : plants attain minimum relative densities when their associated soil biota are dominant.

competitive dominance of the associated plant species. Similarly, we say that there is *negative plant-soil feedback* if the direction of the feasibility arc is downwards and to the right. In that case, dominance of a soil community favors the competitive dominance of the plant species *not* associated with the dominant soil community.

The importance of the sign of the feedback was pointed out by Bever (Bever et al. 1997, Bever 1999) who argued that positive feedback tends to favor competitive dominance and, hence, species-poor plant communities, while negative feedback tends to favor plant coexistence. To quantify plant-soil feedbacks, Bever introduced an *interaction coefficient*  $I_S$  that is defined by

$$I_S = \alpha_A + \beta_B - \alpha_B - \beta_A. \quad (8)$$

As we will show in a moment, there are more reliable indicators for the sign of the feedback. As revealed by the graphical analysis, the feedback is positive if the feasibility arc is directed upwards (as in Fig 3.3A). This means that  $y = \kappa_A / \kappa_B$  is increasing with  $S_A$  or, in other words, that the derivative of  $y$  with respect to  $S_A$  is positive. This derivative is given by  $d_y / dS_A = (K_A K_B / \kappa_B^2) J_S$ , where

$$J_S = (1 + \alpha_A)(1 + \beta_B) - (1 + \alpha_B)(1 + \beta_A) \quad (9)$$

Hence, the plant-soil feedback is positive if  $J_S > 0$  and it is negative if  $J_S < 0$ . In the

absence of plant-soil feedback ( $J_S = 0$ ), the feasibility arc collapses into a single point, and we are in the situation described in section 3.3.2.

There is a simple relationship between  $J_S$  and Bever's coefficient  $I_S$ :

$$J_S = I_S + \alpha_A\beta_B - \alpha_B\beta_A. \quad (10)$$

$J_S$  generalizes Bever's interaction coefficient  $I_S$  to systems without parameter symmetry (i.e.,  $\alpha_A\beta_B \neq \alpha_B\beta_A$ ). The difference between  $J_S$  and  $I_S$  is often small, but it is easy to construct examples where  $J_S$  and  $I_S$  differ in sign, that is, where Bever's coefficient  $I_S$  does not correctly indicate the sign of the feedback.

It will be useful to define a third interaction coefficient. Similar to Lotka-Volterra competition coefficients, the net feedback of soil community effects can be derived from the ratio between effects on the host plant (analogous to intraspecific competition) and cross-effects on the other plant (analogous to interspecific competition):

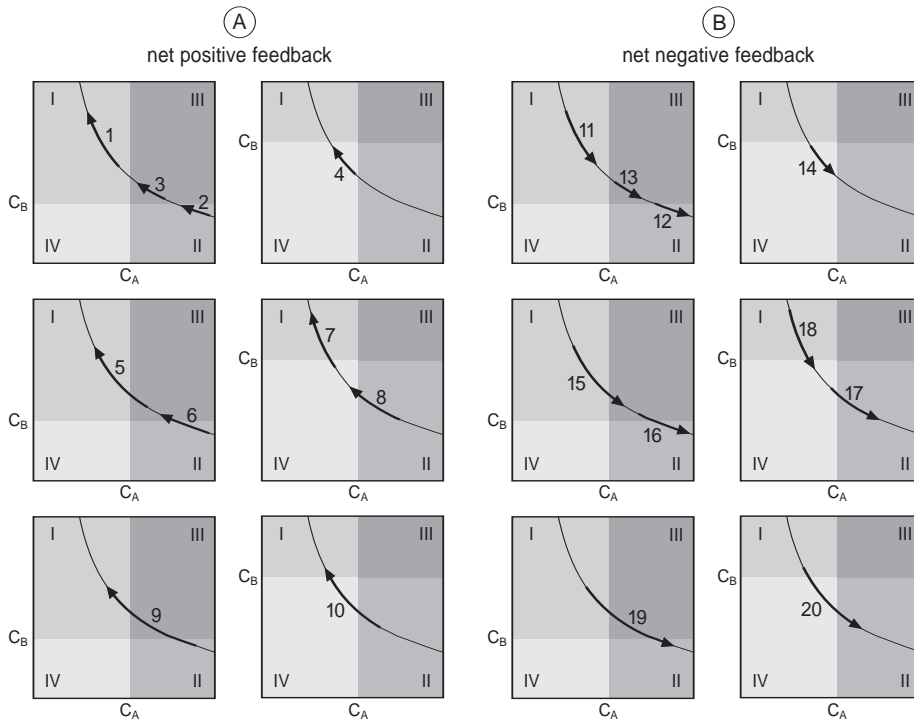
$$H_S = x_{BY}y_A = \frac{(1 + \alpha_A)(1 + \beta_B)}{(1 + \alpha_B)(1 + \beta_A)}. \quad (11)$$

In view of (9),  $H_S > 1$  implies that host-plant effects are more favorable than cross-effects, meaning that plant-soil feedback is positive ( $J_S > 0$ ).  $H_S < 1$  implies that cross-effects are more favorable than host plant effects, meaning that plant-soil feedback is negative. As we will show below, the net feedback effect in the system and the outcome of plant competition thus depend on the strength of interspecific and intraspecific effects of aboveground plant competition (eqn 6) and the strength of soil community effects (eqn 11).

### 3.3.4 Combining plant and soil dynamics

Combining the plots in Figures 3.2 and 3.3 provides us with a graphical method that is often sufficient for a complete characterization of the dynamics of the coupled plant-soil community described by equation (2). (This method is in the same spirit as the "recovery plane" analysis of Eppinga et al. (2006), in which  $\kappa_B/\kappa_A - c_A$  and  $\kappa_A/\kappa_B - c_B$  are plotted against each other.) As shown in Figure 3.4, there are 20 different ways in which the feasibility arc can intersect the four parameter domains corresponding to the plant competition scenarios I to IV, which are characterized by the monoculture invasion criteria (5). Here we will discuss two special cases (Fig 3.5). Some interesting but more complex cases will be highlighted in Section 3.4. A complete overview of all 20 scenarios is given in the Appendix.

Figure 3.5A depicts case 12, where the feasibility arc lies completely within region II where only species B can invade. We can therefore conclude that irrespective of the state of the soil only plant species B can grow in this scenario. Thus the system converges to the monoculture equilibrium  $\mathbf{B_B}$  in which  $S_A = 0$ . In fact, in all



**Figure 3.4** Intersection of the feasibility arc of Fig 3.3 with the invasion zones of Fig 3.2. The arc is represented as an arrow, thus indicating its orientation: the “head” ( $x = x_A$ ,  $y = y_A$ ) corresponds to  $S_A = 1$  and the “tail” ( $x = x_B$ ,  $y = y_B$ ) to  $S_A = 0$ . A monoculture of species A will follow the orientation of the arc ( $S_A$  increases), whereas a monoculture of plant B will move in the opposite sense ( $S_A$  increases). There are 20 intersection “cases”, differing in the relative position and arc orientation with respect to the invasion zones. The location of the end points (head & tail) with respect to the invasion zones determines if a species can invade or not.

cases where the feasibility arc lies within regions I or II (cases 1, 2, 11 and 12) one of the plants will always win, irrespective of the initial conditions. When the arc lies in region III (cases 3 and 13), each plant species can invade the monoculture of the other species, implying that the two species will stably coexist at equilibrium. When the arc lies within region IV (cases 4 and 14) neither plant species can invade when rare. Both plant monocultures are stable, and the winner depends on the initial conditions (founder control; (founder control; Bolker et al. 2003). Summarizing, we can conclude that in the eight scenarios where the feasibility arc lies completely inside one of the four competition regions the qualitative outcome of plant competition does not depend on the sign of the plant-soil feedback.

Figure 3.5B depicts case 15, which is more complex because the feasibility arcs spans two invasion zones. However, the analysis is still straightforward. A monoculture of plant B can never be stable, since the whole arc lies in the parameter

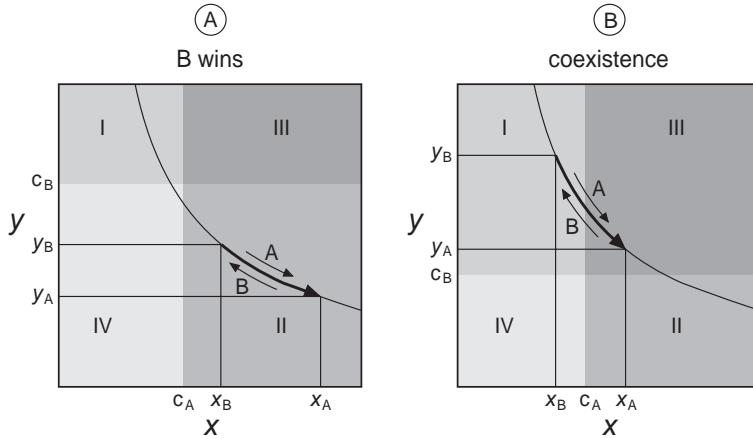


region where A can invade such a monoculture. In parameter regime I, a monoculture of plant A is to be expected in the absence of plant-soil feedback. However, as long as plant A is in monoculture, the associated soil community  $S_A$  will increase. Hence the system will be shifted along the feasibility arc in the direction of the arrow, until the plant coexistence regime III is reached. We conclude that in this case the (negative) plant-soil feedback enables coexistence of the two plant species.

As indicated by Figure 3.5, the position of the end points of the feasibility arc with respect to the invasion zones tells us whether a plant monoculture can be invaded or not. For a monoculture of plant A, the "head" of the feasibility arc ( $S_A = 1$ ) is relevant, which corresponds to the equilibrium state  $\mathbf{A}_A$ . This state can be invaded by plant B if and only if  $c_A < x_A$ . By symmetry, a monoculture of plant B corresponds to the state  $\mathbf{B}_B$ , which is located at the "tail" of the feasibility arc ( $S_A = 0$ ). This state can be invaded by plant A, if and only if  $c_B < y_B$ . Multiplying these two inequalities and noticing that  $x_A y_B = (x_B y_A)^{-1} = H_S^{-1}$  we obtain a necessary condition for mutual invasion of the two monoculture equilibria:

$$c_A c_B < H_S^{-1}. \quad (12)$$

In the absence of plant-soil feedback ( $J_S = 0$  or  $H_S = 1$ ) this coincides with condition (6b) for the stable coexistence of the two plant species in the Lotka-Volterra competition model. In line with the predictions of Bever (e.g. Bever et al. 1997, Bever



**Figure 3.5** Graphical analysis of two cases depicted in Fig 3.4. (A) In case 12 the feasibility arc occurs in a zone where only species B can invade (II) and a coexistence equilibrium is not possible, thus species B always wins. (B) In case 15 the monoculture of species A would evolve towards the  $S_A = 1$  end of the arc, which lies in a zone where species B can invade (III). The monoculture of B will instead evolve towards the  $S_A = 0$ , located in the zone where species A can invade (I). As a consequence both can grow when rare and coexist.

1999), inequality (12) leads to the conclusion that the conditions for plant coexistence are more stringent in the case of a positive feedback (where  $H_S^{-1} < 1$ ), while they are more relaxed in the case of a negative plant-soil feedback.

It is important to realize that, in contrast to standard Lotka-Volterra theory, monoculture invasion conditions as those considered above do no longer provide a complete picture of the outcome of plant competition. For example, we will demonstrate below that the two plant species can stably coexist even in cases where condition (12) for the mutual invasibility of the two plant monocultures is not satisfied.

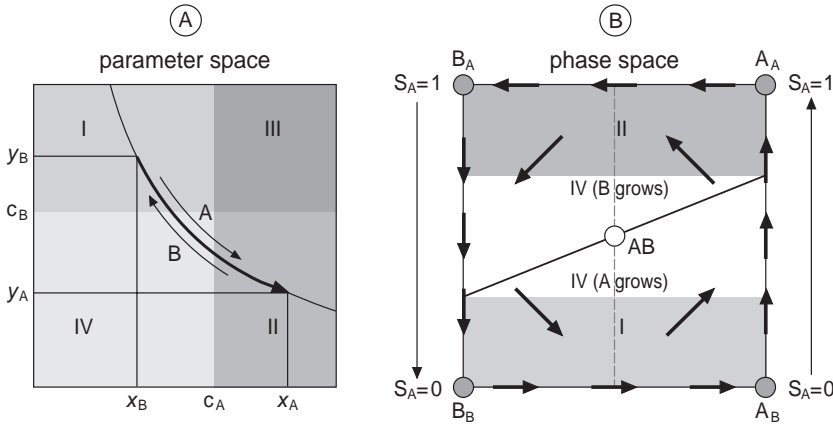
### 3.4. Implications for plant coexistence

In this section we employ the graphical method presented in section 3.3 to address some questions addressed in the literature on the implications of plant-soil interaction. First, we study the mechanism by which competitive oscillations emerge in the Bever model. Second, we discuss the consequences of competitive oscillations for the maintenance of plant diversity. And third, we critically examine the generality of results obtained by means of invasion analysis and our graphical methodology.

#### 3.4.1 When do plant-soil feedbacks drive community oscillations?

Perhaps one of the most interesting results in Bever (2003) is a numerical example showing that plant species that cannot coexist in the absence of feedback, can do so by means of competitive oscillations induced by negative plant-soil feedbacks. When running the same example, we did not find oscillations but instead convergence to a coexistence equilibrium. This is confirmed by a mathematical stability analysis (Revilla 2009). Most probably, Bever's Figure 3.4(C,D) is a numerical artifact due to the use of inappropriate integration methods. We therefore have to investigate anew whether and under what conditions oscillations arise in the Bever model.

Population cycles are frequently associated with predator-prey dynamics, but they are also a common feature in Lotka-Volterra competition models (Gilpin 1975, May and Leonard 1975) and in resource competition models (Huisman and Weissing 2001, Revilla and Weissing 2008). In these models, oscillations require at least three competitors such that species R outcompetes species S, S outcompetes P and P outcompetes R, as in the Rock-Scissors-Paper game. Mathematically, the monocultures of R, P and S are connected by means of heteroclinic orbits, i.e. a sequence of paths  $R \rightarrow P \rightarrow S \rightarrow R$  that forms a cycle. Although the Bever model involves only two plant competitors, it has in fact four monoculture states ( $\mathbf{A}_A, \mathbf{A}_B, \mathbf{B}_B, \mathbf{B}_A$ ). This allows us to construct a heteroclinic cycle as follows.



**Figure 3.6** Competitive oscillations. (A) The monoculture of species A evolves from a non-invadable (I) to an invadable (II) condition, and the monoculture of B from non-invadable (II) to invadable (I); in both directions, the system passes through the zone of unstable community equilibria (IV). (B) The parameter space is mapped into a phase space where circles represent equilibria (white: unstable, gray: saddle) at the corresponding invasion zones (this mapping is explained in the Appendix). The diagonal line represents the A-B nullcline (species A grows towards the right B grows towards the left), and the dashed line is the soil nullcline ( $S_A$  increases in the right, and decreases in the left); their intersection corresponds to the internal equilibrium predicted in (A). Monocultures are competitively or soil stable, but not both: they are saddle points forming a heteroclinic cycle around the internal equilibrium AB which is competitively unstable. The system oscillates permanently.

Consider the configuration corresponding to cases 19 or 20 in Figure 3.4, or to be more specific case 20 as in Figure 3.6A. From the positioning of the end points of the feasibility arc in competition regimes I and II we can conclude that the following inequalities are satisfied:

$$x_B < c_A < x_A \text{ and } y_A < c_B < y_B. \quad (13)$$

As discussed above, the inequalities  $c_A < x_A$  and  $c_B < y_B$  imply that both plant monocultures can be invaded when their associated soil biota are dominant. In other words,  $A_A$  and  $B_B$  are unstable against invasion. The inequalities  $x_B < c_A$  and  $y_A < c_B$  imply that the two monocultures cannot be invaded when the invader's soil biota is dominant. In other words,  $A_A$  and  $B_B$  are stable against invasion. However, as shown in Section 3.3.1, these two equilibria are not soil stable. Figure 3.6B illustrates the potential implications of this configuration:

1.  $A_A$  is competitively unstable: B invades and the system moves towards  $B_A$
2.  $B_A$  is soil unstable: the system moves towards  $B_B$  (where  $S_A = 0$ )
3.  $B_B$  is competitively unstable: A invades and the system moves towards  $A_B$
4.  $A_B$  is soil unstable: the system moves towards  $A_A$  (where  $S_A = 1$ )

Hence one might expect the cyclic sequence  $\mathbf{A}_A \rightarrow \mathbf{B}_A \rightarrow \mathbf{B}_B \rightarrow \mathbf{A}_B \rightarrow \mathbf{A}_A$  of successions or at least oscillations following this sequence.

This is confirmed by Figure 3.7 which shows the time course of the system under the above scenario. For Bever's parameterization (Fig 3.7A), the system exhibits damped oscillations that converge to a coexistence equilibrium. By increasing the intensity of competition,  $c_{ACB}$ , this equilibrium becomes unstable and gives rise to a limit cycle (Fig.7B). With a further increase in competition intensity the cycle becomes heteroclinic, i.e. oscillations attain very low minima and maxima for increasingly longer times (Fig 3.7C).

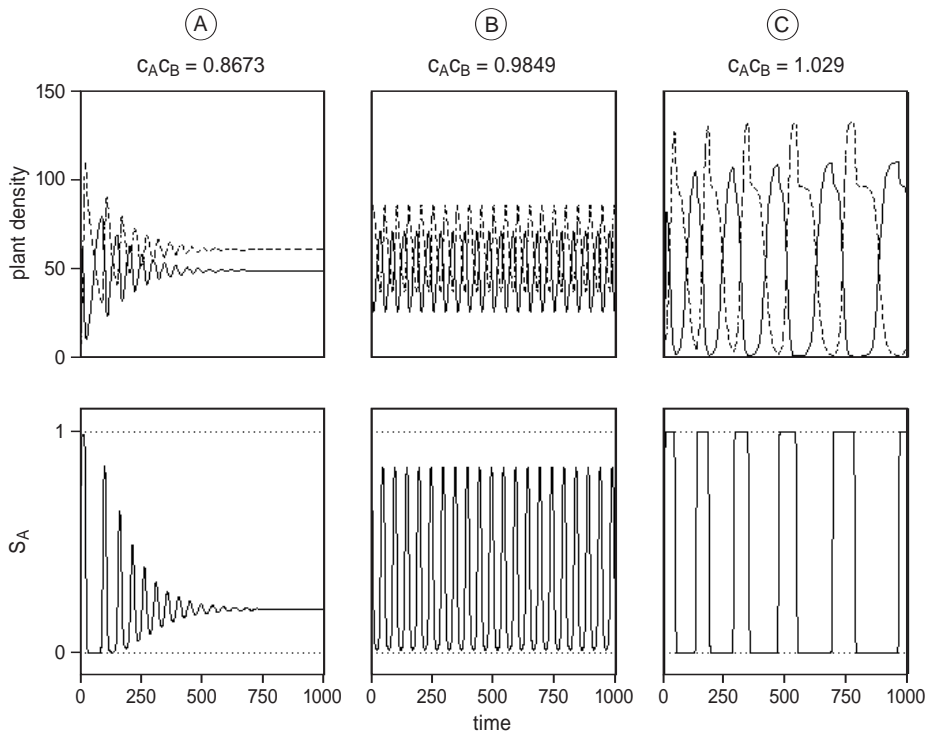
In Figure 3.7C,  $c_{ACB} > 1$ . As a consequence, the parameter configuration corresponds to case 20 in Figure 3.4, where the feasibility arc intersects parameter region IV for mutual competitive exclusion (see also Fig 3.6A). In this region, there exists a coexistence equilibrium, but this equilibrium is unstable. As a consequence, the above considerations concerning the monoculture equilibria tell the whole story, and the system converges to the heteroclinic cycle  $\mathbf{A}_A \rightarrow \mathbf{B}_A \rightarrow \mathbf{B}_B \rightarrow \mathbf{A}_B \rightarrow \mathbf{A}_A$  (see Fig 3.6B).

In Figures 7A and 7B,  $c_{ACB} < 1$  and the parameter configuration corresponds to case 19 in Figure 3.4. Now the feasibility arc intersects parameter region III for stable competitive coexistence. In the case of pure plant competition, there exists a stable coexistence equilibrium, but this equilibrium can be destabilized due to the addition of soil dynamics. This is indeed the case if the competition intensity  $c_{ACB}$  is just below 1 (as in Fig 3.7B). In fact, it can be shown analytically (Revilla 2009) that stable equilibrium coexistence (as in Fig 3.7A) is only possible if  $c_{ACB} < 1 - \delta$ , where is a positive quantity that can be calculated from the remaining system parameters.

Notice that the conditions (13) imply that  $H_S = x_{BYA} < x_{AYB} = H_S^{-1}$ , which is only possible for  $H_S < 1$  or, equivalently,  $J_S < 0$ . As a consequence, the above scenario for the development of oscillations can only occur in case of negative plant-soil feedback. Since the analytical results of Revilla (2009) suggest that this is the only route to oscillations in the Bever model, we conclude that competitive oscillations will only occur if two requirements are met: (a) the plant-soil feedback is negative; (b) the two soil-stable monoculture equilibria are mutually invadable.

### 3.4.2 Does negative soil feedback enhance coexistence?

An important conclusion of (Bever 2003) is that net negative feedbacks enhance coexistence and promote high diversity, while net positive feedbacks lead to exclusion and low diversity. This can be understood graphically as follows. In a monoculture each species evolves towards its soil stable state, which is  $\mathbf{A}_A$  for plant A (located at the "head" of the feasibility arc) and  $\mathbf{B}_B$  for plant B (located at the "tail" of the arc). Coexistence between plant species A and B will be favored when each plant species can invade in the monoculture of the other species, that is when each species is able to grow when rare. Species A can invade a monoculture of species B



**Figure 3.7** Competitive oscillations due to a cyclic succession of competitive instability and soil instability. The top row shows the densities of plant A (solid line) and B (dashed line), respectively; the bottom row depicts the density of the soil biota  $S_A$ . The parameters in (a) are as in Bever's (2003) Figure 3.4(c,d) (see below). From left to right, the competition coefficient  $c_A$  is increased from  $c_A = 0.885$  in (A) via  $c_A = 1.005$  in (B), to  $c_A = 1.050$  in (C), while  $c_B = 0.980$  in all three cases. With increasing intensity of competition, the asymptotic behavior of the system changes from convergence to a stable coexistence equilibrium in (A), via convergence to a limit cycle in (B) to convergence to a heteroclinic orbit in (C). In view of  $K_A = 100$ ,  $K_B = 120$ ,  $\alpha_A = \beta_A = 0.10$ ,  $\beta_B = -0.20$ , the soil-plant feedback is negative ( $J_S = -0.434$ ). Because of  $x_A \approx 1.36$ ,  $y_A \approx 0.73$ ,  $x_B \approx 0.87$ ,  $y_A \approx 1.15$ , the conditions (13) for the cyclical succession  $A_A \rightarrow B_A \rightarrow B_B \rightarrow A_B \rightarrow A_A$  are satisfied. In (A) and (B),  $c_A c_B < 1$ , corresponding to case 19 in Figure 3.4. In (C),  $c_A c_B > 1$ , corresponding to case 20. Remaining parameters:  $r_A = 0.7$ ,  $r_B = 0.5$ ,  $\nu = 0.8$ .

when  $B_B$  (the tail of the arc) is in zones I or III of Figure 3.2. Species B can invade a monoculture of species A when  $A_A$  (the head of the arc) is in zones II or III. Inspection of Figure 3.4 reveals that there are many scenarios of mutual invasion when the net feedback is negative (Fig 3.4 scenarios 11, 12, 13, 15, 16, 19, 20), compared to when it is positive (Fig 3.4 scenarios 1, 2, 3). In fact, if we exclude the scenarios where feedback does not determine invasion (1, 2, 3, 11, 12, 13) we can see that only negative feedback allows mutual invasion. This supports the conclusion (Bever 2003) that negative plant-soil feedback may favour the coexistence of plant species.

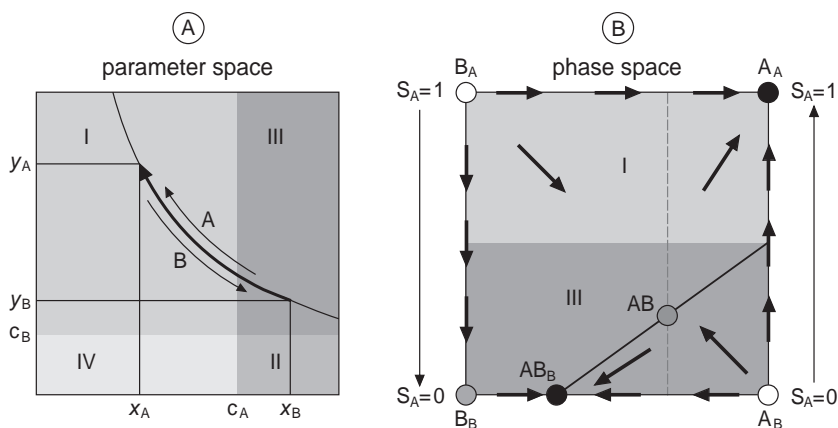
However, this conclusion needs some qualification, since in case of negative feedback mutual invasibility of the plant monocultures does not necessarily lead to equilibrium coexistence. Instead, oscillations can occur. If these oscillations take the form of a limit cycle, the two plant species will still stably persist, be it not in equilibrium. But the oscillations can also take the form of a heteroclinic cycle, where the populations are repeatedly driven to very low densities. In the mathematical model, they still survive, but in the real world extinction would occur. Thus, in case of a heteroclinic cycle mutual invasibility of the two monocultures will not result in long-term coexistence.

To assess the relative importance of limit cycles versus heteroclinic cycles, we investigated the pattern in Figure 3.7 numerically. Using continuation-bifurcation analysis software (Ermentrout 2002) we found that as  $c_{ACB}$  increases, regular oscillations start at  $c_{ACB} = 0.977$  (Hopf bifurcation). Already at  $c_{ACB} = 0.998$  these oscillations turn into a heteroclinic orbit. Oscillations persist until  $c_{ACB} = 1.559$  which is the point at which the requirement for mutual invasion (12) does not hold anymore (the chain of heteroclinic orbits connecting the four monocultures is broken). Thus, while oscillations occur for  $0.977 < c_{ACB} < 1.559$ , a limit cycle does only occur for the much smaller range  $0.977 < c_{ACB} < 0.998$ . About 96.4% of the parameter range for oscillations correspond to the occurrence of heteroclinic cycles, and, hence, eventual extinction. Accordingly, negative feedback has a much smaller potential for the facilitation of plant coexistence than Bever's analysis seems to suggest. One should notice, however, that in a spatial context, the risk of extinction via the large-amplitude oscillations associated with a heteroclinic cycle may be counteracted by the repeated re-immigration of the extinct species. Under such conditions, also a heteroclinic cycle can allow the regional coexistence of the plant species.

### 3.4.3 Invasion requirements and coexistence requirements

When properly used, invasion criteria are very useful to predict the range of dynamics that a dynamical system can possibly display. In the present context, we can list all possible dynamics of Bever's model (Table 3.1) by just considering whether  $\mathbf{A}_A$  and  $\mathbf{B}_B$  can be invaded or not (i.e. whether  $c_A < x_A$ ,  $c_B < y_B$  hold or not), and the direction of the feedback (the sign of  $J_S$ ).

However, as illustrated by the following example an invasion analysis can also have its limitations. Consider the scenario in Figure 3.8a (case 5) where the feedback is positive ( $J_S > 0$ ) and where  $c_A < x_A$  and  $c_B < y_B$ . In this case, criterion (12) for mutual invasibility does not hold: while plant species A is able to invade B (since  $c_B < y_B$ ), plant species B cannot invade A (since  $c_A < x_A$  does not hold). However, coexistence may still be possible. As indicated in Figure 3.8A, the "tail" of the feasibility arc ( $S_A > 0$ ) lies in zone III, which means that for  $S_A > 0$  there is a competitively stable community equilibrium. Following our previous notation, this equilibrium will be denoted by  $\mathbf{AB}_B$  since the soil is dominated by B's soil biota, as



**Figure 3.8** Alternative stable states (case 5 of Fig 3.4). (A) In this configuration a monoculture of species A evolves from an invadable (III) to a non-invadable zone (I), whereas a monoculture of B can be invaded always: coexistence by means of mutual invasion is ruled out. However, there are coexistence equilibria because the feasibility arc intersects the zone of mutual invasion (III). (B) The phase space shows the equilibria (white: unstable, black: stable, gray: saddle) and the invasion zones. The diagonal line representing the A-B nullcline (both species grow towards it) intersects  $S_A = 0$  giving rise to the coexistence equilibrium  $AB_B$  predicted in part (A). If the vertical dashed line representing the soil-nullcline ( $S_A$  increases to the right, and decreases to the left) intersects the A-B nullcline there will be an internal equilibrium  $AB$  that is a saddle point; and depending on the initial conditions the system moves towards the monoculture of  $A_A$  dominated by A's soil biota, or towards the coexistence equilibrium  $AB_B$  dominated by B's soil biota.

**Table 3.1** Outcomes of plant community composition under different scenarios of plant invasion and net plant-soil feedback.

	Net positive feedback $J_S > 0$	Net negative feedback $J_S < 0$
Both plants invade $c_A < x_A, c_B < y_B$	Stable coexistence with $S_A = 1$ Stable coexistence with $S_A = 0$ Stable coexistence with $S_A = 0$ or 1 depending on initial conditions	Stable coexistence with $0 < S_A < 1$ Competitive oscillations, limit cycles or heteroclinic cycles
A invades $c_B < y_B$ , B does not $c_A > x_A$	A wins or stable coexistence, depending on initial conditions	A wins
B invades $c_A < x_A$ A does not $c_B > y_B$	B wins or stable coexistence, depending on initial conditions	B wins
None invade $c_A > x_A, c_B > y_B$	Winner depends on the initial conditions	Winner depends on the initial conditions

seen in Figure 3.8B. This equilibrium must be also soil stable in order to allow plant coexistence, in other words  $S_A$  must be kept at zero around the equilibrium  $\mathbf{AB}_B$ . This can only happen if the influence of plant B on its soil community relative to that of A on its soil community ( $v$ ) is high enough, such that  $dS_A / dt < 0$  (to be more explicit, the ratio of plant densities in the equilibrium must be  $N_A / N_B < v$ ). Thus,  $\mathbf{AB}_B$  becomes a local attractor because it is competitively stable and soil stable. The other end point of the arc ( $S_A = 1$ ), i.e. the “head” lies in zone I, where a coexistence equilibrium is not possible and plant A attains a competitively stable and soil stable monoculture  $\mathbf{A}_A$ . This is another local attractor. As a consequence, the system has two alternative stable states: the coexistence equilibrium  $\mathbf{AB}_B$  dominated by B’s soil community and the soil-stable monoculture  $\mathbf{A}_A$  dominated by A’s soil community, as shown in Figure 3.8B.

In contrast, if at  $\mathbf{AB}_B$   $nu$  is small (the density ratios are  $N_A / N_B > v$ ) then  $\mathbf{AB}_B$  is soil unstable, while  $\mathbf{A}_A$  remains stable in both senses (soil and competitively). In this case A always wins, as predicted by the invasion criterion.

This example illustrates two things. The first is a limitation in our graphical analysis, which cannot address soil stability in all the cases, like it does with respect to competitive stability (in other words we cannot infer the magnitude of  $v$  in the graphs). Second, it shows the limitations of invasion analysis. Invasion analysis is a powerful technique for the analysis of dynamical systems in ecology and evolution (Case 2000), perhaps the best example is the classical Lotka-Volterra model where mutual invasion and stable coexistence are synonymous. But if the model being considered displays multiple equilibria and alternative stable states, as is the present case, it is erroneous to state that mutual invasion is a requisite for coexistence.

### 3.5. Discussion

In this paper we performed a more complete analysis of the Bever model (Bever 2003) to get more insight in plant species coexistence and dynamics and the potential role of plant-soil feedback affecting those. We found that plant species coexistence could be explained by an interaction between net soil feedback ( $J_S$ ) and competition strength ( $c_A c_B$ ) between the plants, i.e., when feedback becomes more positive competition coefficients have to be lower in order to allow plant coexistence. Thus more negative plant-soil feedback results in higher chances for coexistence. However, our analysis showed that negative plant-soil feedback also extended the range of plant coexistence by means of oscillations, in particular for more competitive plant species. Therefore, we conclude that negative plant-soil feedback has the potential to enhance plant coexistence and hence plant community diversity, but for more competitive plant species it may also enhance stochastically driven extinction of one of the plant species.



Our main conclusions, that plant species coexistence is dependent on the interplay between net plant-soil feedback and competition strength and that negative soil feedback can enhance plant coexistence via oscillations, agree with Bever's main conclusions (Bever 2003), however, some of our results disagreed with Bever's findings. First we found that Bever's soil interaction coefficient  $I_S$  does not predict net plant-soil feedback effects right in all cases. We propose to use the soil feedback interaction coefficient  $J_S$ . Second, we found that under positive feedbacks, in general associated with species exclusion and loss of plant diversity, plant species are able to coexist if competition coefficients are low enough. Therefore, we conclude that mutual invasion is not a necessary requirement for coexistence, yet it does increase chances a lot. Third, we found a different range of parameters under which plant-soil interactions led to sustained oscillations in plant abundances. Although most of the insights gained from the Bever model and our new analysis coincide, we still think we have to be careful translating these findings to the real world. To single out the parameters that are crucial for a better understanding of plant-soil interactions and their consequences for community dynamics and diversity, it would be useful to develop a more mechanistic model.

Positive plant-soil feedbacks may enhance the dominance of particular plant species. For example, it has been shown that the presence of arbuscular mycorrhizal (AM) fungi may enhance the abundance of the dominant plant species, thereby reducing plant diversity (Hartnett and Wilson 1999). Moreover, exotic plant species that escaped from species-specific soil pathogens while still encountering mutualistic root symbionts may be favored by positive plant-soil feedbacks in their new range (Klironomos 2002, Callaway et al. 2004) and as a result have the potential become successful invaders. Although positive plant-soil feedbacks are generally not considered to be important for coexistence of competing plant species at the local scale (Bever 2003), several studies have shown that the presence of AM fungi may enhance plant diversity in grasslands (Grime et al. 1987, van der Heijden et al. 1998b) by promoting seedling establishment and enhancing competitive ability of subordinate plant species (Grime et al. 1987, van der Heijden et al. 2008).

Negative plant-soil feedback has been suggested as one of the mechanisms to explain plant species coexistence and consequently to maintain plant community diversity (Bever et al. 1997, van der Heijden et al. 2008). Results of different recent empirical studies, as well as a meta-analysis of more than 300 plant-soil feedback experiments, indeed provide evidence for this hypothesis (Bever 1994, Mills and Bever 1998, De Deyn et al. 2003, Kulmatiski et al. 2008, Bradley et al. 2008). Results from the Bever-model show that negative plant-soil feedback enhanced plant coexistence by increasing the range in which oscillations between the two plant species occurred. That means that plant coexistence was possible under conditions that would otherwise lead to competitive exclusion of one of the two plant species, but also oscillations occurred in situations that would otherwise lead to stable coexis-

tence of the two plant species. We are not aware of any empirical study directly testing the effect of negative plant-soil feedback on plant oscillations. However, there are suggestions that soil-borne pathogens may play an important role determining plant community dynamics (Olf et al. 2000), also during succession (van der Putten et al. 1993, De Deyn et al. 2003). Interestingly, our model suggests that a potential role of soil community effects on plant oscillation could be identified relatively easily with plant-soil feedback experiments (e.g. van der Putten et al. 1993, Bever 1994). More specifically, a necessary requirement for soil community-driven oscillations is that both plant species lose competition when growing in soil occupied by their own soil community.

As illustrated in the above example, the complete set of possible scenarios presented in this study can be used to formulate hypotheses about the way soil community effects drive observed plant competition dynamics. We have to be careful however to translate the findings of the Bever model to natural systems, because this model expresses soil community dynamics in a highly simplified way, i.e., the soil community is always saturated in the Bever model. There will be a need for more mechanistic models that describe soil community dynamics more specifically and can better evaluate the consequences of plant-soil feedback effects for plant and soil community dynamics. Linking results of such models with plant-soil feedback experiments will provide a promising approach for future empirical research.

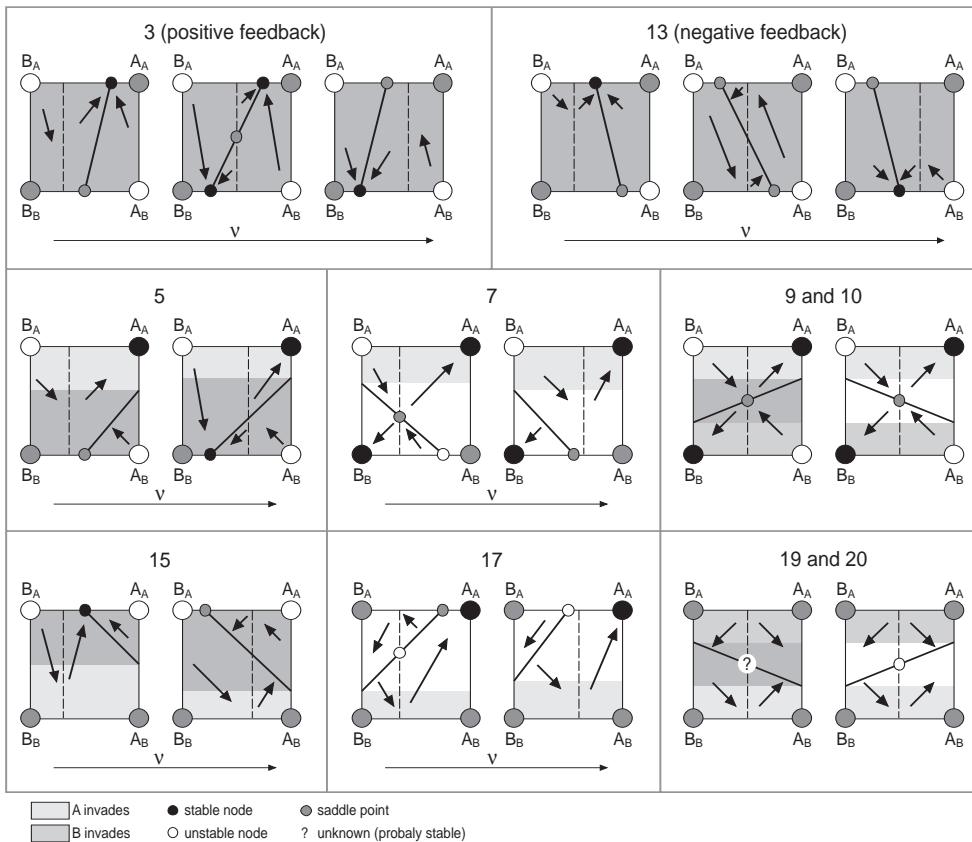
### **Acknowledgements**

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## Appendix: Graphical analysis

Figure 3.4 is useful for classifying and describing the main features of the dynamics in terms of the invasion conditions. For example in cases 1, 2, 11 and 12 it is easy to see that only one species A or B always wins, because only one of them is able to grow for any soil composition. In cases 4 and 14 any resident species will be protected against invasion, and any equilibrium that may exist will be competitively unstable, for any soil composition.

However, cases 3, 5-10, 13, 15-20 are more complicated. In these situations, it is sometimes useful to have a graphical representation in the familiar form of a phase space and nullclines. Although this is possible for a three-dimensional system like the Bever model, the following two-dimensional representation is more convenient:



The phase space is constructed according to the following rules:

- The horizontal axis indicates the plant composition: plant A is dominant on the right, plant B on the left. The vertical axis indicates soil composition: A's soil biota dominates on the top, B's soil biota dominates on the bottom.
- The corners represent the plant monocultures, with  $\mathbf{A_B, B_B}$  corresponding to  $S_A = 0$ , and  $\mathbf{A_A, B_A}$  corresponding to  $S_A = 1$ . Depending on the competitive stability conditions (7, 8, 11, 12) and their soil stability, the corners are classified as stable, unstable or saddle points.
- The invasion zones intersected by the feasibility arc become the domains of attraction in the phase plot, placed in the same order as they are encountered by traversing the arc from  $S_A = 0$  to 1 (and using the same fill patterns).
- If the arc intersects zones III or IV the corresponding domain of attraction in the phase plot is divided by a diagonal line. This line, representing coexistence equilibria, is the *plant nullcline*, i.e. a nullcline for the plant composition, not the plant densities. In case of intersecting zone III, the plant composition moves towards the line (communities are competitively stable). In case of intersection with zone IV, the plant composition moves away from the line (communities are competitively unstable).
- The plane is divided by vertical that represents the *non trivial soil nullcline*:  $S_A$  increases at the right of the line ( $N_A > vN_B$  in eq. 2), and decreases at the left ( $N_A < vN_B$  in eq. 2). The smaller the  $v$  the bigger the portion of the plane where  $S_A$  increases, and vice versa. The top  $S_A = 1$  and the bottom  $S_A = 0$  sides of the plane are *trivial soil nullclines*.
- A coexistence equilibrium corresponds to the intersection of the plant nullcline with a soil nullcline, trivial or not. For this reason, there can be border equilibria where  $S_A = 0, S_A = 1$  or an internal equilibrium where  $S_A$  is intermediate. Depending on its location with respect to the attraction domains and the non trivial plant nullcline, an equilibrium is declared stable, unstable or a saddle point.

Because of symmetry, we do not show cases 6, 8, 16 and 18 because they are qualitatively equivalent to cases 5, 7, 17 and 18 (by swapping the "A" and "B" labels). Cases 3 and 13 are very similar in the stability of their monocultures, and because of having border equilibria. However, they display qualitatively different dynamics. Under net positive feedbacks (case 3) the system can display alternative stable states: coexistence with dominance of plant A and its soil community or coexistence with dominance by plant B and its soil community. On the other hand, under net negative feedbacks (case 13) there cannot be alternative stable states, and oscillations may develop (though we suspect they dampen out given the geometry of the nullclines).

The majority of cases under net positive feedback result in competitive exclusion. However, some can display alternative stable states, and coexistence

depending on the initial conditions (5 and 6). On the other hand, the majority of scenarios under net negative feedback promote mutual invasion and coexistence (17 and 18 are the exceptions), including coexistence through oscillations (19 and 20).

A black and white photograph of a greenhouse filled with rows of potted plants, likely seedlings, with a wooden bench in the background.

# *Chapter four*

# 4

Large vertebrate herbivores overrule  
negative plant-soil feedback effects and  
stabilize spatiotemporal composition  
of plant communities

G.F. (Ciska) Veen, Saskia de Vries, E.S. (Liesbeth) Bakker,  
Wim H. van der Putten, Han Olff

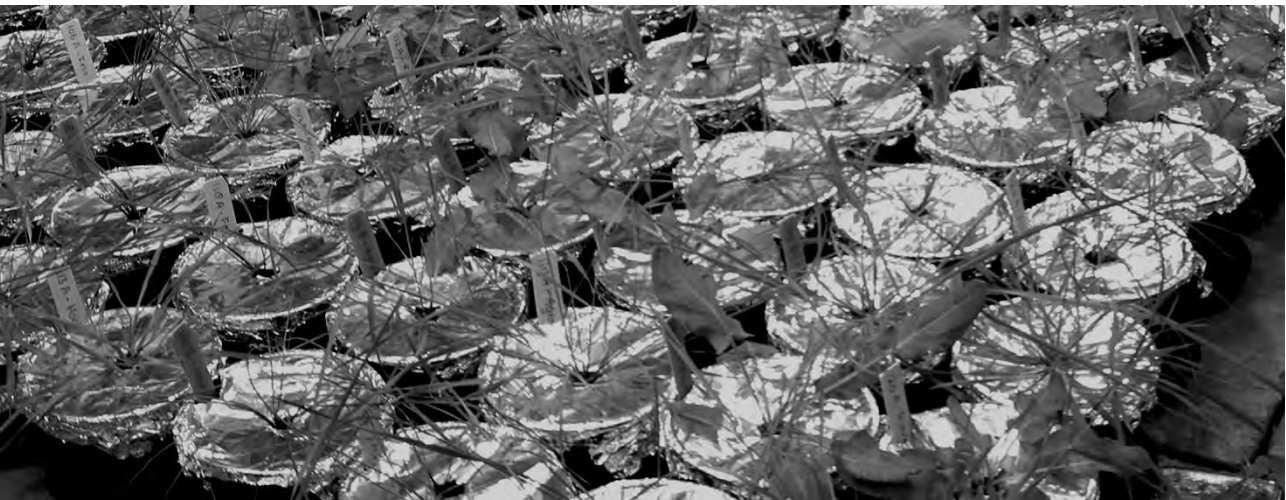


## Abstract

Interactions between plants, aboveground and belowground organisms have important effects on the community composition and functioning of terrestrial ecosystems. Belowground interactions can be captured by measuring feedback between plants and soil biota, however, it is poorly understood how large aboveground vertebrate herbivores affect plant-soil feedback and how this influences plant community dynamics. Here we use a combination of field and greenhouse experiments to study how plant-soil feedback may influence spatiotemporal plant community patterns in the absence and presence of aboveground vertebrate herbivores.

In the greenhouse we found that plants performed worse in field-collected soils pre-cultured by their own species compared to soils pre-cultured by another species, independent of cattle grazing of the soil origin. Such negative plant-soil feedback effects may increase spatiotemporal plant community dynamics by altering interspecific competitive abilities resulting in local species replacements. We observed in the field that plant community composition was most dynamic when large vertebrate herbivores had been excluded, whereas cattle grazing stabilized plant community composition in space and time.

We suggest that plant-soil feedback has the potential to drive strong spatiotemporal plant community dynamics in natural communities, as found in the ungrazed plots of our field experiment. However, large vertebrate herbivores seem to overrule such plant-soil feedback effects by stimulating the dominance of a few grazing tolerant plant species. In other words, plant-soil feedbacks seem to be conditional on the presence of larger herbivores. Therefore, to further understand the relative importance of plant-soil feedback in natural communities, hierarchical relations between plant-soil feedback and other ecological factors (such as environmental stress) need to be considered.





## Introduction

Interactions between aboveground and belowground organisms are important determinants of community composition and dynamics in terrestrial ecosystems (Wardle et al. 2004). These interactions are often mediated by plants that link the spatially separated aboveground and belowground subsystems of terrestrial ecosystems. Plants provide organic carbon for decomposer organisms, as well as resources for root and shoot herbivores, pathogens and symbiotic mutualists. In turn these organisms influence plant performance and thus plant community composition. Most studies that attempt to identify the biotic determinants of the structure and dynamics of whole plant communities have been either focusing on effects of aboveground herbivores (e.g. Huntly 1991, Olff and Ritchie 1998, Adler et al. 2001) or belowground organisms (e.g. van der Putten 2003, Kardol et al. 2007, Harrison and Bardgett 2010), although some studies have considered combined effects of aboveground and belowground biota on plant community structure (Brown and Gange 1989, Brown and Gange 1992, van Ruijven et al. 2005, Ilmarinen and Mikola 2009). However, studies focusing on interactions between vertebrate herbivores aboveground and soil organisms are hardly available. An exception is the work of Bardgett et al. (Bardgett et al. 1998) and Bardgett and Wardle (2003) who present a framework describing how plant communities respond to changes in the soil decomposer food web and accordingly in soil nutrient cycling as a result of vertebrate grazing. Here, we investigate empirically how large aboveground vertebrate herbivores and overall plant-soil feedback effects may interact as drivers of spatiotemporal plant community dynamics. Such plant-soil feedbacks do not only include effects via nutrient cycling, but also take direct effects of for example root herbivores into account.

Large aboveground vertebrate herbivores can enhance or reduce spatial and temporal patterns in the vegetation depending on ecosystem productivity, herbivore selectivity and scale of observation (Glenn et al. 1992, Adler et al. 2001, Bakker 2003). Large herbivores forage at relatively large spatial scales and reduce shoot biomass of competitively dominant plant species in productive habitats (Olff et al. 1997, Knapp et al. 1999). As a result, in these habitats large herbivores may promote locally homogenous, but high diverse plant communities (Glenn et al. 1992, Bakker 2003, Bakker et al. 2006).

Plant species modify their biotic and abiotic environment in a species-specific way (Bever et al. 1997, Yeates 1999), which in turn affects growth and the competitive ability of themselves or their offspring (van der Putten and Peters 1997, Bever 2003, Wardle et al. 2004, Ehrenfeld et al. 2005, Manning et al. 2008). This can be captured by a plant-soil feedback approach, which is a two-step process where plants influence the composition of soil communities and soil physical conditions, which in turn influences the performance plants and the composition of plant

communities (Bever et al. 1997, van der Putten 2003). Plant species experiencing positive plant-soil feedback may outcompete other plant species (Klironomos 2002, Callaway et al. 2004), which reduces local plant community diversity (Hartnett and Wilson 1999, Bever 2003). In contrast, when a plant experiences negative plant-soil feedback competitive abilities decline and other plant species may increase in abundance (van der Putten and Peters 1997, Bonanomi et al. 2005, Kardol et al. 2007). Negative plant-soil feedback can result from the accumulation of plant species specific soil-borne pathogens and root herbivores (van der Putten et al. 1993, Bever 1994, Olff et al. 2000), and can stimulate temporal and spatial replacement of plant species and therefore is generally thought to promote coexistence, diversity and local dynamics in the plant community (Olff et al. 2000, van der Putten 2003, Bever 2003, Kardol et al. 2006, Kulmatiski et al. 2008).

When acting in combination, different hypothesis for the effect of large above-ground herbivores on plant-soil feedback effects are possible (Bardgett and Wardle 2003, Sørensen et al. 2008, Mikola et al. 2009, Ilmarinen and Mikola 2009). On the one hand, large herbivores may suppress the ability of plant species to respond to plant-soil feedback because they relax interspecific competition by removing competitively dominant plant species (Bengtsson et al. 1994, Olff and Ritchie 1998). Thus, large aboveground herbivores may then reduce the potential of plant-soil feedback to drive community dynamics via effects on competitive abilities of plant species. Because large herbivores will generally be regulated in their population and habitat choice on much larger spatial scales, we expect the large herbivores will suppress small-scale effects of plant-soil feedback. Just the plant species that can tolerate aboveground grazing will dominate even though their growth may be suppressed by soil-borne pathogens. On the other hand, herbivores may alter plant-soil feedback indirectly by influencing the quality and quantity of nutrient return to the soil (Bardgett and Wardle 2003). This can change composition (Viketoft et al. 2009, Veen et al. 2010) and activity of the soil community (Holland et al. 1996, McNaughton et al. 1997a, Hamilton and Frank 2001) and may result in altered soil nutrient availability (Sørensen et al. 2008).

We use a combination of greenhouse and long-term field experiments to test effects of aboveground herbivores on the stability of plant communities in time and space and on the potential of soil organisms to drive spatiotemporal patterns via plant-soil feedback. Our hypothesis is that large aboveground herbivores stabilize the plant community in time and space (Bakker 2003), while plant-soil feedback can either enhance or reduce stability, depending on the direction of the feedback effect. Such effects are mostly expected in the absence of large herbivores. Negative feedback may reduce stability (Bever 2003), while we expect positive feedback to have a stabilizing effect. In order to test this hypothesis, we link the abundance of plant species in the field to their direct growth and feedback response to field soil under controlled greenhouse conditions.

## Methods

### Study site

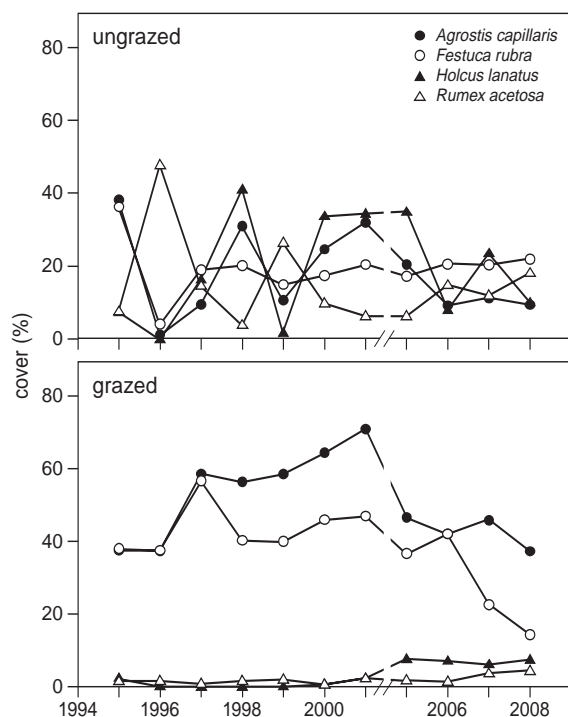
We performed our study in Junner Koeland, a 100-ha nature reserve along the river Overijsselse Vecht in the Northeast of the Netherlands (52°32'N, 6°36'E). We carried out our experiment on a 50-ha floodplain, which used to be flooded regularly until canalization of the river Vecht (around 1910) and regulation of the water level. The Junner Koeland was common grazing land, used by farmers from the village Junne, and has been grazed extensively by livestock for centuries. Nowadays, it is managed as a nature reserve by the National Forestry Service (Staatsbosbeheer), and is grazed by cattle (*Bos taurus*) from April till November at stocking rates of about 0.3 animals/ha. The most abundant natural vertebrate herbivores in the area are European rabbits (*Oryctolagus caniculus*) and common voles (*Microtus arvalis*). Dominant plant species in the grasslands are the graminoids *Agrostis capillaris*, *Festuca rubra*, *Holcus lanatus*, and *Luzula campestris*. Frequently occurring dicots are *Rumex acetosa* and *Trifolium repens*. Also, the grasslands locally host several endangered (red list) plant species, such as *Carex caryophyllea*, *Dianthus deltoids*, *Platanthera chlorantha*, *Mentha pulegium*, *Succisa pratensis*, and *Thymus serpyllum* (nomenclature follows van der Meijden 2005).

### Field experiment

In 1994 we established a long-term enclosure experiment that consisted of 5 randomly established blocks, with 2 different grazing treatments per block to study the plant community response to vertebrate grazing. Each block contained a  $12 \times 12\text{m}^2$  chicken-wire fenced enclosure plot from which cattle and rabbits were excluded ("Ungrazed"), and a plot of  $12 \times 12\text{m}^2$  to which all larger herbivores had free access ("Grazed"). Within each plot 4 permanent subplots of  $2 \times 2\text{m}^2$  were established to record long-term vegetation responses to combined grazing by cattle and rabbits (see Fig 4.1). All subplots were positioned at least 1 m from the fences to avoid edge effects. In each of the  $2 \times 2\text{m}^2$  subplots, we recorded plant species composition from 1995-2001 and from 2005-2008 at peak standing biomass (in July-August). We estimated cover of each plant species visually according to the decimal Londo scale (Londo 1976) once a year in July.

### Greenhouse experiment 1: growth potential on field soil

In greenhouse experiment 1 we tested the growth potential of four plant species that were dominant in the Junner Koeland enclosure experiment. Dominant species were defined as species that had an average cover of  $>10\%$  in one of the grazing treatments averaged over all years and plots. Selected species were the grasses *Agrostis capillaris*, *Festuca rubra*, *Holcus lanatus* and the forb *Rumex acetosa*. Seeds of *Holcus lanatus* and *Rumex acetosa* were collected in the Junner Koeland



**Figure 4.1** Temporal dynamics in cover (%) of four dominant plant species in the different grazing treatments of the enclosure experiment in the Junner Koeland: three grasses *Agrostis capillaris*, *Festuca rubra* and *Holcus lanatus* and one herb *Rumex acetosa*.

area. Seeds for *Agrostis capillaris* and *Festuca rubra* were purchased from the Cruydt-hoeck (Assen, The Netherlands).

We grew all four plant species in undiluted field soil from the permanent subplots of the field experiment to determine their growth potential in field soil. In the fall of 2006 we collected approximately 6 kg of soil of the top 15 cm from each of the “grazed” and “ungrazed”  $2 \times 2\text{m}^2$  subplots from the Junner Koeland enclosure experiment, amounting 40 subplots (5 blocks  $\times$  2 grazing treatments  $\times$  4 subplots). We gently homogenized the 6 kg of soil per subplot and divided it over four 1500 ml pots. In these pots we grew the four different plant species in monoculture, resulting in a total of 160 pots in the greenhouse experiment (40 subplots  $\times$  4 plant species). Four seedlings were transplanted into each pot. We did not apply fertilizer to pots in experiment 1 because we tested for growth potentials in pure field soil, which could have been altered when we added fertilizer. After 12 weeks shoot biomass was harvested. Half of the soil from each pot was used to collect roots by washing with tap water, whereas the other half was used to set up greenhouse experiment 2. We determined shoot and root biomass after drying plant material at 70°C for 48 hours.

### **Greenhouse experiment 2: plant-soil feedback**

In greenhouse experiment 2 we tested plant-soil feedback of the four plant species that were also used in experiment 1. Experiment 2 consisted of two different growing phases: a soil conditioning phase and a feedback phase (Brinkman et al. 2010). During the soil conditioning phase we let the plants build up a species-specific soil community. From the remaining half of each pot from greenhouse experiment 1 we collected 500 g of soil that was gently mixed with 500 g of sterilized field soil ( $\gamma$ -irradiation, 25 kGy, which eliminates all soil biota). A subsample of 25 g was collected to determine soil moisture content of the mixed soil and remaining 975 g of soil was placed in 1500 ml pots. Each pot was planted with the same plant species as in experiment 1 to further build up its species-specific soil community. Plants were planted in monoculture using 3 seedlings per pot. After 10 weeks aboveground biomass was harvested and dried at 70°C for 48 hours. In the soil conditioning phase we added sterilized soil which is known to have enhanced levels of available nitrogen and phosphate (Olff et al. 2000). Therefore, we did not apply fertilizer to the plants in this phase of experiment 2.

In the second phase of experiment 2 we tested for plant-soil feedback effects using soil from the conditioning phase of experiment 2. We grew plants in soils pre-conditioned with their own species ("home") or in soils pre-conditioned with the other three species ("foreign"). Home conditions were created by collecting and gently homogenizing half of the soil from each pot containing the soil community of one specific plant species. Foreign soils were created by gently mixing soils from each of the other three plant species, in a 1:1:1 ratio. During soil preparation, we made sure to keep the soils from the same field subplots together. All these prepared soil samples were placed individually in pots of 750 ml. All plant species were grown in home and foreign soils, using 1 seedling per pot. In total the experiment consisted of 320 pots (40 subplots  $\times$  4 plant species  $\times$  2 soil (home and foreign) origins). In the feedback phase of experiment 2 we added a limited amount of nutrients to prevent nutrient limitation, but to interfere as little as possible with plant-soil feedback effects. From week 4 onwards we added 25 ml 25%-Hoagland solution every 2 weeks, which is relatively low when compared to the plant needs in such growth conditions (van der Putten and Peters 1997). Shoots and roots were harvested after 10 weeks. We determined shoot and root biomass after drying plant material at 70°C for 48 hours.

### **Greenhouse experiment 1 and 2: seedling germination and growth conditions**

Seeds of the plant species used in both greenhouse experiments were germinated on glass beads in plastic containers in the greenhouse three weeks prior to the start of each experiment. After three weeks plants were transplanted into pots that were placed in the greenhouse at day/night rhythm of 15/9h at temperatures of 21/15°C. Soil moisture content in all pots was kept constant at 25% w/w by watering 2–3

times per week using demineralized water. To account for light and temperature differences within the greenhouse the position of the pots was randomized once a week. Pots were arranged in the greenhouse according to the experimental design in the field, i.e. pots containing soil from the same subplot and block were kept close together in the greenhouse.

### **Soil biotic and abiotic properties**

After gentle homogenization of the soil from each subplot we collected one subsample of 100 g to determine soil abiotic properties. We determined soil moisture content as the weight loss after drying soil at 105°C for 24 hrs. To measure pH we dissolved 15g of soil in 20 ml demineralized water and measured the samples with a Sentron pH meter. We determined soil organic matter content (SOM) of dry soil samples (achieved by drying for 24 hrs at 105°C) by loss-on-ignition at 550°C for 3 hrs. SOM was expressed as the relative weight difference before and after ignition. We determined soil texture in freeze-dried soil as volume % particle size classes. The freeze-dried soil was first dispersed by ultrasonification and subsequently analyzed with a coulter counter LS230 (fluid module; Beckman Coulter Inc., USA). Additionally, we collected 2 PVC cylinders filled with soil (diameter 4.3 cm, length 16 cm) to measure potential nitrogen (N) mineralization rates. We homogenized soil from half of all tubes within 24 hrs after collection to measure concentrations of inorganic nitrogen ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ). The remaining tubes were kept at 21°C and constant soil moisture levels for 6 weeks. After 6 weeks the soil was homogenized and extractable levels of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  were measured again. The differences between the first and second analysis yield potential mineralization rates per subplot (Olff et al. 1994). To determine  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations 25g of soil was dissolved in 60 ml 1M KCl. Concentrations were determined colorimetrically with a Flow Solution Autoanalyzer (Skalar, The Netherlands).

### **Data analysis**

We focus our data analysis on the four dominant plant species in the Junner Koeland enclosure experiment to be able to compare between field and greenhouse data. For each of the four dominant plant species we calculated temporal and spatial stability in the field experiment. Temporal stability was the average cover of a plant species over different years in an individual plot divided by the standard deviation (Bezemer and van der Putten 2007). We used a general linear mixed model with temporal stability as response variable and plant species and grazing treatment as fixed factors. Subplot nested within grazing treatment within block was used as a random factor (specified in R as block/grazing). Spatial stability was the average cover of a plant species over the four subplots within one grazing treatment within a block divided by the standard deviation. Hence, we had one value for spatial stability per grazing treatment within a block, resulting in 5 replicate spatial

stability measures per grazing treatment for each year. To test spatial stability we used a general linear mixed model with spatial stability as response variable, grazing treatment and plant species as fixed factors, and block nested within year as a random factor (specified in R as year/block).

The growth potential of each plant species in field soil (greenhouse experiment 1) was determined as total biomass production per pot (root + shoot biomass) in the greenhouse. We used regression analyses to determine the relationship between greenhouse biomass production and field abundance. In a previous study carried out in the same year and same field plots as the present study we determined soil nematode community composition (Veen et al. 2010). We used the nematode abundance data from this study to determine the relationship between biomass production in the greenhouse and the number of plant feeding nematodes in the soil by regression analysis. We used multiple regression analyses to relate biomass production in the greenhouse to soil abiotic properties. Data were log-transformed when necessary to obtain normality.

Plant-soil feedback effects were determined by comparing biomass production on home and foreign soil of each plant species in growing phase 3 of the greenhouse experiment. We calculated plant-soil feedback as:  $\ln(\text{biomass home soil/biomass foreign soil})$  (Brinkman et al. 2010). A negative value indicates that plants performed worse on home soil than on foreign soil, a positive value indicates that plants performed better on home soil than on foreign soil. We performed one-sample t-tests to test whether potential feedback effects were significantly different from zero, i.e. whether there was a significant positive or negative feedback. We performed a general linear mixed model to test whether potential feedback effects were different in grazed and ungrazed soils. We used plant species and grazing as fixed factors and block as a random factor (specified in R as block/ grazing). We used regression analyses to determine the relationship between plant-soil feedback in the greenhouse and stability of a plant species in the field.

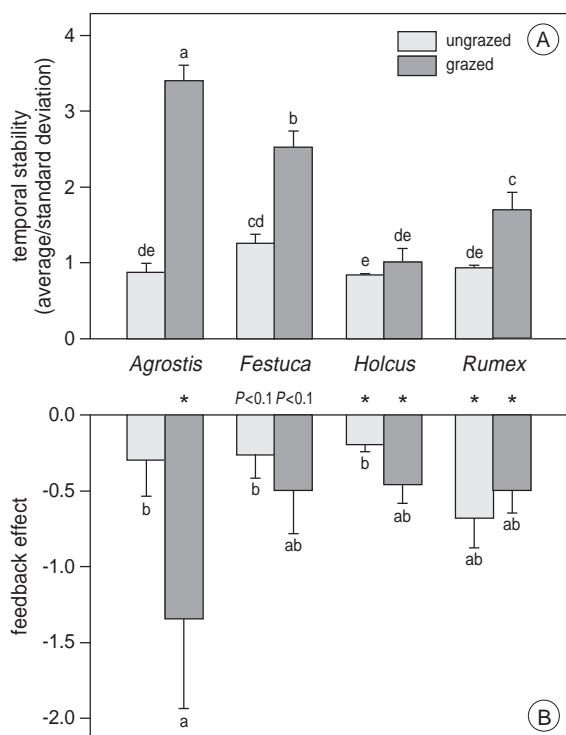
All data were analyzed using R, version 2.10.0. The lme4 package (Bates and Maechler 2009) was used to calculate general linear mixed models, using maximum likelihood estimation. To determine effects of our fixed factors we compared models with and without the variable of interest (Crawley 2007) using a chi-square test statistic. When interaction effects were significant we do not report the effects of the main factors (Crawley 2007).

## Results

### Temporal and spatial plant community stability

Temporal and spatial stability of the four dominant plant species in the field experiment were affected by an interaction between grazing treatment and plant species

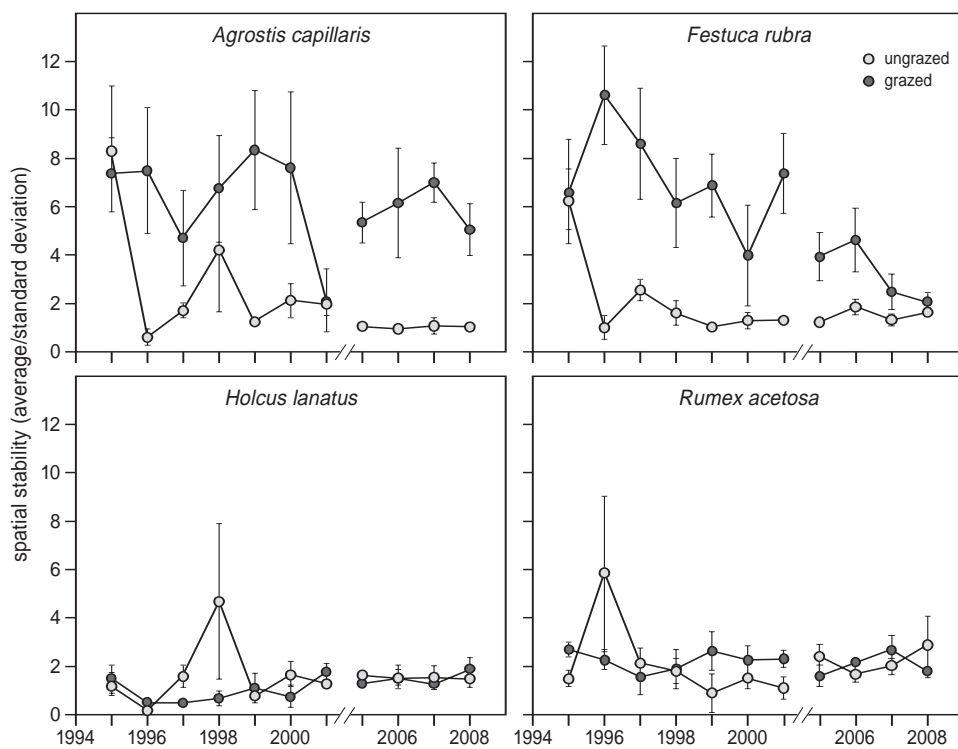




**Figure 4.2** (A) Temporal stability (average over all years/standard deviation over all years) for the four dominant plant species in the different grazing treatments of the enclosure experiment in the Junner Koeland: three grasses *Agrostis capillaris*, *Festuca rubra* and *Holcus lanatus* and one herb *Rumex acetosa*. Error bars display standard errors. Different letters indicate significant differences at  $P < 0.05$ . (B) Feedback effects for the four dominant grass species in the Junner Koeland. Feedback is expressed as the natural logarithm of the division between biomass produced on home soil and on foreign soil. Error bars display standard errors. Different letters indicate significant differences between bars at  $P < 0.05$ . Asterisks above bars indicate feedback effects significantly differing from zero at  $P < 0.05$ .

(temporal Fig 4.2a:  $\chi^2=116.17$ ,  $P < 0.001$ ,  $df=3$ ; spatial Fig 4.3:  $\chi^2=59.85$ ,  $P < 0.001$ ,  $df=3$ ). *Agrostis capillaris*, *Festuca rubra* and *Rumex acetosa* were more stable over time under large herbivore grazing, while *Holcus lanatus* was fluctuating in both grazed and ungrazed plant communities (Fig 4.1 and 2A). In space, *Agrostis capillaris* and *Festuca rubra* were more stable in grazed than in ungrazed subplots. *Holcus lanatus* and *Rumex acetosa* were less stable, irrespective of the grazing treatment (Fig 4.3). These results indicate that aboveground vertebrate grazers stabilized the plant community in time and space, mainly by promoting the stability of *Agrostis capillaris* and *Festuca rubra*. Consequently, small-scale spatiotemporal plant community heterogeneity arises in the absence of grazing.





**Figure 4.3** Spatial stability (average over plots within a year/standard deviation over plots within a year) for the four dominant plant species in the different grazing treatments of the enclosure experiment in the Junner Koeland: three grasses *Agrostis capillaris*, *Festuca rubra* and *Holcus lanatus* and one herb *Rumex acetosa*. Error bars display standard errors.

### Greenhouse experiment 1: growth potential in field soil

In experiment 1 we measured growth potential of the four dominant plant species to field soil. We related biomass production in the greenhouse to field abundance, abundance of plant feeding nematodes and soil properties to determine which factors could drive biomass production in the greenhouse.

We expect to find a negative or positive relationship between greenhouse biomass and field abundance when the accumulation of species-specific soil-borne enemies or mutualists is important in driving plant growth, respectively. We found that biomass of *Holcus lanatus* in the greenhouse was positively related to field cover in the same year (2006) in grazed soils ( $F_{1,17} = 7.98$ ,  $P = 0.011$ ) while it was negatively in ungrazed soils ( $F_{1,18} = 0.11$ ,  $P = 0.003$ ). For *Agrostis capillaris* (grazed  $F_{1,17} = 2.47$ ,  $P = 0.135$ ; ungrazed  $F_{1,18} = 0.19$ ,  $P = 0.666$ ), *Festuca rubra* (grazed  $F_{1,18} = 0.65$ ,  $P = 0.430$ ; ungrazed  $F_{1,17} < 0.01$ ,  $P = 0.966$ ) and *Rumex acetosa* (grazed  $F_{1,18} = 0.36$ ,  $P = 0.557$ ; ungrazed  $F_{1,17} = 2.11$ ,  $P = 0.164$ ) we found no relationship

between greenhouse biomass and field abundance. We also tested whether greenhouse performance was related to field abundance in the year before (2005) and after (2007) the experiment, to quantify potential legacy effects of the soil community, or impacts still-to-come. Using the field data from the year before soil sampling, we found a positive relationship between biomass in the greenhouse and percentage cover in the field in grazed soils for *Holcus lanatus* ( $F_{1,17} = 13.84$ ,  $P = 0.002$ ) and *Rumex acetosa* ( $F_{1,17} = 9.65$ ,  $P = 0.006$ ), and a negative relationship for *Agrostis capillaris* ( $F_{1,17} = 7.33$ ,  $P = 0.015$ ). In contrast, in ungrazed soils *Holcus lanatus* biomass production was negatively related to field cover ( $F_{1,18} = 11.5$ ,  $P = 0.003$ ), while *Agrostis capillaris* biomass production was positively related to field cover ( $F_{1,18} = 5.20$ ,  $P = 0.035$ ). For 2007, we only found a positive relationship between biomass production and field cover of *Holcus lanatus* ( $F_{1,17} = 7.30$ ,  $P = 0.015$ ). Lumping all species together, we did not find a general relationship between biomass production in the greenhouse and plant abundance in the field.

In soils from grazed plant communities, we found a negative relationship between greenhouse biomass and the number of plant feeding nematodes in the soil for *Holcus lanatus* ( $F_{1,17} = 4.56$ ,  $P = 0.048$ ) and for *Rumex acetosa* ( $F_{1,18} = 8.15$ ,  $P = 0.011$ ). However, *Agrostis capillaris* ( $F_{1,17} = 2.44$ ,  $P = 0.136$ ) and *Festuca rubra* ( $F_{1,17} = 2.16$ ,  $P = 0.159$ ) did not show a clear relationship. In soils from ungrazed plant communities the relationships between plant biomass and plant-feeding nematodes were less strong for all four species ( $P > 0.05$ ). Again, these data did not show a general link between greenhouse and field results for all plant species,

**Table 4.1** Results of multiple regression analysis on the relationship greenhouse biomass and key soil abiotic properties.

Plant species	pH		SOM		Potential N min.	
	t	P	t	P	t	P
Grazed soils						
<i>Agrostis capillaris</i>	<b>2.16</b>	<b>0.047 *</b>	-1.26	0.229	1.62	0.126
<i>Festuca rubra</i>	1.74	0.102	-1.54	0.142	0.12	0.904
<i>Holcus lanatus</i>	0.74	0.469	<b>-3.69</b>	<b>0.002 **</b>	-1.77	0.098
<i>Rumex acetosa</i>	<b>2.97</b>	<b>0.009 **</b>	<b>-2.75</b>	<b>0.014 *</b>	0.03	0.980
Ungrazed soils						
<i>Agrostis capillaris</i>	<b>-2.12</b>	<b>0.050 *</b>	<b>-2.84</b>	<b>0.012 *</b>	0.80	0.437
<i>Festuca rubra</i>	0.32	0.752	0.40	0.697	<b>2.19</b>	<b>0.045 *</b>
<i>Holcus lanatus</i>	<b>3.45</b>	<b>0.003 **</b>	<b>-4.92</b>	<b>&lt;0.001 ***</b>	-0.52	0.608
<i>Rumex acetosa</i>	1.04	0.315	-1.69	0.113	-0.35	0.728

Degrees of freedom (df) = 16 for *Festuca* and *Rumex* in grazed soils and for *Agrostis* and *Holcus* in ungrazed soils. For other tests df = 15, because of missing data due to plant death in the greenhouse experiment.

however they indicate that in some cases the presence of soil-borne biota may reduce plant production.

We found species-specific relationships between growth potential of the plant species and soil abiotic properties (Table 4.1). The biomass of *Agrostis capillaris* and *Rumex acetosa* in soils from grazed plant communities and *Holcus lanatus* in both soils was increasing towards higher pH soils, while biomass of *Agrostis capillaris* in ungrazed soil decreased towards higher pH. We found a negative relationship between SOM content and greenhouse biomass in grazed soils for *Holcus lanatus* and *Rumex acetosa* and in ungrazed soils for *Agrostis capillaris* and *Holcus lanatus*. Potential N mineralization rate showed a positive relationship with *Festuca rubra* biomass in soils from ungrazed plant communities only.

### Greenhouse experiment 2: plant-soil feedback

In greenhouse experiment 2 we tested for plant-soil feedback effects in pre-conditioned soil from the different grazing treatments. We found that plant-soil feedback was affected by an interaction between grazing treatment and plant species ( $\chi^2 = 9.24$ ,  $P = 0.026$ ,  $df = 3$ ). For *Agrostis capillaris* plant-soil feedback effects were stronger in grazed than in ungrazed soils, while for the other three plant species there were no significant differences between grazing treatments (Fig 4.2B).

We found significant negative plant-soil feedback effects for *Agrostis capillaris* ( $t = -3.48$ ,  $P = 0.003$ ,  $df = 19$ ) in soils from grazed plant communities and for *Holcus lanatus* (grazed,  $t = -5.16$ ,  $P < 0.001$ ,  $df = 19$ ; ungrazed,  $t = -4.29$ ,  $P < 0.001$ ,  $df = 18$ ) and *Rumex acetosa* in both soils (grazed,  $t = -4.66$ ,  $P < 0.001$ ,  $df = 18$ ; ungrazed,  $t = -4.06$ ,  $P < 0.001$ ,  $df = 19$ ). Feedback effects for *Festuca rubra* also tended to be negative in both soils (grazed,  $t = -1.94$ ,  $P = 0.067$ ,  $df = 19$ ; ungrazed,  $t = -1.74$ ,  $P = 0.099$ ,  $df = 18$ ). Feedback effects in soils from ungrazed plant communities for *Agrostis capillaris* ( $t = -1.13$ ,  $P = 0.272$ ,  $df = 19$ ) were not significantly different from zero (Fig 4.2B). The results from experiment 2 show that all plant species experience negative plant-soil feedback, which is generally not dependent on the grazing origin of the soil.

## Discussion

In the greenhouse experiment we found that all plant species performed worse in soils incubated with their own plant species compared to soils incubated with other plant species. This indicated a general negative plant-soil feedback (Brinkman et al. 2010) for all species in both grazing treatments. Negative plant-soil feedback may result from the accumulation of species-specific plant pathogens and herbivores and can reduce competitive abilities of specific plant species (van der Putten et al. 1993, Bever et al. 1997, Olff et al. 2000). Therefore, in both grazing treatments soil

organisms are potential drivers of small-scale spatiotemporal plant community patterns by locally enhancing competitive replacement of plant species (Olf et al. 2000, van der Putten 2003, Bever 2003). Yet, our field results showed that local plant community composition was highly stabilized by cattle grazing, despite the observation that the dominant species clearly were subject to negative plant-soil feedback. Such stabilizing effects of large herbivores on plant community composition are well known (Glenn et al. 1992, Adler et al. 2001, Bakker 2003), but have not been viewed before in the light of plant-soil feedback. Our results showed that cattle grazing apparently overruled the negative plant-soil feedback effects that were present in this community. The grazing-tolerant plant species *Agrostis capillaris* and *Festuca rubra* reached high abundances and remained the most stable over space and time in grazed plots although both species differed strongly in their soil feedback. Plant-soil feedback for *Festuca* did not differ significantly from zero and this plant species was relatively stable over time and space under grazed conditions. In contrast, *Agrostis* was also stable in grazed plots, even more than *Festuca*, while it experienced the strongest negative plant-soil feedback of all plant species tested in soils originating from grazed plots (Fig 4.2B). Therefore, the experimentally inferred feedback strength did not explain the temporal stability of these species in the field.

Our results suggest that vertebrate grazers overrule plant-soil feedback effects; so that species with a strong negative plant-soil feedback can still become abundant and remain stable when tolerant to aboveground grazing. This may be because their successors in the community (species that are insensitive to the soil community of the species they replace) are more sensitive to aboveground biomass loss (grazing intolerant). Large vertebrate grazers often exert a homogeneous grazing pressure over relatively large spatial scales (Ritchie and Olf 1999, Haskell et al. 2002), at least compared to the scale at which soil organisms and individual plants usually operate. At these large scales aboveground vertebrate grazers have been found to prevent competitive exclusion between plants by removal of some potential dominant grassland species (Olf et al. 1997, Olf and Ritchie 1998) and promote the abundance of several grazing-tolerant (Olf et al. 1997) or subordinate plant species (Knapp et al. 1999). When grazers exert a strong control on the plant community composition, tolerance of plants to aboveground grazers may thus be more important as a factor determining plant abundance than plant tolerance to negative feedback with soil communities. Therefore, grazing-tolerant plants may perform better under grazing than plant species that are less grazing-tolerant, even when they experience a reduction in competitive ability by negative plant-soil feedback, as in the case of *Agrostis capillaris*.

Alternatively, tolerance to negative plant-soil feedback may be dependent on the plant species identity (Verschoor et al. 2002, De Deyn et al. 2003). Consequently, plants that become dominant under grazing could be tolerant to plant-soil feed-

back. Similar mechanisms, such as high plant growth rates and flexible resource allocation (Strauss and Agrawal 1999) can be favorable to become tolerant to both aboveground grazers as well as root herbivores and pathogens. Therefore, it may well be that the same species can grow well under grazing conditions by both aboveground and belowground herbivores, which may result in stable abundances of these species in grazed grasslands. Our results showed that biomass production of the grazing-intolerant plant species *Holcus lanatus* and *Rumex acetosa* was reduced when the number of plant feeding nematodes increased, while this was not the case for the more grazing-tolerant species *Agrostis capillaris* and *Festuca rubra*. This indicates that plant species which are tolerant to aboveground grazing may also be more tolerant to belowground herbivores. *Agrostis capillaris* and *Festuca rubra* are also longer-lived than *Holcus lanatus* and *Rumex acetosa*, as a result of which it becomes more useful to be tolerant to soil-borne herbivores.

Large vertebrate grazers may not only overrule plant-soil feedback effects; they may also modify the strength of plant-soil feedback effects (Sørensen et al. 2008), at least in the case of *Agrostis*. This could be due to alteration of the quality and quantity of resource input into the soil, which changes the activity and composition of the soil community (Holland et al. 1996, McNaughton et al. 1997a, Bardgett and Wardle 2003). Also, aboveground grazers may alter the soil community composition indirectly through modifying the plant community composition (Veen et al. 2010), or by modifying soil physical conditions (Hobbs 1996). Plant species may respond to the altered soil community because they are more or less sensitive to the soil organisms that become more abundant. Moreover, an altered composition of the soil community may lead to changes in top-down control on belowground herbivores or pathogens by their enemies, resulting in a different pressure of soil-borne pathogens.

In spite of aboveground vertebrate herbivores which exert a strong control on plant community stability (Bakker 2003), negative plant-soil feedback may still contribute to drive plant community dynamics in grasslands in the absence of aboveground vertebrate grazing. We found that in ungrazed plots the plant community was less stable, with alternating high and low abundances between years and plots for the four dominant plant species. Particularly, plant species that become dominant in ungrazed situations, i.e. *Holcus lanatus* and *Rumex acetosa*, experienced negative plant-soil feedback in soils originating from ungrazed situations. Negative plant-soil feedback may contribute to the replacement of plant species (van der Putten 2003, Bever 2003). Previous studies showed that root herbivores can reduce the total net primary production (NPP) in grasslands up to 30% (Stanton 1988), and that this may vary between 1–40% depending on local abundances of root feeders (Verschoor 2002). Therefore, soil-borne herbivores may contribute to reduced spatiotemporal plant community stability by locally removing primary production.

When negative plant-soil feedback is important in driving natural plant communities (Kulmatiski et al. 2008), we expected plants in the greenhouse to perform worse in soils collected from field plots where their own species had been abundant due to accumulation of species-specific soil-borne pathogens and root herbivores (van der Putten et al. 1993, Olff et al. 2000, Klironomos 2002, Bever 2003). However, we could not establish a direct, clear link between plant cover in the field and biomass production in the greenhouse. This may be due to differences in scale between the field and greenhouse study. Field data were collected on the plot level, while plant-soil feedback effects take place at the scale of individual plants. Also, effects that play out over longer time scales such as the influence of arbuscular mycorrhizal fungi and decomposers were not included in our experiment (Hart et al. 2003, Casper and Castelli 2007, Harrison and Bardgett 2010). Alternatively, in the field plant-soil feedback effects may be modified by variation in biotic and abiotic environmental variables, such as soil type (Bezemer et al. 2006), soil nitrogen availability (Manning et al. 2008) and competitive interactions between plant species (Kardol et al. 2007, Casper and Castelli 2007). Our greenhouse data indeed revealed that soil abiotic properties affected plant biomass production, and hence may be interfering with effects of soil biota on plant growth. The responses of plant species to soil abiotic properties were not consistent across grazing treatments, which implies that also other factors in the soil, such as the presence of soil organisms, may affect plant growth as well. Consequently, the relative importance of plant-soil feedback in grazed, natural grasslands remains poorly understood. More field studies are needed quantifying the role of plant-soil feedback structuring natural grasslands (Casper and Castelli 2007, Harrison and Bardgett 2010), particularly in the presence of vertebrate grazers.

## Conclusion

Our findings support the idea that negative plant-soil feedback can be important for structuring plant communities (Wardle et al. 2004, Kulmatiski et al. 2008, Mangan et al. 2010), but we show that in the field plant-soil feedback effects may be modified or overruled by aboveground vertebrate herbivores. The strong stabilizing control of aboveground herbivores on the local plant community may rule out the opportunities for soil organisms to enhance small-scale spatiotemporal plant community dynamics in grazed situations. Consequently, a strong negative plant-soil feedback does not necessarily result in replacement of plant species and hence in cyclic plant community dynamics (Bever 2003) in the presence of aboveground vertebrate grazers. This suggests that in grassland ecosystems soil organisms have more opportunities to drive spatiotemporal dynamics in the absence of aboveground vertebrate herbivores than when these herbivores are present.

## **Acknowledgements**

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# Chapter five





# 5

## Interactive effects of soil-dwelling ants and aboveground vertebrate grazers on local plant community composition

G.F. (Ciska) Veen, H. Olff

## Abstract

Interactions between aboveground and belowground organisms have been acknowledged as important drivers of plant community structure and composition. Most studies focused on effects of such interactions on single plant species, while the consequences for mixed plant communities are less well examined. In this study we investigate the consequences of interactions between subterranean yellow meadow ants (*Lasius flavus*) and simulated cattle grazing on biomass production and species composition in plant communities. We set up a greenhouse experiment using 10-l pots with intact soil cores with their associated vegetation. In a full factorial design, we studied effects of simulated grazing (clipping), ant mound presence and the actual presence of ants themselves on changes in plant community structure and composition.

We found that plant biomass production on the short term was affected by an interaction between simulated grazing and ant mound presence. Grazing homogenized production on and off ant mounds, while production was increased off mounds only after the cessation of grazing. Biomass production on the longer term, as well as plant species richness and plant community composition during the whole experimental period were explained by simulated grazing only. Simulated grazing reduced long-term biomass production, enhanced the cover of grazing-tolerant plant species, and maintained higher plant species richness. The actual presence of yellow meadow ants did not affect biomass production and plant community composition.

We conclude that simulated grazing may overrule effects of ant mounds on plant biomass production. However, since biomass production shortly after the cessation of grazing was affected by ant mound presence, our results suggest that under low intensity grazing ant mounds may become important determining plant production. In such situations the interaction between ant mounds and grazing can potentially drive small-scale plant community structure and composition.



## Introduction

Aboveground and belowground components of terrestrial ecosystems are linked by plants. Consequently, the interaction between aboveground and belowground organisms is an important driver of plant community composition and structure (Wardle et al. 2004). Aboveground and belowground herbivores directly remove plant biomass, thereby altering the competitive ability of plant species and hence plant community composition (van der Putten and Peters 1997, Olff and Ritchie 1998). Moreover, aboveground-belowground interactions modify soil nutrient cycling which feeds back to plant growth and community composition (Bardgett and Wardle 2003).

Most studies on aboveground-belowground interactions have focused organisms affecting single plant species (e.g. Gange and Brown 1989, Masters and Brown 1992, Masters 1995, Gange and Nice 1997, Bezemer et al. 2003, Soler et al. 2005, Soler et al. 2007). However, recent work suggests that interspecific competition between plants can modify effects of belowground organisms (Kardol et al. 2007, Casper and Castelli 2007). Therefore, to understand the importance of aboveground-belowground interactions structuring plant communities it is essential to study whole communities (Harrison and Bardgett 2010).

In our study we focus on interactive effects of subterranean yellow meadow ants (*Lasius flavus*) and aboveground vertebrate herbivores on structure and composition of plant communities. Yellow meadow ants are ecosystem engineers that modify biotic and abiotic soil properties (Jones et al. 1994). They dig up fresh sand to build nest mounds and thereby they create open, competition-free space that can be colonized by plants (King 1977b). Moreover, digging modifies abiotic soil conditions (such as pH, organic matter content, temperature and water availability), changes soil community composition and reduces abundances of plant-parasitic pathogens (Blomqvist et al. 2000, Dauber and Wolters 2000, Dostal et al. 2005). Also, ants 'farm' root aphids (which are root sap suckers) in their nest mounds, from which they harvest honey dew excretion (Pontin 1978), and which they consume as well. All these changes in biotic and abiotic environmental conditions induced by yellow meadow ants affect plant species directly and indirectly and can alter the plant community composition on mounds (e.g. King 1977a, 1977b, Dean et al. 1997, Blomqvist et al. 2000). Both King (1977b) and Blomqvist et al (2000) showed that yellow meadow ants interact with aboveground vertebrate grazers. When tall grasses become dominant in the absence of vertebrate grazing ants become more active and build higher mounds to keep up with the vegetation height. This is probably done to maintain sufficient solar radiation reaching the soil surface, and thus regulate the temperature in the mound. In turn, altered ant activity and mound structure may influence plant growth. We expect that the interaction between vertebrate grazing and yellow meadow ants will be important in

determining small-scale plant community structure and composition. We tested our hypothesis by addressing the following questions (1) how does the interaction between ants and aboveground biomass removal affect structure and composition of a grassland plant community?, and (2) do ants alter plant community structure predominantly by building nest mounds (and their associated effects on soil properties) or does the actual presence of the ants themselves affect the plant community as well (for example by tending root aphids)?

We set up a greenhouse experiment by collecting intact soil cores and their associated plant communities from the field. In a full factorial design, we studied effects of simulated grazing (clipping), ant mound presence and the actual presence of ants themselves (ants were removed or not by poison addition) on changes in plant community structure and composition. During 9 months we measured biomass production and plant community composition.

## Methods

### Study site

We set up a greenhouse experiment using soil cores from Junner Koeland, a 100-ha nature reserve along the river Overijsselse Vecht (52°32'N, 6°36'E). The Junner Koeland consists of a 50-ha floodplain area and of a heathland area. The floodplain area was flooded regularly until 1910 when the river Vecht was canalized. The area has been used as common grazing land by farmers from the village Junne, and has been grazed extensively by livestock for centuries. Nowadays, it is managed as a nature reserve by the National Forestry Service (Staatsbosbeheer), and is grazed by cattle (*Bos taurus*) from April till November at stocking rates of about 0.3 animals/ha. The most abundant natural vertebrate herbivores in the area are European rabbits (*Oryctolagus caniculus*) and common voles (*Microtis arvalis*). Dominant plant species in the grasslands are the graminoids *Agrostis capillaris*, *Festuca rubra*, *Holcus lanatus*, and *Luzula campestris*. Frequently occurring dicots are *Rumex acetosa* and *Trifolium repens*. Also, the grasslands locally host several endangered plant species, such as *Dianthus deltoids*, *Succisa pratensis*, *Thymus serpyllum* and *Mentha pulegium* (nomenclature follows van der Meijden 2005). Yellow meadow ants (*Lasius flavus*) are abundant subterranean invertebrates in the floodplain areas of Junner Koeland nature reserve. The soil consists of the loamy sand that has been deposited there by the river. The site is flooded on an irregular basis (once every few years). The ants build obvious nest mounds by digging up fresh sand from deeper soil layers thereby changing biotic and abiotic soil properties (e.g. Dean et al. 1997, Blomqvist et al. 2000). In their nests, ants mutually live together with certain species of root aphids, which are root herbivores that feed on phloem sap of plants (Pontin 1978, Stadler and Dixon 2005). This can be viewed as an obligate

symbiosis. The aphids excrete honeydew that serves as an important food source for yellow meadow ants, and in return the ants tend these aphids in their nests by protecting them from enemies, cleaning them from microbes and moving them to suitable root sites (Pontin 1978, Stadler and Dixon 2005).

### **Experimental set up**

In the Junner Koeland nature reserve we laid out a randomized block design for the collection of soil samples, consisting of 6 blocks of 5 by 5 meter in the cattle-grazed floodplain grassland. In September 2006 we randomly collected 4 paired soil cores of 10-l with a depth of approximately 30 cm, totaling 48 soil cores (6 blocks  $\times$  4 pairs). One of the soil cores consisted of a whole ant mound and the other of matrix soil. The cores were left intact and put into 10-litre pots.

We used these soil cores to set up a full factorial greenhouse experiment testing three factors. (1) Clipping of aboveground biomass. In half of our pots aboveground biomass was clipped homogeneously every 2 weeks to a height of approximately 4 cm to simulate repeated cattle grazing during the growing season. In the other half biomass was clipped every 3 months to prevent pots becoming limited for plant growth. (2) Presence of ant mounds. Half of the soil cores collected in the field contained an ant colony. We collected small, probably young colonies, including the queen, to be able to maintain complete colonies with its associated vegetation in the 10-l pots. The other half of the pots contained soil cores that consisted of matrix soil with its associated vegetation. (3) Presence of ants. To half of the ant mounds we added ant poison baits (0.1% trichlorofenylaldehyde). For this we placed 5 15-ml plastic cups on top of the soil at 2 and 4 weeks after the start of the experiment. The ants came to collect the poison themselves, consuming it and feeding it to their offspring. Therefore the poison specifically removed the ants without affecting other soil fauna directly. To control for potential effects of ant poison on other soil organisms (if it would be collected by those at all), we also added poison baits to half the pots containing matrix soil cores. To check whether our ant removal treatment was successful, we collected soil cores of the top 5-cm of the soil at the end of the experiment and separated all insects from the soil using a Tullgren (van Straalen and Rijninks 1982). In these samples we counted the number of ants. We did not find any ants in pots that originally contained an ant mound and received poison bait, while all pots with mounds that did not receive poison bait still did contain ants. However, we also found a few pots that originally contained matrix soil but were colonized by yellow meadow ants. Regarding digging activity in the pots, colonization seemed very recent and happened probably towards the end of the experiment.

Our full-factorial set up resulted in 8 different treatments, each replicated 6 times (i.e. 6 random blocks): (a) not clipped + no mounds + no poison, (b) not clipped + no mounds + poison, (c) not clipped + mounds + no poison, (d) not clipped + mounds + poison, (e) clipped + no mounds + no poison, (f) clipped + no

mounds + poison, (g) clipped + mounds + no poison, (h) clipped + mounds + poison.

The experiment was maintained in the greenhouse for 9 months at a day/night rhythm of 15/9 hours at a temperature of 23/17°C. Pots were watered automatically with demineralized water. Pots with soil cores originating from the same random block were kept together in the greenhouse. To control for differences in microclimate in the greenhouse the spatial arrangement of the pots was randomized once every 2 weeks.

### Measurements

Clipped plant material was dried at 70°C for 24h and weighed. Plant community composition was measured in weeks 4, 6, 8, 10, 12, 14, 22, 28, and 39 of the experiment using a point quadrat method (Levy and Madden 1933, Goodall 1952). A frame with 52 holes was placed on top of the 10-l pots. Through each hole a metal pin was lowered slowly onto the vegetation. The first plant that was touched by the pin was recorded.

### Data analysis

We calculated cumulative biomass production over the first 3 months and over the whole experimental period of 9 months by adding up all biomass measures for these periods respectively. Plant species richness was calculated as the number of plant species per pot using the point quadrat data. We calculated plant species richness after 3 and 9 months. Treatment effects on cumulative biomass production and plant species richness were tested using a general linear model with clipping, presence of ant mounds and poison addition as fixed factors. Block was used as a random factor (specified in R as block/clipping/mound). When interactions between the fixed factors were strong we do not report the main effects (Crawley 2007). Data were distributed normally (Shapiro-Wilk normality test) and had homogeneous variances (Levene's test). Analyses were performed using R version 2.10.0. We implemented the lme4 package to perform general linear mixed models (Bates and Maechler 2009).

We used redundancy analysis (RDA) and principle response curves (PRC) to analyze the extent and direction of plant community changes over time (van den Brink and ter Braak 1999, Lepš and Šmilauer 2003). RDA analysis is a constrained form of principle component analysis (PCA). RDA and PCA find the best set of predictor variables (summarized in ordination axes) that explain a multivariate data set. In contrast to PCA, in RDA the ordination axes are constrained to linear combinations of predictor (environmental) variables. We used RDA analysis to test the effect of our experimental treatments (clipping, mound presence and poison addition) on plant community development. We used treatment × sampling time interactions as explanatory (i.e. environmental) variables. Sampling time and block



were used as covariables. Additionally, to test explanatory power of the separate treatment levels on temporal changes in plant community composition we performed a detailed RDA analysis where we used treatment  $\times$  time interaction for one of our treatments as environmental variable, and treatment  $\times$  time interactions for the other two as covariables.

We used principle response curves (PRCs) to graphically present plant community changes over time for each of our experimental treatments (van den Brink and ter Braak 1999). In PRC analysis one of the treatments is used as reference treatment against which the changes in the other treatments are plotted. We used clipped pots without mounds and poison as a reference, because this represents the dominant situation in the Junner Koeland nature reserve. PRC analysis is based on a partial RDA where the interactions between time and each treatment combination (i.e. our full-factorial combination of all treatment levels), except for the reference treatment, are used as explanatory variables. Sampling time was added as covariable. The vertical scores of the first-order PRCs are based on the scores of environmental variables on the first axis of the RDA analysis. In the PRC diagram we included a 1-D plot that shows the plant species scores on the first RDA axis. A combination of the PRCs with the 1-D species plot illustrates changes in species abundance in the different treatments over time.

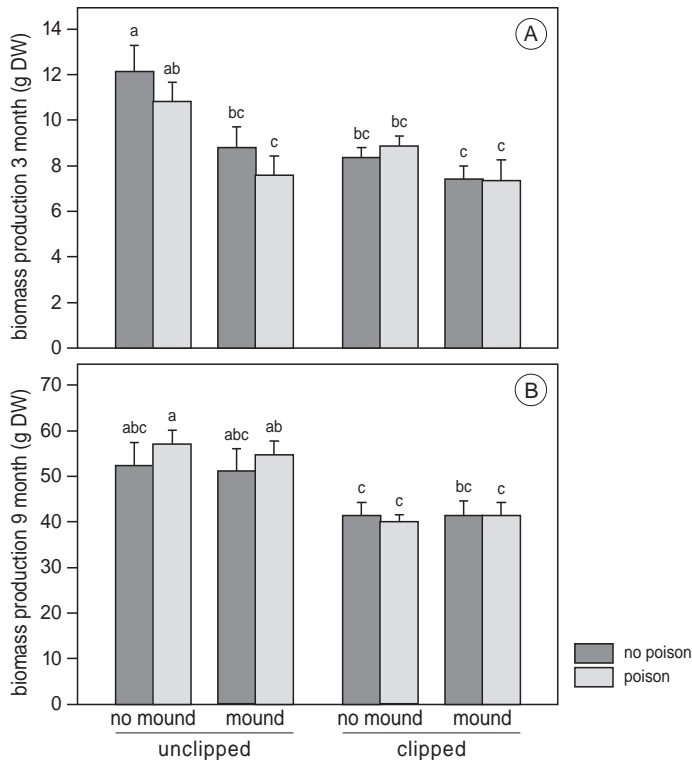
The significance of treatment  $\times$  sampling time interactions in all RDA analyses was tested using Monte Carlo permutation tests with 999 permutations. Within blocks permutations were restricted by a split-plot design that represented the temporal structure of the data. We allowed whole plots (data that were collected in the same pot at the same time) to permute at random, while split-plots (different pots at different times) were not permuted. To test the significance of higher order ordination axes we added the lower-order axes as covariables. We only present analyses results for the first ordination axes, because in all cases higher-order axes were found to be not significant ( $P > 0.05$ ).

RDA and PRC analyses were carried out using CANOCO version 4.5 for Windows (ter Braak and Šmilauer 2002).

## Results

### Biomass

After 3 months, cumulative biomass production was affected by an interaction between clipping and the presence of ant mounds ( $\chi^2 = 4.16$ ,  $df = 1$ ,  $P = 0.041$ , Fig 5.1A). Only in pots without mounds that were left unclipped biomass production was increased. This indicates that under clipping there was neither an effect of the presence of mounds nor of poison on biomass production, while in the absence of clipping it did matter whether mounds were present or not. Both clipping ( $\chi^2 = 8.25$ ,

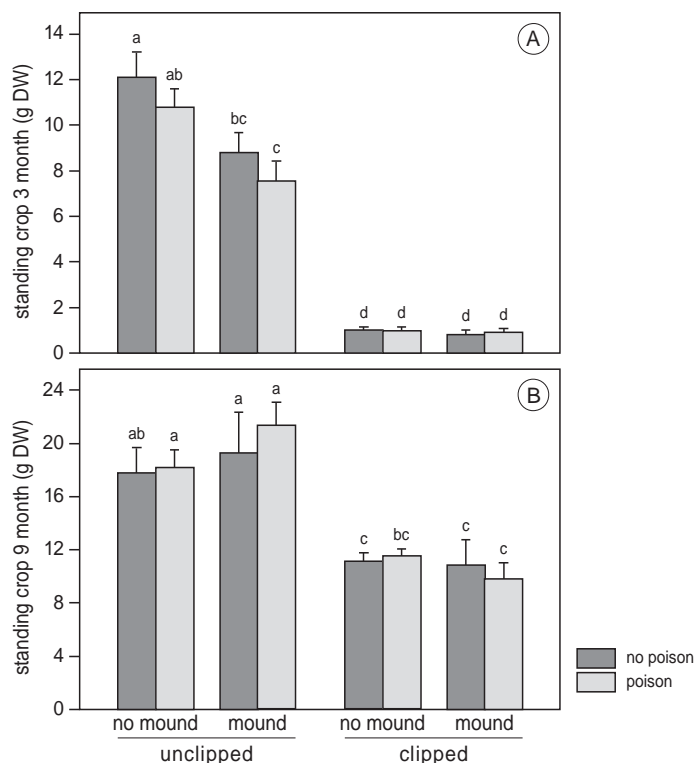


**Figure 5.1** Cumulative biomass production (g dry weight) per treatment after (A) 3 months and (B) 9 months. Treatments represent a full-factorial design with three factors: aboveground biomass was left or removed by clipping (unclipped vs. clipped), ant mounds were absent or present (no mound vs. mound), and ants were left or removed (no poison vs. poison). Error bars display standard errors. Different letters above bars indicate significant differences at  $P < 0.05$ .

$df = 1$ ,  $P = 0.004$ , Fig 5.1A) and the presence of ant mounds ( $\chi^2 = 14.31$ ,  $df = 1$ ,  $P < 0.001$ , Fig 5.1A) reduced biomass production. The addition of ant poison did not affect biomass production after 3 months ( $\chi^2 = 1.02$ ,  $df = 1$ ,  $P = 0.313$ , Fig 5.1A). After 9 months, cumulative biomass production was affected by clipping only. In clipped pots less biomass was produced after 9 months ( $\chi^2 = 15.28$ ,  $df = 1$ ,  $P < 0.001$ , Fig 5.1B). Neither the presence of ant mounds ( $\chi^2 = 0.036$ ,  $df = 1$ ,  $P = 0.850$ , Fig 5.1b), nor of poison ( $\chi^2 = 1.107$ ,  $df = 1$ ,  $P = 0.292$ , Fig 5.1B) affected total biomass.

Standing crop after 3 and 9 months showed similar patterns as cumulative biomass production after 3 and 9 months. After 3 months standing crop was affected by an interaction between clipping and ant mounds ( $\chi^2 = 11.98$ ,  $df = 1$ ,  $P < 0.001$ , Fig 5.2A). Clipping reduced standing crop and in clipped treatments there was no effect of ant mounds on standing crop. In unclipped treatments ant mounds reduced standing crop. After 9 months standing crop was affected by the clipping





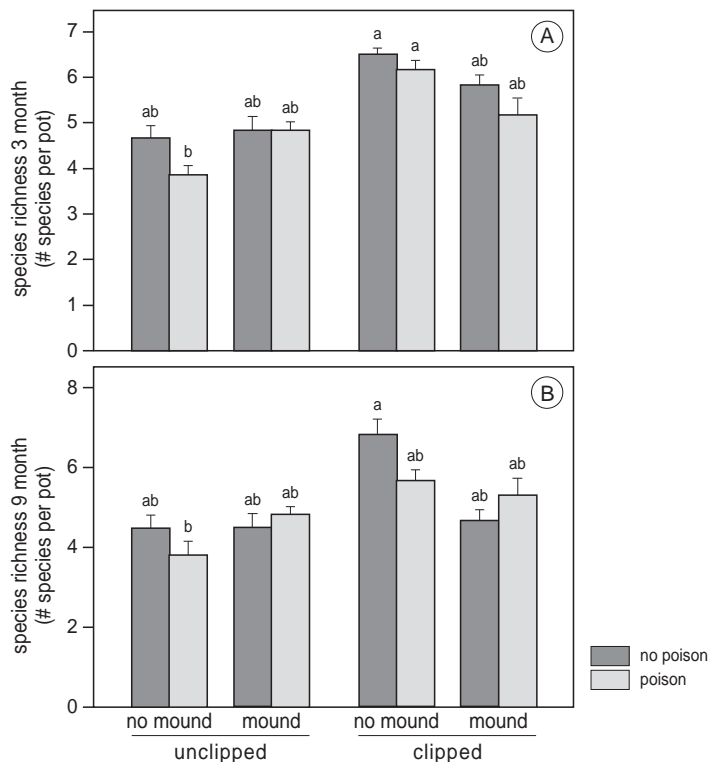
**Figure 5.2** Standing crop (g dry weight) per treatment after (A) 3 months and (B) 9 months. Treatments represent a full-factorial design with three factors: aboveground biomass was left or removed by clipping (unclipped vs. clipped), ant mounds were absent or present (no mound vs. mound), and ants were left or removed (no poison vs. poison). Error bars display standard errors. Different letters above bars indicate significant differences at  $P < 0.05$ .

treatment only ( $\chi^2 = 18.97$ ,  $df = 1$ ,  $P < 0.001$ , Fig 5.2B), while there was no effect of ant mound ( $\chi^2 = 0.48$ ,  $df = 1$ ,  $P = 0.504$  Fig 5.2B) or poison addition ( $\chi^2 = 0.27$ ,  $df = 1$ ,  $P = 0.606$ , Fig 5.2B).

Our results indicate that on the short term (3 months) clipping overruled effects of ant mounds on biomass production and standing crop. Only in unclipped situations the ants may have acted as a “consumer” in reducing the total biomass production, similar to clipping. However, on the longer term clipping was the only determinant of biomass production and standing crop, because effects of ant mounds in unclipped situations disappeared.

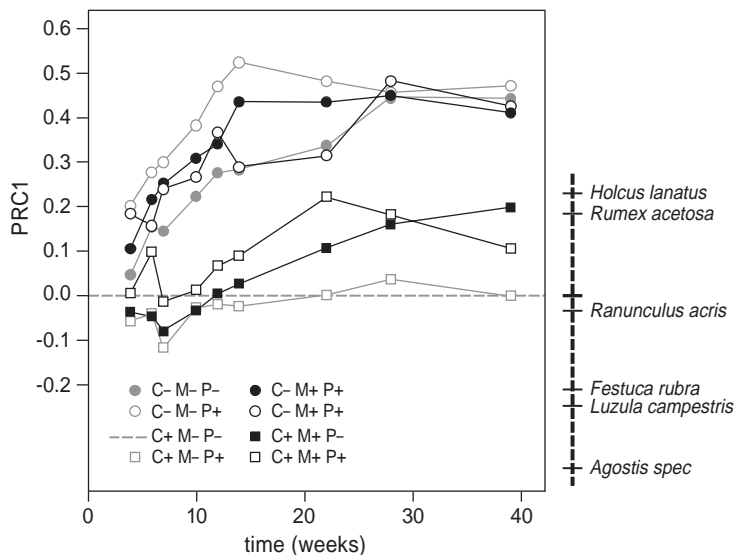
### Plant species richness

After 3 months species richness was affected by an interaction between clipping and the presence of ant mounds ( $\chi^2 = 3.96$ ,  $df = 1$ ,  $P = 0.047$ , Fig 5.3A). Only in pots



**Figure 5.3** Plant species richness (number of plant species per pot) after (A) 3 months and (B) 9 months. Treatments represent a full-factorial design with three factors: aboveground biomass was left or removed by clipping (unclipped vs. clipped), ant mounds were absent or present (no mound vs. mound), and ants were left or removed (no poison vs. poison). Error bars display standard errors. Different letters above bars indicate significant differences at  $P < 0.05$ .

with clipping and without mounds we found a higher number of species. Clipping significantly increased plant species richness ( $\chi^2 = 9.27$ ,  $df = 1$ ,  $P = 0.002$ , Fig 5.3A) from 4.5 species in unclipped pots to 5.9 species on average in clipped pots. Nor the presence of ant mounds ( $\chi^2 = 0.106$ ,  $df = 1$ ,  $P = 0.744$ , Fig 5.3A), neither the actual presence of the ants themselves ( $\chi^2 = 1.56$ ,  $df = 1$ ,  $P = 0.211$ , Fig 5.3A) affected plant species richness after 3 months. After 9 months we found a very similar pattern. The interaction between clipping and the presence of mounds tended to affect species richness ( $\chi^2 = 3.45$ ,  $df = 1$ ,  $P = 0.063$ , Fig 5.3B), because species richness was higher in the pots with clipping and without mounds only. Again, clipping increased species richness from 4.4 species in unclipped pots to 5.6 species clipped pots ( $\chi^2 = 5.86$ ,  $df = 1$ ,  $P = 0.015$ , Fig 5.3B). Neither the presence of ant mounds ( $\chi^2 = 0.617$ ,  $df = 1$ ,  $P = 0.432$ , Fig 5.3B), nor of poison affected species richness ( $\chi^2 = 0.20$ ,  $df = 1$ ,  $P = 0.653$ , Fig 5.3B). These results show that clipping is the main



**Figure 5.4** Principle response curves showing the extent and direction of plant community changes in our greenhouse experiment along the first RDA axis (PRC1:  $F = 33.704$ ,  $P = 0.002$ ). Curves are plotted relative to changes in C+M-P- (clipping, no mound, no poison), which serves as the reference treatment (striped line). Different symbols represent the different treatments: C-M-P- unclipped, no mound, no poison (closed grey circle); C-M-P+ unclipped, no mound, poison (open grey circle); C-M+P- unclipped, mound, no poison (closed black circle); C-M+P+ unclipped, mound, poison (open black circle); C+M-P+ clipped, no mound, no poison (open grey square); C+M+P- clipped, mound, no poison (closed black square); C+M+P+ clipped, mound, poison (open black square). The vertical 1-D plot represents the scores the plant species on the first RDA axis. A positive value means that a species increases as compared to the control treatment (C+M-P-); while a negative value means a decrease. For clarity, only species with the best fit to the first ordination axis are included in the diagram ("Lower axis minimum fit" was set to 4).

determinant of the number of plant species, because the effect of the interaction between clipping and mound was weak and we did not find consistently different effects of mounds under certain clipping treatments.

### Plant community composition

Our experimental treatments significantly explained plant community changes over time ( $F = 33.80$ ,  $P < 0.001$ ). Detailed analysis showed that this was due to the effect of clipping on temporal plant community patterns ( $F = 34.89$ ,  $P = 0.036$ ). Neither the presence of mounds ( $F = 16.06$ ,  $P = 1.000$ ) nor the addition of poison ( $F = 5.91$ ,  $P = 0.555$ ) could significantly explain plant community development. Figure 5.4 clearly shows that the principle response curves (PRCs) separate out based on the clipping treatments only, while there is little effect of the presence of ant mounds or poison addition. PRCs of clipped pots do not differ much from the reference

treatment that was clipped as well, while unclipped pots have a divergent community composition. Clipped treatments had higher abundances of grazing-tolerant plant species such as *Festuca rubra* and *Agrostis capillaris* while unclipped pots had higher abundances of plant species that become dominant in ungrazed situations, such as *Holcus lanatus* and *Rumex acetosa* (Fig 5.4).

## Discussion

We found that simulated grazing overruled effects of yellow meadow ants on small-scale plant community structure on the short term. Simulated grazing homogenized biomass production and standing crop on and off mounds. In the absence of simulated grazing biomass production and standing crop were enhanced and were higher off than on ant mounds. On the longer term, biomass production and standing crop were only affected by simulated grazing because the influence of ant mounds in unclipped situations disappeared. Consequently, plant species richness and plant community composition were mainly driven by simulated grazing, while ant mound presence and poison addition had little influence.

Effects of aboveground grazers on plant species richness and plant community composition have been well-established (Milchunas and Lauenroth 1993, Olff and Ritchie 1998, Proulx and Mazumder 1998). In general, in productive ecosystems grazers have been shown to increase plant diversity (Bakker 1989, Collins et al. 1998, Bakker et al. 2006). The removal of aboveground biomass enhances light availability and relaxes competition for light between plant species (Olff and Ritchie 1998). This promotes the cover of subordinates (Glenn and Collins 1990, Olff et al. 1997, Knapp et al. 1999, Veen et al. 2008) and may enhance regeneration opportunities for plant species (Grubb 1977, Bakker and de Vries 1992, Bakker and Olff 2003).

In addition to the clear and well-established effects of biomass removal on plant community composition, we expected to find altered plant species richness and community composition on and off ant mounds as well. Previous field studies showed that plant community composition on ant mounds is different from the surroundings due to more open vegetation and changes in soil biotic and abiotic properties (King 1977a, Dean et al. 1997, Blomqvist et al. 2000). Ant mounds provide altered abiotic conditions (Dean et al. 1997, Blomqvist et al. 2000, Dostal et al. 2005) but also a reduction in the abundance of soil-borne pathogens and herbivores (Blomqvist et al. 2000) and changes in the soil microbial community (Dauber and Wolters 2000) which may favor different plant species than in surrounding vegetation. Species richness on ant mounds may either increase when the reduction in biomass that we measured on the mounds favors subdominants (Grime 1973, Goldberg and Miller 1990) or it may decrease when plants that are sensitive to ant

activity decline (Dean et al. 1997). However, we did neither find differences in plant species richness nor in plant community composition on and off ant mounds.

Different factors may explain the lack of an effect of yellow meadow ants on plant community composition. It could be that our grazing treatments were too extreme so that the effect of ants and ant mounds did not become expressed. Simulated grazing may have been too intensive resulting in the promotion of grazing-tolerant plant species thereby overruling other factors driving plant community composition. Indeed we did find a clear increase in *Festuca rubra* and *Agrostis capillaris* in the clipped pots. In contrast the cessation of simulated grazing resulted in the increase of tall grasses, such as *Holcus lanatus*. An increase of tall grasses may stimulate ant activity, but when the ants cannot keep up with the grass height colonies get extinct (King 1977b). In our experiment the ants had little opportunities to access new soil to keep up with vegetation changes in the absence of simulated grazing, which may explain the disappearance of their effects in this situation. Therefore, our experimental pots may have been too limited to be able to quantify ant and mound effects.

Even if the previous explanations for the absence of ant effects hold, we would have expected to find differences on and off mounds at the start of our experiment (Dean et al. 1997, Blomqvist et al. 2000). However, also in the first weeks plant communities on and off mounds were very similar (Fig 5.3). The mounds we collected in the field were very small, but soil heaping of yellow meadow ants in young mounds smothers existing vegetation and leads to the invasion of a new set of plant species directly (King 1977b). Therefore we suggest that in the field cattle grazing may have homogenized the local plant community (Glenn et al. 1992, Adler et al. 2001, Bakker 2003) by locally exerting a strong grazing pressure, thereby overruling effects of yellow meadow ants. Under conditions of more extensive grazing effects of ants on plant community structure and composition may become expressed however. Our data confirm this, because cessation of simulated grazing resulted in altered plant productivity on and off mounds, but only on the short term. Moreover, in field sites in central Germany that were mown once a year, resembling extensive removal of biomass, mounds of yellow meadow ants had an altered plant community composition (Dean et al. 1997).

The effects of yellow meadow ants that were expressed in our experiment, i.e. a reduction in biomass production and standing crop, were governed by the presence of ant mounds and not by the actual presence of the ants (and therefore also root aphids) themselves. Effects of mounds on the vegetation may operate through soil turbation by the ants (Jones et al. 1994). Digging activities alter biotic and abiotic soil conditions, such as water availability, pH and nutrient availability, which influence plant community composition (Dean et al. 1997, Blomqvist et al. 2000). In contrast, effects of ants themselves may run via mutualistic relationships between ants and aphids. In European grasslands about one-third of the aphid species is

tended by ants (Stadler and Dixon 2005). Aphids feed on the phloem sap of plants, which is rich in sugar but low in nitrogen. To obtain enough nitrogen the aphids ingest large quantities of sap and excrete the excess as sugar-rich honeydew which is a main food source of some ant species. In turn, ants may provide protection for the aphids (Stadler and Dixon 2005), keep their eggs in their nests and carry aphids to good feeding places (Pontin 1978). Yellow meadow ants are amongst the ant species that mutually live together with root aphids which can result in increased number of aphids in their nest mounds (Pontin 1978). Associations between yellow meadow ants and aphids may be species-specific and obligatory for some of the aphid species (Pontin 1978). Therefore, removal of yellow meadow ants may reduce aphid performance and affect plant growth and composition. However we could not identify such effects in our experiment. It can be that aphid numbers were not increased in the mounds that we collected, that aphid performance only declined after longer periods without ant attendance, or that aphid-induced changes in the plant community composition take more time. To investigate effects of interactions between ants and aphids on plant community structure and composition it will be useful to set up long-term ant removal experiments in the field or to perform a detailed full-factorial greenhouse experiment where single plants or mixed plant communities are grown on soils with known numbers of aphids in the absence and presence of ants.

## **Conclusion**

In intensively grazed or ungrazed situations effects of yellow meadow ants and ant mounds were not expressed in plant community structure and composition. However, our results show that shortly after the cessation of grazing biomass production is different on and off ant mounds. Therefore, in situations of extensive vertebrate grazing differences in biomass production on and off mounds may become expressed and yellow meadow ants may then become important drivers of small-scale plant community structure and composition.

## **Acknowledgements**

We thank Renate Otter, Jacob Hogendorf and Nelly Eck for assistance during the greenhouse experiment. Matty Berg helped with the Tullgren analysis. We are grateful to Staatsbosbeheer (National Forestry) for permission to collect soil cores in the Junner Koeland nature reserve.







# *Chapter six*



# 6

Small-scale plant community composition affected by interactions between above- and belowground organisms that operate on different spatial scales

G.F. (Ciska) Veen, Elzemiek Geuverink, Han Olff

## Abstract

Aboveground and belowground organisms influence plant community composition by local interactions and their scale of impact may vary from millimeters belowground to kilometers aboveground. However, it is still poorly understood how differences in spatial scale of operation affect the outcome of aboveground-belowground interactions on plant community heterogeneity. Here, we investigate the formation of small-scale heterogeneity in vegetation composition created by subterranean yellow meadow ants (*Lasius flavus*) in the presence of aboveground herbivores that make foraging decisions on a fine grain (European brown hares – *Lepus europaeus*) and on coarse grain (cattle – *Bos taurus*). We hypothesize that small aboveground vertebrate herbivores (hares), with local and selective diet selection, will enforce heterogeneity in vegetation composition that is created by soil-dwelling ants, while large aboveground herbivores (cattle) will homogenize such heterogeneity.

Ant mounds that were only grazed by hares had a very different community composition compared to their surroundings; the cover of the grazing-intolerant grass *Elytrigia atherica* was reduced, whereas the cover of the more grazing-tolerant and palatable grass *Festuca rubra* was enhanced. Combined grazing by hares and cattle, on the other hand, resulted in homogenization of the plant community composition on and off ant mounds, with high overall cover of *Festuca rubra*.

We conclude that the smaller, more selectively foraging aboveground herbivore that we studied promotes small-scale plant community heterogeneity by responding at the same spatial scale to local ant-soil-vegetation interactions. When exposed to both a selective and a larger, less selective aboveground herbivore, patterns of small-scale vegetation heterogeneity triggered by ant-soil-vegetation interactions disappeared.



## Introduction

It is widely acknowledged that aboveground and belowground communities interact using plants as intermediates (van der Putten et al. 2001, Wardle et al. 2004). However, where many studies have shown that aboveground or belowground organisms may influence the spatial and temporal composition of plant communities (e.g. Milchunas and Lauenroth 1993, Dobson and Crawley 1994, Olff and Ritchie 1998, van der Heijden et al. 1998b, Olff et al. 2000, Bradford et al. 2002, Klironomos 2002, De Deyn et al. 2003), relatively few studies have examined their combined effects in a community context. Most previous research on aboveground-belowground interactions has focused on single plant species with specific aboveground and belowground organisms affecting the plant and each other (e.g. Gange and Brown 1989, Masters and Brown 1992, Masters 1995, Gange and Nice 1997, Bezemer et al. 2003, Soler et al. 2005, Soler et al. 2007). However, in natural situations these interactions occur in diverse communities.

The available studies that did investigate above-belowground interactions in diverse plant communities showed that the interactive above-belowground effects on plant community structure are not always simply additive (Brown and Gange 1992, Blomqvist et al. 2000, van Ruijven et al. 2005). For instance, in an experimental community, neutral effects of aboveground herbivores and positive effects of belowground herbivores on plant diversity became negative when these herbivores were added in combination (van Ruijven et al. 2005). Also, it has been shown that mounds of subterranean yellow meadow ants (*Lasius flavus*) harbor different plant communities than their surroundings and that mound building activity was higher with than without large vertebrate herbivores (King 1977b, Blomqvist et al. 2000). Hence, the potential of belowground ant activities to drive small-scale plant patterns is dependent on the activities of aboveground organisms.

An important aspect of combined aboveground-belowground effects in natural communities is the huge difference in spatial scale at which most soil biota affect plants compared to some aboveground organisms, such as vertebrate grazers, due to limited mobility belowground and fewer restrictions aboveground (van der Putten et al. 2009). However, it is not yet clear to what extent aboveground herbivores that operate at a very different spatial scale than soil organisms influence the outcome of above-belowground interactions. We expect that aboveground organisms that operate across much larger scales than soil organisms will interact less with the soil organisms which may result in lower small-scale spatial heterogeneity in plant community composition. In contrast, aboveground organisms that affect plants on a more comparable scale to soil organisms may interact to increase small-scale plant community heterogeneity. Here, we investigate how interactions between soil organisms that operate at small spatial scales and two aboveground vertebrate herbivores that forage at relatively small and large spatial scales respectively affect plant community heterogeneity.

The scale at which aboveground herbivores affect plants and the plant community is generally related to their body size, food quality and quantity requirements, and associated selectivity. Aboveground herbivores in grasslands vary in an order of magnitude in body mass from arthropods (0.01 – 10 g) to small rodents (10 – 100 g) to lagomorphs (1 – 5 kg) to large grazing mammals (50 – 1500 kg). Larger species are expected to have larger home ranges (Haskell et al. 2002), neglect detail in their environment and food when foraging (Ritchie and Olff 1999), and require a lower quality diet and less energy per unit body mass than small herbivores which have a shorter residence time of food in their digestive system (Demment and Van Soest 1985, Gordon and Illius 1994). This forces small herbivores to choose their forage selectively while large herbivores can be less selective in their diet selection (Olff et al. 2002, Hopcraft et al. 2010). Therefore, effects of small herbivores on vegetation composition are more localized, specific and small scale, while effects of larger herbivores are often more generic, less specific and large scale (Ritchie and Olff 1999, Haskell et al. 2002, Cromsigt and Olff 2006, Mouissie et al. 2008, Laca et al. 2010). We will summarize these patterns through the phrase that larger herbivores ‘operate on larger spatial scales’.

In a field experiment on the island of Schiermonnikoog (the Netherlands) we studied plant community patches on mounds of subterranean yellow meadow ants (*Lasius flavus*) in the presence of European brown hares (*Lepus europaeus*) and cattle (*Bos taurus*). Yellow meadow ants are subterranean social insects that locally alter abiotic and biotic soil properties by digging up fresh soil to build nest mounds (Dean et al. 1997, Blomqvist et al. 2000, Dauber and Wolters 2000, Dostal et al. 2005) of 0.1 to 1 m in diameter, up to 0.5 m tall. They tend root aphids in their nest for honey dew exploitation (Pontin 1978). This results under some conditions in a different plant community composition on and off yellow meadow ant mounds and hence in small-scale plant community patches (e.g. Dean et al. 1997, Blomqvist et al. 2000). The social nature of ants causes them to operate at larger spatial scales than many other soil biota and therefore their effects are easier to study in a natural situation.

We expect that hares and cattle respond differently to plant patches created by yellow meadow ants, because they operate on a different scale with different selectivity, as outlined before. Consequently, when plant quality is increased on mounds of yellow meadow ants, e.g. due to increased mineralization rates (Blomqvist et al. 2000), hares may be attracted to these small high-quality patches, whereas cattle may not select for them particularly. This can affect plant community heterogeneity at the local scale (Olff et al. 1999, Bakker 2003, Cromsigt and Olff 2008). We tested the hypothesis that small-scale plant community patches created by below-ground organisms will be more distinct in the presence of selective-feeding above-ground vertebrate herbivores, while plant community patches will more homogeneous when aboveground vertebrates forage on a coarser grain, i.e., operate across larger spatial scales.

## Methods

### Site description

Our study was conducted at the salt marsh of Schiermonnikoog, a barrier island in the Dutch Wadden Sea (53°30'N, 6°10'E). The salt-marsh consists of two parts. One part is grazed by cattle (approx. 400 ha) from May till October at stocking rates of about 1.0 animal/ha and the other part is not grazed by cattle (approx. 1800 ha). Natural vertebrate herbivores grazing the salt marsh are brown hares and rabbits. In our research area rabbits have been reduced to permanently low densities since the outbreak of the Rabbit Hemorrhagic Disease (RHD) in the early 1990's. Therefore, we will refer to the salt marsh grazed by cattle as cattle-grazed marsh and to the cattle-excluded salt marsh as hare-grazed marsh, because cattle and hares were the largest vertebrate grazers present respectively. In the absence of cattle the salt marsh is dominated by the grass *Elytrigia atherica* (Sea couch), while the cattle-grazed marsh has a more diverse plant community with the grass *Festuca rubra* (Red fescue) as one of the dominant plant species. Yellow meadow ants (*Lasius flavus*) are abundant subterranean invertebrates at the salt marsh.

### Data collection

#### PLANT COMMUNITY

In the fall of 2006, we randomly located 5 blocks of 20m × 20m along the cattle fence line on the salt marsh such that half of each block was grazed by cattle and hares and half was grazed exclusively by hares. Elevation, and hence sea flooding frequency, within each block was similar. In each grazing treatment within a block we randomly selected 4 *Lasius flavus* mounds. We set up paired plots of 50cm × 50cm each. One subplot was situated on top of a mound, the other 1 m off that mound. In each pair of plots we recorded vegetation composition by visually estimating cover of the plant species.

The height of the vegetation on and off ant mounds was determined in the summer of 2007. We randomly selected ant mounds and paired control plots in hare- (n = 25 pairs) and cattle-grazed (n = 25 pairs) salt marsh. We recorded settling height of a Styrofoam disc (20 cm diameter, 70 gram weight) that was dropped on top of the vegetation (e.g. Bransby et al. 1977, Sharrow 1984).

To determine nutrient content in plant tissue, we collected green biomass of *Elytrigia atherica* and *Festuca rubra* on and off 6 paired ant mounds and control plots in hare-grazed salt marsh. Samples were collected at the end of summer in 2009 and dried (70°C for 24 hours), grinded and analyzed for C and N content with an autoanalyzer (Interscience, EA 1110, New York, USA).

#### GRAZING FREQUENCY OF VERTEBRATE HERBIVORES

In 2007, we measured grazing frequency of vertebrate herbivores on random pairs

of ant mounds and control plots ( $n = 25$  pairs per grazing treatment). We set up one plot of  $1\text{m} \times 0.1\text{m}$  on an ant mound and the other 1 meter away from that ant mound. Each plot consisted of 10 subplots of  $0.1\text{m} \times 0.1\text{m}$ . In all subplots we randomly counted 10 tillers (or all tillers when there were less than 10) of *Elytrigia atherica* and *Festuca rubra* (which were the dominant plant species) and recorded how many of those were grazed. Moreover, we recorded the number of hare droppings present in each plot, which is regarded a good measure for hare grazing pressure within a specific habitat (Langbein et al. 1999). Finally, in each subplot we recorded plant species cover to determine plant species richness and diversity.

#### ABIOTIC SOIL PROPERTIES AND ANT MOUND CHARACTERISTICS

In 2006 and 2007 we collected one soil sample from the top 5 cm of the soil in the paired plots ( $n = 9$  and  $n = 24$  pairs per grazing treatment, respectively). Soil moisture content was determined by the weight difference between fresh samples and samples that were dried at  $105^\circ\text{C}$  for 24 hours. We determined soil organic matter content (SOM) of dry soil samples (achieved by drying for 24 hrs at  $105^\circ\text{C}$ ) by loss-on-ignition at  $550^\circ\text{C}$  for 3 hrs. SOM was expressed as the relative weight difference before and after ignition.

In 2007 only we dissolved 15 grams of soil in 37 grams of water and samples were homogenized regularly by shaking. After 24 hours we measured pH with a Sentron pH meter (Sentron, Roden, The Netherlands) and EC ( $\text{mS}\cdot\text{m}^{-1}$ ) with a portable EC meter (WTW, Retsch, The Netherlands) in these samples.

Soil temperature was measured in 2007 using I-buttons (Thermochron DS1921K, MAXIM Inc., Sunnyvale CA, USA) that were placed in the top 5-cm of the soil ( $n = 26$  paired plots per grazing treatment). The I-button recorded a temperature measure every 10 minutes for 24 hours. For data analysis we used the minimum and the maximum soil temperature measured.

We measured the thickness of the organic top layer of the soil in the 2007 paired plots ( $n = 26$  paired plots per grazing treatment). The top layer is defined as a fine-grained layer on top of the continuous base layer of coarse sand. In the plots containing ant mounds we also measured ant mound diameter and height, the latter defined as the distance between the highest point of the mound to the soil surface.

In 2009 we measured the diameter, *Festuca rubra* cover percentage and number of hare droppings of more than 400 mounds in the hare-grazed area to establish the relationship between mound size, plant cover and hare visitation.

#### Data analysis

In our experiment *Elytrigia atherica*, *Festuca rubra* and bare soil represented the most dominant groups with their cover percentages adding up to 82% (cattle-grazed, off mound), 93% (cattle-grazed, on mound), 102% (hare-grazed, off mound)



and 89% (hare-grazed, on mound) in the four treatments. Therefore, we focus data analysis of the plant community on the dominant plant species *Elytrigia atherica* and *Festuca rubra* only.

To determine plant community structure we calculated plant species richness (S, the total number of plant species per subplot) and Shannon's diversity index ( $H'$ ,  $H' = -\sum p_i \cdot \ln(p_i)$  where  $p_i$  is the relative cover or abundance of species  $i$  (Magurran 2003).

For the temperature data we used the relative difference between soil ( $T_{\text{soil}}$ ) and air ( $T_{\text{air}}$ ) temperature:  $(T_{\text{soil}} - T_{\text{air}})/T_{\text{air}}$  as response variable to be able to compare measurements that were collected on different days with different air temperatures. We calculated the differences between maximum and minimum soil and air temperature respectively. Air temperature data were obtained from the KNMI (Royal Dutch Meteorological Institute) database using the data from the Lauwersoog weather station.

We analyzed the cover values of the dominant plant species and the number of hare droppings with a generalized linear mixed model assuming a Poisson error distribution and log-link function, because data were counts. Grazing frequency was analyzed with a logistic regression with a logit link function, because tillers counted were either grazed or ungrazed. Plant species richness, plant diversity, vegetation height, plant quality (expressed as percentage N measured in leaves and as C/N ratio), and the metrics for abiotic (soil) properties and mound characteristics were analyzed using a general linear mixed model. Data on ant mound height and SOM content were log-transformed to improve normality (Shapiro test) and homogeneity of variances (Levene's test). In all analyses grazing treatment and ant mound presence were used as fixed factors and the experimental setup was described by a random factor. For plant community analyses the random factor was described by nesting paired plot in grazing treatment in block, i.e., block/grazing treatments/paired plot. For data on grazing frequency the random factor was described by nesting subplot within paired plot within grazing treatment, i.e., grazing treatment/paired plot/subplot. For the other analyses we only used paired plot nested within grazing treatment as a random factor, i.e., grazing treatment/paired plot, because data were not collected according to the randomized block design.

To analyze the relationship between ant mound diameter and *Festuca rubra* cover and between mound diameter and number of hare droppings, we used linear regression analysis. Mound diameter was used as a predictor variable, and *F. rubra* cover and number of hare droppings as a response variable. We included data from hare-grazed salt marsh only because here we observed a plant community shift.

All data were analyzed using R, version 2.10.0. The lme4 package (Bates and Maechler 2009) was used to calculate general and generalized linear mixed models and logistic regression, using maximum likelihood estimation. To determine effects

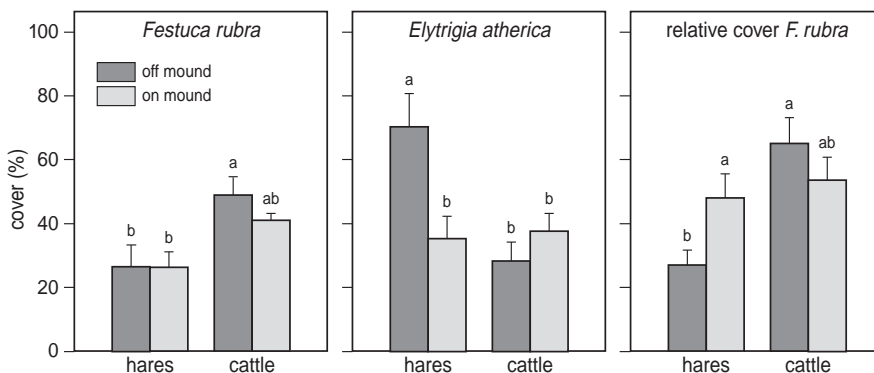
of our fixed factors we used a likelihood ratio test to compare models with and without the variable of interest (Crawley 2007) using a chi-square test statistic. When interaction effects were significant we do not report the effects of the main factors (Crawley 2007). Post-hoc comparisons were made using a Tukey's test.

## Results

### Plant community responses

An interaction between the presence of yellow meadow ants and grazing treatment affected the cover of the dominant salt marsh plants. *Elytrigia atherica* cover was higher off ant mounds in hare-grazed salt marsh only ( $\chi^2 = 184.61$ ,  $df=1$ ,  $P < 0.001$ ; Fig 6.1). *Festuca rubra* cover was increased in the cattle-grazed salt marsh in general, with a maximum cover off ant mounds under cattle grazing ( $\chi^2 = 4.92$ ,  $df=1$ ,  $P = 0.026$ ; Fig 6.1). The relative cover of *Festuca rubra* (cover *Festuca*/(cover *Festuca* + *Elytrigia*)) was significantly lower off ant mounds in hare-grazed salt marsh only ( $\chi^2 = 131.43$ ,  $df=1$ ,  $P < 0.001$ ; Fig 6.1), indicating a shift in plant dominance from *Festuca rubra* to *Elytrigia atherica* off ant mounds under hare grazing. In contrast, in cattle-grazed salt marsh ant mounds and surroundings were both dominated by *Festuca rubra*.

Plant species richness was higher in cattle-grazed than in hare-grazed salt marsh plots ( $\chi^2 = 8.76$ ,  $df=1$ ,  $P = 0.003$ ; table 6.1). Plant community diversity ( $H'$ ) was determined by an interaction between grazing treatment and the presence of ant mounds ( $\chi^2 = 4.24$ ,  $df=1$ ,  $P = 0.040$ ; table 6.1). Diversity was higher in cattle-



**Figure 6.1** Cover of the dominant plant species on and off ant mounds in hare-grazed and cattle-grazed salt marsh is represented in the first two graphs. The third graph represents the relative of *Festuca rubra* compared to the sum of the cover of *Festuca rubra* and *Elytrigia atherica* (cover *Festuca*/(cover *Festuca* + *Elytrigia*)). Error bars represent the standard error. Different letters above the bars indicate significant differences at  $P < 0.05$ .

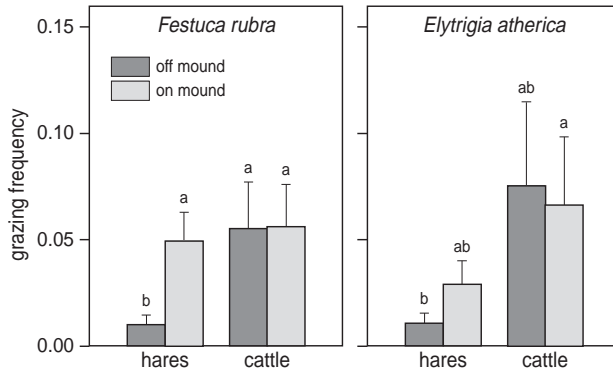


grazed salt marsh, regardless of the presence of ant mounds. In hare-grazed salt marsh diversity was generally lower and was most reduced on ant mounds compared to off mounds.

Vegetation height also was affected by an interaction between grazing treatment and mound presence ( $\chi^2 = 31.41$ ,  $P < 0.001$ ,  $df = 1$ ; table 6.1). Vegetation was higher off mounds in hare-grazed marsh only, while there was no difference between

**Table 6.1** Overview of effects of grazing treatment (hare grazing vs. cattle grazing) and ant mound presence (on vs. off ant mounds) on plant community characteristics, vertebrate grazers, soil abiotic properties and mound properties. Mean values are displayed with SE between brackets. Different letters behind mean values indicate significant differences at  $P < 0.05$ .

	Hare grazed, off mound	Hare grazed, on mound	Cattle grazed, off mound	Cattle grazed, on mound
<b>Plant community characteristics</b>				
Species richness	1.71 (0.13) <sup>b</sup>	1.26 (0.15) <sup>b</sup>	3.84 (0.16) <sup>a</sup>	3.95 (0.29) <sup>a</sup>
Diversity ( $H'$ )	0.42 (0.04) <sup>b</sup>	0.29 (0.04) <sup>b</sup>	0.99 (0.04) <sup>a</sup>	1.01 (0.07) <sup>a</sup>
%N <i>F. rubra</i>	1.57 (0.01) <sup>b</sup>	2.20 (0.08) <sup>a</sup>	ND <sup>1)</sup>	ND <sup>1)</sup>
C/N ratio <i>F. rubra</i>	26.8 (0.68) <sup>b</sup>	20.4 (1.83) <sup>a</sup>	ND <sup>1)</sup>	ND <sup>1)</sup>
%N <i>E. atherica</i>	1.19 (0.03) <sup>a</sup>	1.38 (0.03) <sup>a</sup>	ND <sup>1)</sup>	ND <sup>1)</sup>
C/N ratio <i>E. atherica</i>	37.7 (2.71) <sup>a</sup>	32.5 (2.05) <sup>a</sup>	ND <sup>1)</sup>	ND <sup>1)</sup>
Vegetation height	28.9 (1.24) <sup>a</sup>	8.8 (1.41) <sup>b</sup>	8.39 (1.34) <sup>b</sup>	6.31 (0.90) <sup>b</sup>
<b>Soil abiotic properties</b>				
pH	8.53 (0.07) <sup>ab</sup>	8.44 (0.05) <sup>ab</sup>	8.21 (0.08) <sup>b</sup>	8.57 (0.07) <sup>a</sup>
EC (mS·m <sup>-1</sup> )	0.72 (0.11) <sup>a</sup>	0.59 (0.08) <sup>ab</sup>	1.46 (0.21) <sup>a</sup>	0.53 (0.06) <sup>b</sup>
Soil organic matter (%) May 2006	13.8 (1.50) <sup>ab</sup>	4.12 (0.82) <sup>c</sup>	26.5 (2.80) <sup>a</sup>	5.00 (0.50) <sup>bc</sup>
Soil organic matter (%) July 2007	4.13 (0.30) <sup>b</sup>	4.15 (0.23) <sup>b</sup>	10.1 (0.93) <sup>a</sup>	6.64 (0.85) <sup>b</sup>
Soil moisture content (%) May 2006	48.1 (2.30) <sup>b</sup>	20.7 (2.18) <sup>c</sup>	56.3 (2.89) <sup>a</sup>	23.4 (2.09) <sup>c</sup>
Soil moisture content (%) July 2007	32.2 (2.77) <sup>ab</sup>	28.1 (3.05) <sup>ab</sup>	37.6 (2.00) <sup>a</sup>	32.7 (1.84) <sup>b</sup>
Relative maximum temp. temperature	-0.09 (0.02) <sup>c</sup>	0.17 (0.03) <sup>a</sup>	0.08 (0.01) <sup>b</sup>	0.17 (0.02) <sup>a</sup>
Relative minimum temp.	0.09 (0.02) <sup>b</sup>	0.03 (0.02) <sup>c</sup>	0.18 (0.02) <sup>a</sup>	0.09 (0.01) <sup>bc</sup>
Top layer thickness (cm)	7.35 (0.99) <sup>b</sup>	19.6 (0.87) <sup>a</sup>	7.48 (0.58) <sup>b</sup>	14.3 (0.81) <sup>a</sup>
<b>Mound properties</b>				
Mound height (cm)	ND <sup>1)</sup>	14.6 (0.83) <sup>a</sup>	ND <sup>1)</sup>	10.4 (0.87) <sup>a</sup>
Mound diameter (cm)	ND <sup>1)</sup>	97.7 (5.90) <sup>a</sup>	ND <sup>1)</sup>	50.8 (3.77) <sup>b</sup>
<sup>1)</sup> No data available				



**Figure 6.2** Grazing frequency by vertebrate herbivores on and off ant mounds in hare-grazed and cattle-grazed salt marsh. Error bars represent the standard error. Different letters above the bars indicate significant differences at  $P < 0.05$ .

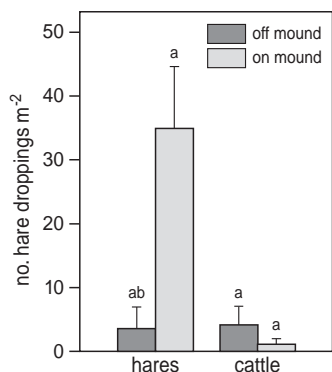
vegetation height on hare-grazed mounds and on and off cattle-grazed mounds. This indicates that plots off mounds in hare-grazed salt marsh were grazed less often than all other plots.

Plant quality on ant mounds was enhanced (table 6.1). For *Festuca rubra* percentage of nitrogen in leaf tissue was higher on ant mounds ( $\chi^2 = 8.67$ ,  $df = 1$ ,  $P = 0.003$ ) and C/N ratio was reduced ( $\chi^2 = 8.88$ ,  $df = 1$ ,  $P = 0.003$ ). For *Elytrigia atherica* percentage of nitrogen also tended to be increased in leaf tissue on ant mounds ( $\chi^2 = 3.61$ ,  $df = 1$ ,  $P = 0.057$ ) and C/N ratio tended to be lower ( $\chi^2 = 3.23$ ,  $df = 1$ ,  $P = 0.072$ ).

### Grazing frequency

Grazing frequency on *Festuca rubra* was lower off than on ant mounds in hare-grazed salt marsh only ( $\chi^2 = 16.59$ ,  $P = 0.001$ ,  $df = 1$ ; Fig 6.2). This shows that hares alone were exerting high grazing pressure on ant mounds in hare-grazed marsh, resembling that of cattle and hares together in cattle-grazed marsh. Cattle-grazing pressure did not differ significantly on and off ant mounds. *Elytrigia atherica* tended to be grazed more frequently in cattle-grazed marsh and tended to be grazed least frequent off mounds in hare-grazed salt marsh ( $\chi^2 = 2.83$ ,  $P = 0.092$ ,  $df = 1$ ).

Counts of hare droppings revealed a similar pattern as grazing frequency data (Fig 6.3). We found most hare droppings per  $m^2$  on ant mounds in hare-grazed salt marsh ( $\chi^2 = 15.39$ ,  $P < 0.001$ ,  $df = 1$ ), while almost no droppings were observed both off ant mounds in the hare-grazed marsh and in the cattle-grazed marsh. This indicates that hares are specifically attracted to ant mounds in the absence of cattle.



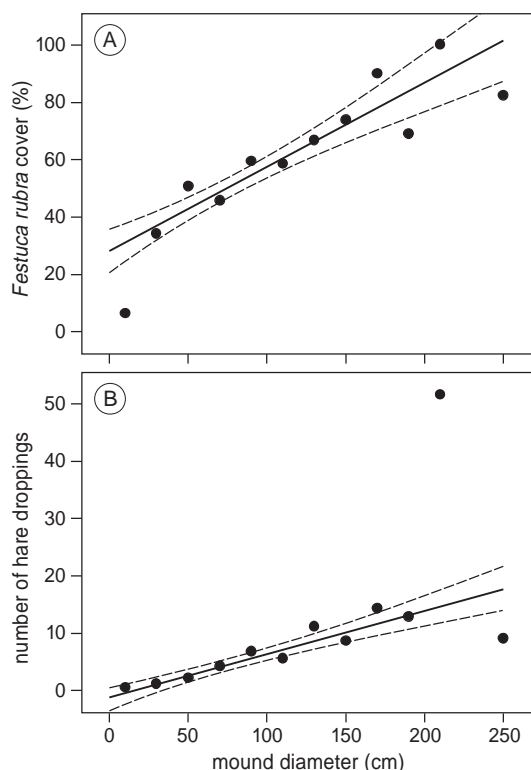
**Figure 6.3** Number of hare droppings on and off ant mounds in hare-grazed and cattle-grazed salt marsh. Error bars represent the standard error. Different letters above the bars indicate significant differences at  $P < 0.05$ .

### Soil properties and mound characteristics

Soil abiotic properties were affected by grazing treatment and the presence of ant mounds in different ways (table 6.1). An interaction between grazing treatment and ant mounds presence affected pH ( $\chi^2 = 22.37$ ,  $P < 0.001$ ,  $df = 1$ ), electric conductivity (EC;  $\chi^2 = 19.57$ ,  $P < 0.001$ ,  $df = 1$ ), and soil organic matter content in 2007 (SOM;  $\chi^2 = 8.26$ ,  $P = 0.004$ ,  $df = 1$ ), with pH being lower, and EC and SOM being higher off ant mounds in cattle-grazed marsh only. SOM content in 2006 was affected by ant mounds presence only, and was lower on than off mounds in both grazing treatments ( $\chi^2 = 52.43$ ,  $P < 0.001$ ,  $df = 1$ ). Soil moisture content was affected by an interaction between grazing treatment and ant mounds presence in 2006 ( $\chi^2 = 6.65$ ,  $P = 0.010$ ,  $df = 1$ ) and by ant mound presence in 2007 ( $\chi^2 = 9.33$ ,  $P = 0.002$ ,  $df = 1$ ). In both years surrounding soils were wetter than ant mound soils, and in 2006 surrounding soils in cattle-grazed marsh were wetter than in hare-grazed marsh. Almost all relative temperature differences were positive, indicating that soil temperature was generally higher than air temperature. Relative differences for maximum temperature were affected by an interaction between grazing treatment and presence of ant mounds ( $\chi^2 = 20.00$ ,  $P < 0.001$ ,  $df = 1$ ; table 6.1). Soil in ant mounds warmed up most, while soil off ant mounds in hare-grazed marsh was coldest. Relative minimum temperature differences were smaller on than off ant mounds, indicating that soil on ant mounds cooled down more at night than surrounding soils, particularly in cattle-grazed marsh where vegetation height is low ( $\chi^2 = 30.33$ ,  $P < 0.001$ ,  $df = 1$ ; table 6.1).

### Mound diameter relationships

Ant mounds had a larger diameter ( $F = 38.04$ ,  $P < 0.001$ ,  $df = 1$ ) in hare-grazed than



**Figure 6.4** Relationship between ant mound diameter and (A) *Festuca rubra* cover and (B) the total number of hare droppings per mound for  $n = 412$  ant mounds. The regression line represents a linear fit, and the dotted lines represent a 95% confidence interval. For clarity in the graph data were averaged for size classes of ant mound diameters (0–20 cm, 21–40 cm, 41–60 cm etc). Dots represent averages of the measurements within each size class.

in cattle-grazed marsh, but did not differ in height ( $F = 2.20$ ,  $P = 0.141$ ,  $df = 1$ ; table 6.1). Mounds with a larger diameter in hare-grazed marsh had a higher percentage of *Festuca rubra* cover ( $F_{1,410} = 51.02$ ,  $P < 0.001$ ,  $R^2 = 0.11$ , Fig 6.4A). Moreover, mound diameter was positively related with the total number of hare droppings per mound ( $F_{1,410} = 49.35$ ,  $P < 0.001$ ,  $R^2 = 0.11$ , Fig 6.4B). This is not only caused by larger mounds having more space, because also the number of hare droppings per  $m^2$  increased with ant mound diameter ( $F_{1,410} = 158.5$ ,  $P < 0.001$ ,  $R^2 = 0.28$ ). This indicates that hares spend more time foraging on larger ant mounds.

## Discussion

Our study showed that small-scale plant community mosaics depend on the type of aboveground herbivores that interact with soil organisms. This has been shown for model systems where it was found that root herbivore identity determined plant-mediated interactions between root and shoot herbivores (Wurst and van der Putten 2007), but has not been shown in a field situation. We found that grazing by hares alone resulted in distinct plant community patches on mounds of yellow meadow ants, while grazing by cattle and hares in combination resulted in more homogeneous plant community composition.

Yellow meadow ants are ecosystem engineers (Jones et al. 1994, 1997) that modify biotic and abiotic soil properties which can enhance small-scale plant community mosaics (Dean et al. 1997, Olff et al. 2000, Blomqvist et al. 2000). In our study site soil abiotic properties, such as soil moisture content, soil organic matter content, top layer thickness and temperature, were consistently different on and off ant mounds, regardless of vertebrate grazing treatment. However, these changes in soil abiotic conditions did not per se lead to clear plant community patches, because the interplay between ants and aboveground herbivores was important driving small-scale plant community composition on ant mounds as well.

Small vertebrate herbivores need higher amounts of energy per unit body mass than large vertebrate herbivores (Demment and Van Soest 1985, Hopcraft et al. 2010). As a results, small grazers, such as hares, depend on high quality food and select their forage on a fine grain, while large herbivores, such as cattle that are restricted more by food quantity and operate on a coarser grain (Ritchie and Olff 1999, Haskell et al. 2002, Laca et al. 2010). Ants speed up local mineralization rates through higher soil aeration (Blomqvist et al. 2000) and fragmentation of soil organic matter which can lead to a enhanced nitrogen content in plants growing on mounds. We showed that plant nitrogen content on mounds was higher and that hares specifically selected for these small-scale patches with better food quality. Increased grazing pressure of hares on ant mounds locally reduced the dominance of the grazing-intolerant and unpalatable plant species *Elytrigia atherica* and enhanced *Festuca rubra*, a grazing-tolerant plant species of higher nutritional value (Kuijper et al. 2004). As *Festuca rubra* is a preferred food plants of hares (Kuijper et al. 2008) ant mounds will become increasingly attractive to hares. This results in a progressive shift in community composition towards a domination of *Festuca rubra*. Therefore, ants and hares interact through a positive feedback, because both affect plant quality in the same local patch, which ultimately leads to clearly pronounced small-scale plant community patches of high nutritional value that are dominated by more grazing-tolerant plant species.

In contrast, in cattle-grazed salt marsh vertebrate grazing frequency on and off ant mounds was not different, indicating that neither cattle nor hares selected plant

patches on ant mounds particularly. Cattle have been shown to reduce the cover of *Elytrigia atherica* and enhance the cover of high-quality and grazing-tolerant *Festuca rubra* on a landscape scale (Olf et al. 1997). As a result, cattle facilitate hares by increasing food quality on the landscape scale (Kuijper et al. 2008) and therefore hares do not have to select for specific small patches on ant mounds to meet their energy requirements. The more homogeneous grazing pressure of vertebrates across large spatial scales leads to less pronounced small scale plant community patches on ant mounds. Hence, herbivores that select their forage on larger spatial scales than patches created by soil organisms, may overrule the effects of soil organisms on plant community composition. How strong the overruling effects of aboveground vertebrates are will probably also depend on herbivore type and herbivore density.

We showed that differences in the scale of operation between above- and belowground organisms are important determining the outcome of their interactions for vegetation patterns. On the one hand, when aboveground organisms, such as hares, selectively choose patches created by belowground organisms, plant community patterns driven by aboveground-belowground interactions may become enforced. On the other hand, when aboveground organisms operate on a much larger scale than belowground organisms, such as cattle, aboveground-belowground interactions may become less important determining plant community patterns. In this situation aboveground herbivores may overrule and homogenize plant community patterns created by soil organisms. In other studies it has been shown that organisms that operate at relatively similar spatial scales can also influence each others patch selection which may drive plant community patterns. For example, prairie dogs, which are digging, social, vertebrate herbivores, build colonies that can range over more than 100 hectares. At these scales the prairie dogs alter plant community composition and increase the nutritional value of the vegetation (Coppock et al. 1983a). Bison, which are large vertebrate grazers, have been shown to selectively forage in habitats affected by prairie dog colonies, probably due to changes in plant community structure and increased food quality (Coppock et al. 1983b).

To further investigate the importance of differences in the scale of operation between aboveground and belowground organisms it would be useful to perform a full-factorial field experiment. Our current experiment was lacking a treatment without vertebrate herbivores, because long-term hare exclosures were not available near to our study plots. However, previous work on Schiermonnikoog showed that exclusion of all aboveground vertebrate grazers (i.e. cattle and hares) resulted in a homogeneous stand of *Elytrigia atherica* (Olf et al. 1997, Bos et al. 2002, Kuijper et al. 2004, Kuijper and Bakker 2005). This confirms the idea that an interaction between aboveground and belowground organisms both operating at a relatively small scale can drive local plant community mosaics.

## Conclusions

The interaction between subterranean yellow meadow ants and hares resulted in a shift in plant community composition, because hares specifically selected for high quality patches on ant mounds. In contrast, cattle exerted a more homogeneous grazing pressure on and off ant mounds thereby overruling effects of ants on plant community composition. We conclude that the promotion of small-scale plant community heterogeneity as a result of aboveground-belowground interactions is dependent on the selectivity of aboveground herbivores.

## Acknowledgements

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# *Chapter seven*

# 7

## Contrasting effects of large herbivores on nitrogen mineralization across ecosystems: integrating effects of soil physical properties.

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## Abstract

The general view of the role of large herbivores in grassland ecosystem functioning is that they speed up nutrient cycling, especially nitrogen (N) mineralization. This classic 'grazing lawn' effect is thought to arise because faecal and urine inputs by herbivores increase soil microbial activity and thus N mineralization, which promote plants with leaf N concentrations, which finally again attracts herbivores. However, field experiments increasingly show that large herbivores often reduce soil N mineralization rates instead of increasing them.

We explain this through effects of large herbivores on soil physical processes, which under some conditions are found to outweigh positive effects on N mineralization. We expect these effects to be strongest in wet, fine-textured soils. In such soils compaction causes periods of soil hypoxia during which aerobic decomposers (both microbes and soil macrofauna) are inhibited which may result in a reduction in N mineralization.

We use long-term field data from experiments in temperate grasslands with different grazing and mowing treatments to substantiate this proposed role of soil compaction in slowing N turnover and promoting soil hypoxia. These experiments reveal that plant species adapted to periods of soil hypoxia and to low N availability generally have higher abundances in more compacted soils.

We conclude that the effect of large herbivores on N mineralization depends on a critical balance between a set of positive and negative effects. Including herbivore effects on soil physical properties into current theories on N cycling in grassland will advance our understanding of contrasting results of herbivores on mineralization rates.

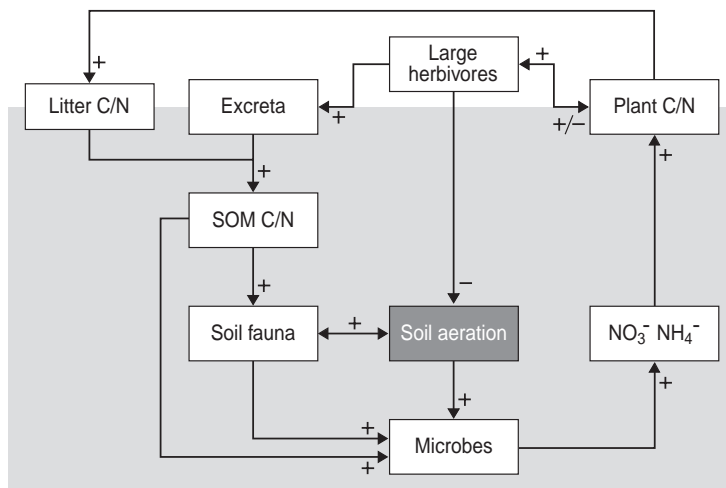


## Introduction

Large vertebrate herbivores play a key role in determining the community structure and functioning of grassland ecosystems. They influence nitrogen (N) mineralization rates, thereby altering plant productivity (Georgiadis et al. 1989, Frank and McNaughton 1993, Frank et al. 2000), as this is often limited by N availability (Schlesinger 1991, Vitousek and Howarth 1991). The prevailing theory is that large herbivores locally enhance the productivity of grasslands through promoting N mineralization. As herbivores enhance the primary production and improve grass quality of the grass, which again attracts more herbivores, this can be viewed as a grazer-induced positive feedback loop on their own density (McNaughton 1984, Pastor et al. 2006). Increased N availability caused by large herbivores has therefore been proposed as a form of self-facilitation where herbivores improve the availability and quality of their food plants in specific locations that are inherently not very different from their surroundings (McNaughton 1984, Belsky 1986, Fryxell 1991, McNaughton et al. 1997a). Indeed, several studies where large herbivores were excluded from plots demonstrate a decreased availability of N in the absence of large herbivores (McNaughton et al. 1997b, Frank and Groffman 1998, Knapp et al. 1999). However, other field studies show an increase of N mineralization in the absence of large herbivores (Pastor et al. 1993, Ritchie et al. 1998, van Wijnen et al. 1999, Bakker et al. 2004), indicating that the positive feedback loop of herbivore-induced increased N availability is not universal.

Several authors have tried to explain contrasting effects of herbivores on N mineralization as a function of herbivore-induced changes on the quality of resources entering the soil food web (Pastor et al. 1993, Hobbs 1996, Ritchie et al. 1998, Pastor et al. 2006). Yet field tests could not confirm the predictions of these theories (Bakker et al. 2006, Bakker et al. 2009). Apparently, the effect of large herbivores on N mineralization in grasslands is still not fully understood.

Existing hypotheses on contrasting effects of grazing on N cycling in natural grasslands have so far largely ignored the physical effects of large herbivores on soil parameters. For example by trampling and the removal of aboveground plant biomass herbivores can have an important influence on e.g. soil moisture, soil aeration and soil temperature. Changes in these soil properties may have major consequences for nutrient cycling, and hence for plant productivity and plant N concentrations, and thus again for herbivores attraction. Recent evidence suggests that this may be a promising avenue for explaining many of the seemingly contradictory results (Sankaran and Augustine 2004, Hamza and Anderson 2005, Startsev and McNabb 2007, Sørensen et al. 2009). Empirical studies that have taken trampling effects of herbivores into account have done so in a very narrow window of abiotic soil parameters and have therefore not been able to show effects on N cycling (Curll and Wilkins 1983, Mikola et al. 2009). Here we propose a general framework that



**Figure 7.1** Overview of the influence of large herbivores on the nitrogen (N) cycle in grasslands. This diagram depicts three main pathways via which herbivores can influence soil N mineralization. Prevailing theories mainly focus on the two pathways via which herbivores alter the quality of resources entering the soil food web, i.e., by excreting dung and urine and by changing plant quality. The third pathway focuses on herbivore effects on soil physical properties. For example, trampling-induced soil compaction may decrease aeration and consequently alter soil N mineralization. In this figure we integrate the three pathways which will lead to a better understanding of N cycling in terrestrial ecosystems.

incorporates effects of herbivores on soil physical parameters into current theories of N cycling (Fig 7.1). This will advance our understanding of contrasting results of grazing on N mineralization rates in natural grasslands. To illustrate how large herbivores can modify N cycling by means of trampling induced soil compaction, we use data on soil parameters and vegetation composition from long term field experiments.

### Explaining contrasting effects of herbivores on mineralization

Several mechanisms have been suggested to explain contrasting effects of large herbivores on N mineralization (see Fig 7.1). The main focus has been on herbivore effects on the quality of resources entering the soil food web. First, herbivores may speed up or slow down N mineralization by returning excreta to the soil. On the one hand, herbivore excreta provide easily available N which stimulates the activity of soil microbes (McNaughton et al. 1997a, 1997b). On the other hand, herbivores return N in different forms, i.e. as urea and faeces which have different decomposition rates, also dependent on microclimate conditions (Hobbs 1996). Urea provides rapidly degradable N, whereas faeces are much slower to decompose, sometimes even slower than plant detritus. Therefore, N excreted in faeces may reduce N mineralization rates (Pastor et al. 2006).

Second, a combination of plant quality and herbivore selectivity may explain contrasting results of herbivores on N mineralization (Ritchie et al. 1998, Pastor et al. 2006). When quality of the dominant plant species is high, herbivores can forage unselectively and return N in urea, which favours growth of fast-growing grazing-tolerant plants (Olff and Ritchie 1998). This can lead to the formation of grazing lawns and to a general increase in N mineralization. However, when the dominant plants are of poor quality, herbivores selectively feed on the high quality subordinate species (as legumes vs. grasses in grasslands, or deciduous tree saplings vs. conifers in forests), which then leads to a shift in community composition towards poor quality species (Pastor et al. 2006). A decrease in plant quality will lead to reduced litter quality and hence may slow down decomposition and mineralization rates (see Table 7.1, Berendse et al. 1989, Wardle et al. 2004, Bardgett 2005). Consequently, herbivores may affect N mineralization in contrasting ways along a gradient of soil fertility. At high fertility sites, where plant quality is high, herbivores may change community composition through inducing a positive feedback on their own abundance by increasing N mineralization and at low fertility sites with low plant quality they may induce a negative feedback (Pastor et al. 1993, Ritchie et al. 1998, Augustine et al. 2003). Whereas examples can be found that support this theory (Pastor et al. 1993, Ritchie et al. 1998), contrasting results are found in other studies. For example, at sites with high soil fertility and high plant quality, such as salt marshes and river floodplains, herbivores have been shown to reduce N mineralization instead of improve it (van Wijnen et al. 1999, Bakker et al. 2004). Hence, both mechanisms described above do still not satisfactorily explain contrasting effects of herbivores on N cycling and neither explicitly take effects of large herbivores on soil physical parameters into account.

Changes in soil physical properties can have a major influence on nutrient mineralization (Hamza and Anderson 2005, Startsev and McNabb 2007) and the effects may be dependent on environmental conditions. For example, in arid environments biomass removal by herbivores can reduce plant cover and litter input to the soil, which may lead to reduced water holding capacity of the soil and increased soil evaporation (van de Koppel et al. 1997). Moreover, in these systems soil compaction by trampling can reduce infiltration of water into the soil. Consequently, in arid environments soil organic matter and water may become limiting for N mineralization in grazed conditions. Herbivores may also affect soil temperature by modifying the insulating capacities of the vegetation through the removal of biomass (Gornall et al. 2009). In cold systems reduced vegetation cover may lead to cooling down of the soil in cold periods, thereby decreasing N mineralization, while in warm periods, soils with low vegetation cover may heat up quickly, which favours mineralization. These examples show that herbivores can modify soil physical properties, which can have important consequences for soil N cycling.

Therefore, we propose that adding herbivore impact on soil physical properties to current models of herbivore effects on N mineralization rates will strongly improve our understanding of the contrasting effects of herbivores on N mineralization (Fig 7.1). From here onwards we will discuss our proposed integrated approach using consequences of soil compaction in temperate grassland as an example showing how this integrated approach may advance our understanding of N cycling in grazed ecosystems.

### **How trampling-induced soil compaction can reduce N mineralization in grasslands**

In every soil some pores will contain water and others will contain air. The amount of water a soil can hold depends on many different factors. Water holding capacity is increased when clay and organic matter content are high or when the fraction of small pores is large (see Bardgett 2005). When soils get compacted, for example as a result of trampling by large herbivores, pore size is reduced, which enhances the fraction of small pores and hence increases water holding capacity (Wolkowski 1990). Generally, when more than 60% of pores are water-filled, diffusion of oxygen is severely hampered because diffusion in water happens 10,000 times slower than in air (Russell 1973). Consequently, in compacted soils oxygen may become limiting for microbial processes, but also for soil fauna and metabolism of plant roots. This may lead to lower N availability because on the one hand nutrient mineralization is slowed down (Breland and Hansen 1996, Jensen et al. 1996, De Neve and Hofman 2000) and on the other hand because denitrification is sped up under anaerobic conditions (e.g. Haunz et al. 1992, De Klein and Van Logtestijn 1994, Lipiec and Stepniewski 1995). Wollersheim et al (1987) showed experimentally that increased bulk density resulted in reduced soil aeration which led to elevated soil moisture levels and denitrification rates.

As the amount of small pores increases in compacted soils, pores available to soil fauna are reduced (Breland and Hansen 1996). Moreover, organic material and microbial biomass become physically protected against attack by soil fauna and microbial feeders, respectively (Breland and Hansen 1996). Consequently, soil fauna densities are found to decrease in more compacted or trampled soils (Whalley et al. 1995, Bouwman and Arts 2000, Sørensen et al. 2009). Higher trophic level soil fauna do not directly mineralize nutrients, i.e. convert organic matter into inorganic forms, but they may affect mineralization rates of microbes and hence the availability of mineral nutrients in the soil (Table 7.1, Osler and Sommerkorn 2007). Several studies report a contribution of soil fauna to nutrient mineralization rates (Verhoef and Brussaard 1990, de Ruiter et al. 1993, Bardgett and Chan 1999, Berg et al. 2001). Particularly, in situations where the microbial community is N limited, the contributions of soil fauna to net N mineralization rates may be relatively high (Osler and Sommerkorn 2007).



**Table 7.1** Overview of processes determining nutrient mineralization. Direction in which these processes are influenced by soil compaction is indicated using signs.

Factors affecting mineralization	main direction <sup>a</sup>	explanation
<b>Plants</b>		
plant quality	+	enhanced availability of nutrients stimulates decomposer activity a)
oxygen transport through roots	+	marsh plants use aerenchym to transport oxygen, locally increasing bacterial production b)
root exudates	+	input of carbon into the soil causes higher microbial production c)
<b>Large herbivores</b>		
defoliation of vegetation	+	defoliation results in higher root exudation and litter quality causing higher decomposition d)e)
dung and urine production	+	nitrogen in wastes becomes easily available to microorganisms and plants f) g)
changing plant species community	+/-	preferential feeding on high/low quality results in high/low quality vegetation and litter i)
trampling	+/-	+ litter availability to decomposers increases h) - high trampling results in anoxic soil which inhibits decomposition (this paper)
<b>Soil fauna</b>		
increased organic matter availability to microbes	+	soil fauna pellets serve as favourable mineralization setting j)
stimulating mineralization in gut	+	guts of earthworms, termites, microarthropods stimulate soil microbial decomposition k), l)
excreting excess nutrients	+	by foraging on microbes, soil fauna accelerates nutrient mineralization ("microbial loop") m)
bioturbation	+	termites, mice, earthworms, ants create macropores by digging and burrowing activities n), o)
<b>Soil parameters</b>		
soil fertility	+/-	high soil fertility yields high mineralization, low soil fertility leads to lower mineralization p)
compaction in clay soil	-	compaction causes lower soil aeration resulting in high denitrification and low mineralization q)
compaction in sandy soil	+	compaction causes higher litter infiltration and therefore higher nutrient availability to soil detritivores q)
soil moisture	+/-	mineralization works optimal under intermediate moisture conditions. Both in dry and very wet soils mineralization is impeded. r)
<sup>a</sup> Indicates whether mineralization is generally enhanced (+) or decreased (-)		
a) (Wardle 1998); b) (Vartapetian and Jackson 1997); c) (Bardgett et al. 1998); d) (Briske et al. 1996); e) (Ferraro and Oosterheld 2002); f) (Afzal and Adams 1992); g) (Bogaert et al. 2000); h) (Zacheis et al. 2001, 2002); i) (Ritchie et al. 1998, Wardle 2002); k) (Trigo et al. 1999); l) (Daniel and Anderson 1992); m) (Clarholm 1985); n) (Lavelle et al. 1997); o) (Brown et al. 2000); p) (Sankaran and Augustine 2004); q) (Rasiah and Kay 1998); r) (Bardgett 2005)		

### **Effects of trampling on plant growth**

Soil compaction may have severe consequences for the growth of plants. Most studies report negative effects of grazer-induced soil compaction on plant growth (Abdelmagid et al. 1987b, Menneer et al. 2005, Hamza and Anderson 2005, Drewry et al. 2008). On the one hand, N mineralization rates in compacted soils may be reduced which will result in a decrease in plant production and a higher dominance of plant species performing better on soils with lower N availability. On the other hand physical effects of soil compaction may lead to oxygen limitation and waterlogging, resulting in increased abundance of plant species better adapted to these circumstances. When the available oxygen gets used up by soil microbes other electron acceptors will be used for bacterial metabolism. Anoxic oxidation processes result in a lower redox potential because of the production of reduced compounds (Laanbroek 1990). These compounds ( $\text{Mn}^{2+}$ ,  $\text{H}_2\text{S}$ ,  $\text{Fe}^{2+}$ ) together with the lack of oxygen may severely harm root metabolism. This often leads to root death (Jackson and Armstrong 1999), sometimes within a few hours (Elzenga and van Veen 2010). Plant species may adapt to hypoxia and waterlogging in compacted soils by forming aerenchym, which serves as an alternative pathway for supply of oxygen to the root other than diffusion through soil pores (Table 7.1, Jackson and Armstrong 1999). Consequently, on wet soils that have been compacted, we expect an increase in plant species with aerenchym in their roots, as well as a shift towards plants performing better under conditions of lower nutrient availability.

### **Soil compaction along environmental gradients**

Effects of soil compaction on nitrogen mineralization are often reported to be negative (Hamza and Anderson 2005). However, the strength and direction of soil compaction effects depend on different soil properties including soil type and soil moisture content (Table 7.1, Kiehl et al. 2001, Hamza and Anderson 2005). Therefore, contrasting effects of herbivores on N mineralization may be explained by contrasting effects of large herbivores on soil compaction along environmental gradients of soil type, rainfall and temperature.

In sandy soils, compaction may enhance soil nutrient availability as a result of higher nutrient diffusion rates (Voorhees et al. 1985, Rasiah and Kay 1998, Xu et al. 2008), less leaching of nutrients (Xu et al. 2008) and better water retention. The positive consequences of compaction in sandy soils become negative when considering soils that are rich in silt and clay (Rasiah and Kay 1998), because fine-textured soils are much more compactable by trampling than sandy soils. This yields an interesting effect where the positive effects of finer texture on nutrient availability can be undone by the negative effects of soil compaction under grazing. Soil moisture content is also an important determinant of effects of soil compaction on nutrient cycling. In very dry soils herbivores may enhance water availability because compaction may increase water retention and disturbances of the soil top

layer can increase water infiltration (Abdelmagid et al. 1987a) which results in higher mineralization rates. In contrast, in wet soils compaction may reduce oxygen availability thereby decreasing mineralization rates. The effect of soil type often interacts with hydrological conditions, because clay and peat soils commonly occur under wetter conditions (e.g. high groundwater tables, flooding) than sandy soils.

The effects of soil compaction can be organized along the axes of soil type and soil moisture. Across these axes, soil compaction in dry and sandy soils has small, stimulating effects on N mineralization. In wet and clay soils effects will be larger because they are more susceptible to compaction and compaction will often lead to a reduction of N mineralization. Consequently, we expect that the effect of trampling-induced soil compaction on mineralization rates varies across environmental gradients. Trampling may have little or no effect on mineralization in habitats with non-compactable sandy soils, while they may strongly reduce mineralization on wet and clayey soils.

### **Herbivore effects on plant community composition through modification of soil physical properties– examples**

Empirical studies that try to explain contrasting results of herbivores on N cycling in natural systems through herbivore effects on soil physical properties are still lacking. Most recent studies that have attempted to quantify the relative importance of soil compaction on N mineralization rates focused on agricultural systems with supposedly proper drainage facilities (Curll and Wilkins 1983, deBruyn and Kingston 1997, Mikola et al. 2009). However, effects of soil compaction via water-logging and oxygen limitation on plant communities may play a more important role in areas without artificial drainage. Therefore, we analyzed data from existing long-term management experiments in Dutch grassland systems to get first insight in effects of herbivores on soil compaction and hence on plant community composition. From the plant community responses we aim to imply the consequences for N mineralization, as these were not directly measured in these studies. We specifically address (1) how herbivores affect soil compaction, (2) how increased soil compaction results in a shift in plant community composition, and (3) what these changes in soil compaction and plant community composition imply for N mineralization rates.

### **Experimental set up and data analysis**

We used data from a grazing experiment on the salt marsh of Schiermonnikoog (53°30'N, 6°10'E), which was set up in the 1970s on a clay soil. We studied soil physical properties and plant community composition in a cattle-grazing treatment (stocking rate 1 cow ha<sup>-1</sup>), and a hand-mowing treatment. In both treatments aboveground biomass was removed, but the treatments potentially differed in levels of soil compaction. We collected soil cores in 2010 to measure herbivore effects on soil physical properties (Table 7.2). In addition, we used data from three

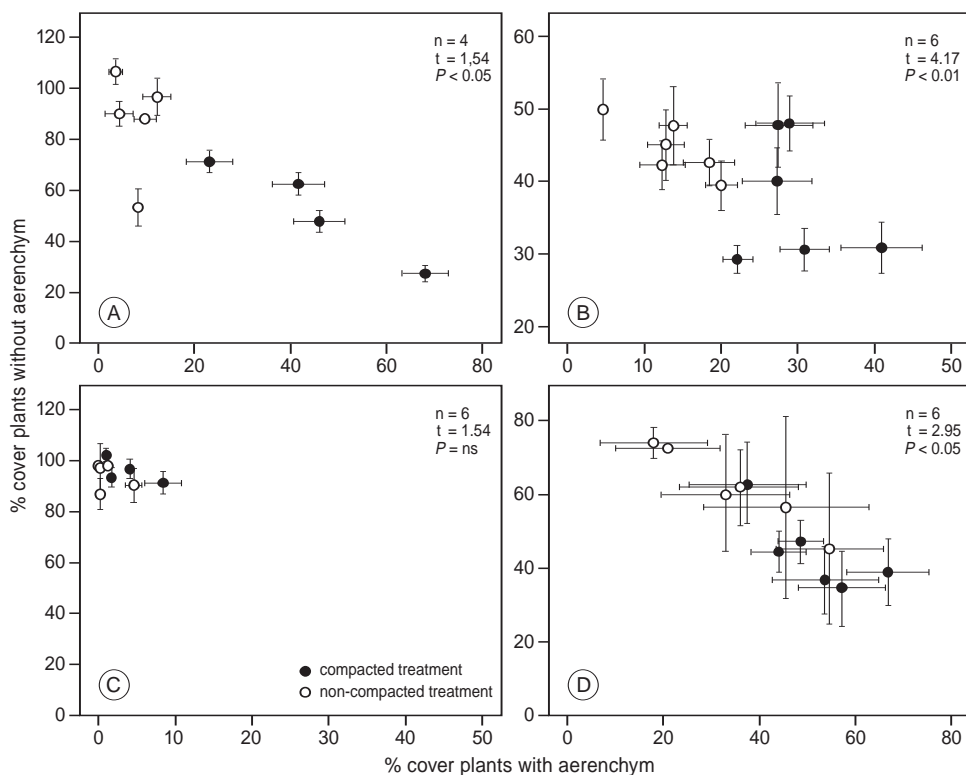
mowing experiments located in the river valley of the Drentsche Aa (53°N, 6°43'E), also set up around 1970. In these experiments no cattle-grazing treatments were available, but there were plots that were mown with heavy machines and plots that were hand mown. Therefore, we could use these experiments to confirm whether soil compaction affected plant community composition, because on both mowing treatments aboveground biomass was removed, while levels of soil compaction potentially differed. The three sites were located on different soil types, i.e. an organic soil (Lammertshem), a sandy soil (Loefvledder B), and an intermediate (Loefvledder A). Experiments in Loefvledder A and B are still maintained, but the experiment at Lammertshem was terminated in 1983 because the site was rewetted. We collected soil cores in 2010 in Loefvledder A and B to measure soil physical properties in the different mowing regimes. More details on setup of the four field experiments that we used can be found in (Bakker 1989).

In wet areas, like our study sites, soil compaction usually leads to higher soil moisture and hypoxia. Therefore we tested vegetation responses by comparing the cover of aerenchym and non-aerenchym producing plant species in the different management regimes (Justin and Armstrong 1987). For Schiermonnikoog, and the Loefvledder A and B sites we used recent plant cover data (1994-2003) because it may take some time for plant communities to respond to the management regimes. For Lammertshem we used data from 1980-1983 since the experiment was not continued after 1983. To study the potential impact of soil compaction and hypoxia on N mineralization we used Ellenberg indicator values for nitrogen (Ellenberg et al. 1992) for the data from Loefvledder A and B only, because in these sites Ellenberg indicator values are well calibrated and commonly used for wet river valley sites (Ozinga et al. 2009). A shift towards lower Ellenberg N values would indicate a reduction in the cover of nitrophilous plant species and hence may imply lower availability of mineral N in the soil and possibly slower nutrient cycling.

## Results

On the salt marsh of Schiermonnikoog we found significantly increased levels of soil bulk density (i.e. the herbivores compacted the soil) in the grazed sites, soil moisture and soil organic matter content (Table 7.2). The plant community responded to the altered soil physical conditions with a shift in community composition. In grazed, i.e. more compacted, plots we found significantly higher coverages of aerenchym producing plant species (Fig 7.2A), which are adapted to periods of low oxygen availability in the soil.

In Loefvledder A we did find increased levels of soil bulk density in machine-mown plots, but differences in weight per volume between soil organic matter and sand masked the much higher degree of compaction probably present at the site



**Figure 7.2** Cover of plant species without aerenchym plotted against the cover of plant species with aerenchym in four different study sites under different management regimes and hence different levels of soil compaction. a) salt-marsh Schiermonnikoog, b) Loefvledder A in the Drentsche Aa river valley, c) Loefvledder B in the Drentsche Aa river valley, and d) the Lammertshem in Drentsche Aa river valley. Closed circles represent compacted treatments (cattle-grazed or machine-mown) and open circles represent non-compacted treatments (hand-mown). In a), b) and c) each data point represents the average cover of plant species per plot over the last 10 years of the experiments (1994–2003). In d) each data point represents the average cover of plant species over the last 4 years (1980–1983).

**Table 7.2** Soil physical properties for Schiermonnikoog in cattle-grazed and hand-mown plots. Values are averages  $\pm$  standard error. Asterisks indicate level of significance: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

	Cattle-grazed	Hand-mown	N	t
<b>Soil physical properties</b>				
Soil moisture content (%)	55.1 $\pm$ 3.6	42.7 $\pm$ 4.5	6	3.7 **
Soil organic matter content (%)	8.4 $\pm$ 2.4	4.2 $\pm$ 0.8	6	2.3 *
Bulk density (g dry weight/ml soil)	0.60 $\pm$ 0.05	0.52 $\pm$ 0.03	6	4.0 **

(Table 7.3). In Loefvledder B soil physical properties were not different between the management regimes (Table 7.3), probably because this site was situated on a more sandy soil, which is less compactable. The observed soil physical conditions were reflected in the plant community composition. In Loefvledder A (Fig. 7.2B), as well as the Lammertshem (Fig. 7.2D) we found a significant shift towards higher coverage of aerenchym species in machine-mown compared to hand-mown plots, while in Loefvledder B there was no difference between the mowing treatments (Fig 7.2C). Similarly, Ellenberg N indicator values at the Loefvledder A site were significantly lower in machine-mown than in hand-mown plots (Table 7.3), while we did not find a difference in Loefvledder B plots (Table 7.3).

### Conclusion of the examples

The long term experiment on Schiermonnikoog shows that herbivores alter soil physical conditions in such a way that lower N mineralization rates are expected; and these changes in soil properties are reflected in the plant community. The analysis of the Drentsche Aa data confirmed that changes in soil physical properties as a result of soil compaction may show up in the plant community. It also showed that cover of nitrophilous plant species was reduced suggesting that N mineralization may be lower in compacted soils. Moreover, it implied that the influence of compacting agents (in this case heavy machines) on levels of compaction, plant community composition, and N mineralization rates depends on environmental conditions like soil type and soil moisture content, because on sandy, thus less-compactable, soils we did not measure a response to soil compaction.

**Table 7.3** Soil physical properties and Ellenberg N indicator values for Loefvledder A and B in machine-mown and hand-mown plots. Asterisks indicate level of significance: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ . We present Ellenberg indicator values as a weighted average per mowing treatment. For each plot we multiplied the N indicator value by the % vegetation cover with that value.

	Machine-mown	Hand-mown	N	t	
<b>Soil physical properties Loefvledder A</b>					
Soil moisture content (%)	73.7 ± 2.3	66.7 ± 2.4	6	7.6	***
Soil organic matter content (%)	46.7 ± 5.2	38.5 ± 4.9	6	4.5	**
Bulk density (g dry weight/ml soil)	0.42 ± 0.05	0.41 ± 0.03	6	0.6	NS
<b>Soil physical properties Loefvledder B</b>					
Soil moisture content (%)	59.7 ± 1.7	59.9 ± 2.6	6	-0.1	NS
Soil organic matter content (%)	13.2 ± 0.9	11.6 ± 1.0	6	2.5	NS
Bulk density (g dry weight/ml soil)	0.67 ± 0.05	0.68 ± 0.05	6	0	NS
<b>Ellenberg N indicator values</b>					
Loefvledder A	17.3 ± 4.5	28.6 ± 1.8	6	-3.5	**
Loefvledder B	36.7 ± 5.2	32.8 ± 4.2	6	0.6	NS

## Conclusion

Combination of the modification of soil physical properties by herbivores with the longer known effects of reducing organic matter C/N ratios allows for the understanding of contradictory results observed so far of large herbivore impacts on nutrient cycling in grasslands. An integrated framework allows us to study the relative importance of herbivore effects on nutrient cycling via changes in plant communities, return of dung and urine to the soil and trampling (Fig 7.1). Whether overall effects of herbivores on N mineralization are positive or negative depends on the balance between different mechanisms affecting nutrient cycling (Fig 7.1). When positive effects of grazing on soil organic matter quality via dung, urine and litter return outbalance negative effects via modification of physical properties, we expect an overall increase in mineralization rates. On the other hand, negative effects may outbalance positive effects, for example in the case of trampling on fine-textured soils. We propose that in temperate ecosystems, the outcome of the balance is too a large extent predictable because it depends on the interaction between soil type and soil moisture content. In systems where soils are not very compactable, i.e. systems with dry or sandy soils, it is likely that positive effects of grazing will dominate. In contrast, in systems where soils are compactable, i.e. systems with organic or clay soils, negative effects may be most important. Different effects are expected when large herbivores have strong impacts on soil temperature or drought as in arctic or arid ecosystems, but this requires further analysis. To single out whether changes in plant community composition as a result of soil compaction by herbivores are really related to a lower N mineralization and whether such effects of herbivores are different along gradients of soil compactability there is a need for field experiments. Such experiments should directly investigate herbivore effects on nutrient cycling, mineralization rates and other soil physical properties in soil types with different susceptibility to compaction.

The framework presented allows us to understand some of the contradictory results in N mineralization that have been found in grasslands previously, but could not be explained with the current theories. Analysis of four long-term data sets confirmed that soil compaction may play a crucial role in explaining nutrient cycling and plant community composition in natural grasslands. However, there is a need for field experiments directly investigating the relative importance of trampling-induced soil compaction for nutrient cycling along a gradient of soil types.

## Acknowledgements

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# *Chapter eight*



# 8

## General discussion

G.F. (Ciska) Veen

## Introduction

Aboveground and belowground organisms structure plant communities (e.g. Grime et al. 1987, Crawley 1989, Huntly 1991, Milchunas and Lauenroth 1993, Olff and Ritchie 1998, van der Putten and van der Stoep 1998, van der Heijden et al. 1998b, Klironomos 2002, Wardle 2002, De Deyn et al. 2003, Bardgett and Wardle 2010). They both influence competitive interactions between plant species, plant growth and plant community composition directly by the removal of plant nutrients and biomass (Olff et al. 1997, van der Putten and Peters 1997), as well as indirectly through the regulation of soil nutrient cycling (Bardgett and Wardle 2003, Wardle et al. 2004). As a result, aboveground and belowground organisms interact with each other using plants as mediators, which has major consequences for the structure and functioning of terrestrial ecosystems (Wardle et al. 2004). For example, interactions between invertebrate aboveground and belowground herbivores have been found to be important drivers of species composition and diversity in the plant community (e.g. Brown and Gange 1989, Brown and Gange 1992, van Ruijven et al. 2005). The consequences of interactions between vertebrate herbivores and soil organisms are much less well studied. In my thesis I therefore explored how the interplay between aboveground vertebrate herbivores and invertebrate soil organisms affected plant community composition, structure and diversity in grassland ecosystems. In this chapter I discuss the main results. I address via which pathways interactions between aboveground vertebrates and soil organisms can be mediated and I discuss the role of soil organisms to drive plant community composition and ecosystem processes in grasslands grazed by vertebrate herbivores. At the end of this chapter I will indicate challenges for future aboveground-belowground research and synthesize the key findings of my thesis.

## Pathways of aboveground-belowground interactions

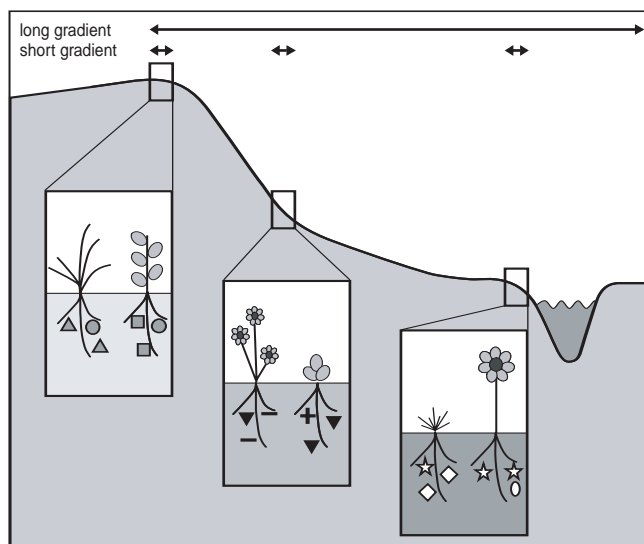
### Aboveground effects on the soil community

Grazing by aboveground vertebrate herbivores can affect the activity of soil organisms and may change soil community composition (Bardgett et al. 1997, Wall-Freckman and Huang 1998). Such effects of grazing can be mediated via different pathways. On the one hand aboveground herbivores remove shoot biomass which alters competitive interactions between plant species and can drive changes in plant community composition (Olff et al. 1997, Olff and Ritchie 1998, Knapp et al. 1999). Altered plant community composition may lead to a different soil community composition due to species-specific associations between plants and soil organisms (Yeates 1999, De Deyn et al. 2004b, Viketoft et al. 2005, 2009). On the other hand aboveground herbivores alter soil physical conditions and nutrient availability as a

result of trampling, local deposition of dung and urine and changes in plant litter quality and quantity (chapter 7, Steinauer and Collins 1995, Hobbs 1996, Bardgett and Wardle 2003). This in turn can affect soil community composition (Yeates and Bongers 1999, Bardgett and Wardle 2003). In chapter 2 I investigated the relative importance of these pathways via which herbivores can affect the nematode community composition, a diverse and often highly responsive group of soil biota. I showed that herbivore-induced changes in plant community composition were more important determining the community composition of nematodes than pathways that involved changes in soil physical conditions. This suggests that the association between plants and soil organisms is, at least to some extent, species specific, so that the presence (De Deyn et al. 2004b, Viketoft et al. 2005, 2009) or absence (Wardle et al. 1999) of certain plant species can alter the soil community composition.

Other studies showed that effects of plant species identity on soil microbial community properties can vary with soil fertility, (Bardgett et al. 1999, Innes et al. 2004) and may even be predominantly be determined by soil type and fertility (Harrison and Bardgett 2010). Therefore, it is still preliminary to generalize that changes in soil community composition are mediated by plant species composition, instead of by soil physical conditions. Yet, recently Bezemer et al (2010) studied entire soil food webs and showed that individual plants in a multi-species community develop their own species-specific soil food web. Zooming in on the nematode community revealed that the same plant species in different communities had more similar nematode communities, than different plant species in the same community (Bezemer et al. 2010). Although this study did not investigate the relative importance of soil physical conditions it does confirm that plant species identity, probably as a result of species-specific differences in root traits (e.g. root morphology, exudates, fungal symbionts), is a very important factor driving the composition of entire soil communities.

Effects of herbivore-induced changes in soil community composition may be affected by changes in soil abiotic conditions, as well as in plant community composition. Effects of these two pathways may be hierarchically ordered; hence their relative importance for driving soil community composition will be dependent on the scale of observation (Ettema and Wardle 2002). At smaller spatial scales as used in many field experiments, (chapter 2, De Deyn et al. 2004b, Viketoft et al. 2005, 2009) soil physical conditions may vary too little to allow a vastly different subset of the soil community to develop and plant species identity is the most important factor driving soil communities (Fig 8.1, chapter 2, Ettema and Wardle 2002). Nevertheless, over longer environmental gradients or time scales changes in soil physical properties will certainly be important selecting a subset of the soil community which can persist under the specific environmental conditions (Fig 8.1). For example, a recent meta-analysis found broad-scale patterns in microbial



**Figure 8.1** Pathways driving soil community composition over long and short environmental gradients. The symbols belowground indicate soil organisms. Symbols with a different color are soil organisms belonging to different soil communities (selected for by soil abiotic conditions). Symbols with different shapes are different species of soil organisms (selected for by different plant species).

biomass and community composition across seven of the major biomes on earth (Fierer et al. 2009). At the ecosystem level distributions of soil biota are often clustered according to large-scale landscape gradients (Ettema and Wardle 2002). Large aboveground vertebrate herbivores could drive such large-scale variation in soil physical conditions by grazing relatively homogeneously across large scales, thereby affecting large-scale patterns of soil abiotic conditions and resource input into the soil food web. This will in turn have an important influence on soil community composition and activity (chapter 7). Thus, depending on the scale of observation, vertebrate herbivores are expected to determine large-scale patterns in soil community composition and activity by altering large-scale soil physical conditions (chapter 7), while small-scale changes in plant growth and community composition become more important determining small-scale species composition in the soil community (chapter 2, Ettema and Wardle 2002, De Deyn et al. 2004b, Viketoft et al. 2005, 2009, Bezemer et al. 2010).

### Plant-soil feedback effects

Plant-induced changes in soil community composition and activity, as well as in soil abiotic conditions, may feed back to change plant performance and plant community composition, a process referred to as plant-soil feedback (Bever et al. 1997). Plant-soil feedback effects can be either positive or negative, depending on

the balance between mutualistic and parasitic interactions in the soil and will be mediated by direct and indirect soil community interaction pathways to plants (Wardle et al. 2004). Direct pathways involve interactions with root herbivores, pathogens and symbiotic mutualists and indirect pathways encompass changes in soil nutrient cycling driven by the decomposer food web (Wardle et al. 2004), as well as changes in soil abiotic conditions. While direct associations between plants and root herbivores and pathogens can be highly species-specific (van der Putten et al. 1993), it is generally assumed that indirect interactions in the decomposer food web are less species-specific (Yeates et al. 1993, Didden 1993). Such generalist feeders will therefore be less sensitive to changes in plant and soil community composition. However, plant species identity has been found to also affect general soil microbes and decomposers (Grayston et al. 1998, Bezemer et al. 2010). This may be because the identity of a plant species is important for determining the quality and quantity of resources entering the soil food web (Bardgett and Wardle 2003). Consequently, even indirect associations between plants and soil decomposers can be species-specific (Bardgett and Wardle 2010).

The relative importance of direct and indirect pathways mediating plant-soil feedback is still not fully understood. Also, the contribution of biotic plant-soil feedback effects for shaping plant communities relative to soil physical conditions remains largely unknown (Ehrenfeld et al. 2005, Casper et al. 2008), due to the complexity of the systems involved. A few recent studies aimed to unravel part of these open questions. It has been found that the strength and direction of plant-soil feedback can be dependent on environmental factors such as soil type (Bezemer et al. 2006) and soil nutrient availability (Manning et al. 2008). These results show that plant-soil feedback effects can be highly context dependent. Instead, another study showed that plant-soil feedback effects were generally independent of soil type and soil fertility (Harrison and Bardgett 2010). Therefore, to better understand the consequences of plant-soil feedback for plant community structure it will be necessary to focus on interactions between biotic and abiotic, and between direct and indirect, components of plant-soil feedback.

## **Consequences of aboveground-belowground interactions for the plant community**

Interactions between aboveground and belowground organisms, either via direct or via indirect pathways, can have important consequences for plant community structure and composition. Spatially separated herbivores may modify each others impact on the plant community, because different herbivores can affect plant species performance in a specific way. Many studies on consequences of aboveground-belowground interactions for the plant community have been focusing on



invertebrate herbivores (Brown and Gange 1989, Brown and Gange 1992, van Ruijven et al. 2005). These studies revealed that interactive effects of invertebrate aboveground and belowground herbivores on plant community diversity were different from the sum of the separate effects. For example, van Ruijven et al. (2005) found that neutral effects of aboveground grasshoppers on plant diversity and positive effects of belowground wireworms became negative when plant communities were exposed to a combination of these herbivores. The impact of belowground invertebrate herbivores on plant community structure was modified by the presence of aboveground organisms, and vice versa, probably as a result of changes in indirect interactions within the plant community.

Such non-additive effects of aboveground-belowground interactions can also be expected when considering aboveground vertebrate herbivores and soil organisms. Both vertebrate herbivores and soil organisms can remove plant biomass and nutrients which affects competitive abilities of plant species (Olf et al. 1997, van der Putten and Peters 1997). Moreover, they may influence soil decomposer activity by changing the quality and quantity of resource input into the soil food web (Bardgett and Wardle 2003). These changes ultimately result in altered plant growth, species composition in the plant and soil community and patterns of soil nutrient cycling, which in turn may modify net plant-soil feedback effects (chapter 3; Bardgett and Wardle 2003), as well as foraging patterns of aboveground vertebrate herbivores (McNaughton 1984, McNaughton et al. 1997a). In chapter 4 – 7 I investigated how aboveground vertebrate grazing modified effects of plant-soil interactions on plant community structure and composition.

Plant-soil feedback effects are mediated via indirect interactions between plants and soil physical properties, soil decomposers and soil nutrient cycling, as well as via direct interactions between plants and their root herbivores, pathogens and symbiotic mutualists (Bever et al. 1997). The net effect of all interactions between plants and their associated soil community can be either positive or negative. In general, plants that experience an overall positive effect (e.g. via interactions with arbuscular mycorrhizal fungi), may be competitively dominant over other species. This will reduce plant diversity (chapter 3, Hartnett and Wilson 1999, Klironomos 2002, Bever 2003, Callaway et al. 2004) and will lead to a relatively stable species composition in the plant and soil community (chapter 3). In contrast, negative plant-soil interactions reduce the competitive abilities of a plant species (van der Putten and Peters 1997), thereby promoting coexistence, diversity (De Deyn et al. 2003), spatial heterogeneity (Olf et al. 2000), year-to-year oscillations (chapter 3 and 4, Olf et al. 2000, Bever 2003) and succession (van der Putten et al. 1993, Kardol et al. 2006, 2007) in the plant community.

Studies investigating how aboveground vertebrates influence the outcome of plant-soil feedback have been mainly focusing on changes in soil decomposer activity and soil nutrient cycling (Bardgett and Wardle 2003). It has been widely

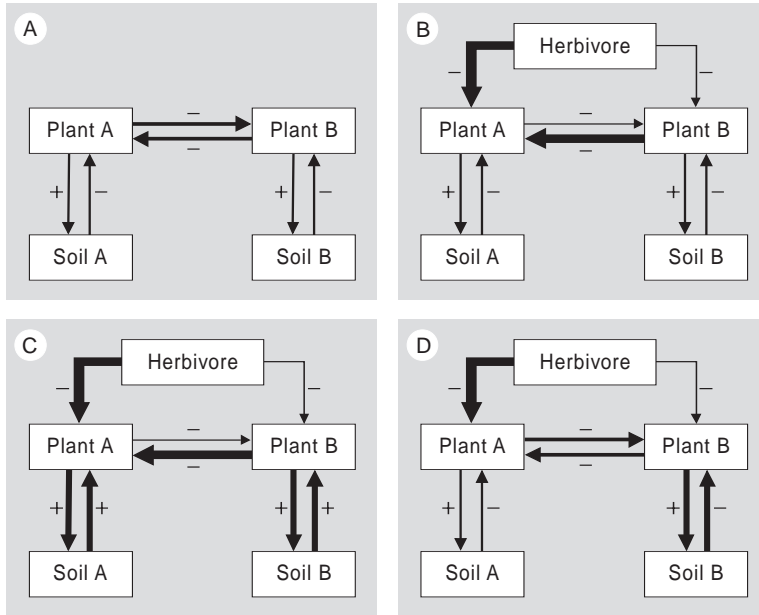


accepted that vertebrate herbivores alter the quality and quantity of resources entering the soil food web by affecting root exudation patterns, litter quality and quantity and the deposition of dung and urine. Moreover, vertebrate herbivores change soil physical conditions, e.g. soil aeration and soil temperature (chapter 7). These herbivore-induced changes in resource quality and the abiotic environment can influence the activity and species composition in the soil food web, which drives soil nutrient cycling and determines soil nutrient availability and plant growth (chapter 7, Holland and Detling 1990, McNaughton et al. 1997a, Frank and Groffman 1998, Bardgett and Wardle 2003, Sørensen et al. 2008). In many systems vertebrate herbivores have been suggested to speed up nutrient cycling by providing easily available resources to decomposers in the form of dung and urine (McNaughton 1984). In contrast, vertebrate herbivores can have a negative influence on nutrient cycling because selective grazing on palatable plants can lead to a reduction in plant and litter quality (Ritchie et al. 1998, Pastor et al. 2006). Moreover, depending on soil texture and soil moisture levels, trampling by vertebrate herbivores may compact the soil which can reduce the abundance of soil organisms and may result in soil hypoxia. Such changes in plant quality and soil physical conditions can accordingly inhibit decomposition and mineralization rates (chapter 7). Whether herbivores stimulate or slow down soil nutrient cycling depends on the balance between positive and negative effects of grazing on the decomposer food web and soil mineralization rates (chapter 7). The net effect of herbivores on soil nutrient cycling potentially alters the overall strength and direction of plant-soil feedback effects, because a stimulation of soil nutrient cycling will result in more positive plant-soil feedback, and vice versa. Thus, herbivores can modify plant-soil feedback interactions via the indirect pathway of soil nutrient cycling, which has important consequences for plant growth on the short term (Bardgett and Wardle 2003, Sørensen et al. 2008) and for plant community composition on the long term (chapter 7; Bardgett and Wardle 2003).

Vertebrate herbivores not only modify plant-soil feedback via indirect associations between plants and soil decomposers; they may also change the species composition and abundance of root herbivores, pathogens and symbiotic mutualists that are directly associated with plant roots. Direct interactions between roots and soil organisms can be highly species-specific (van der Putten et al. 1993), and therefore plant species identity can be a major determinant of species composition in the soil community (De Deyn et al. 2004b, Viketoft et al. 2005, 2009, Bezemer et al. 2010). Consequently, herbivore-induced changes in plant species composition may alter soil community composition (chapter 2). Moreover, in response to grazing, plants can alter biomass allocation among roots and shoots (e.g. van der Maarel and Titlyanova 1989), which affects the availability of resources for root consumers and hence the abundance of root mutualists and antagonists in the soil. Changes in the species composition in the soil community and in the abundances of root herbi-

vores, pathogens and mutualists may affect the strength and direction of plant-soil feedback. For example, when the abundance of deleterious soil pathogens is stimulated plant soil feedback effects can become more negative, while it becomes more positive when pathogen pressure is reduced.

The previous examples indicate that vertebrate herbivores have the potential to modify the strength and direction of plant-soil feedback effects. However, how vertebrate herbivores affect net plant-soil feedback interactions and how plant-soil feedback effects in turn drive plant community composition in different vertebrate grazing regimes is still largely unknown, particularly because direct interactions between plants and root consumers have been rarely considered in this context. I used a greenhouse experiment to investigate the response of plant species to soils that had a different vertebrate grazing history and that were either incubated with conspecific or with heterospecific plant species (chapter 4). I found that all plant species performed worse in soils from conspecifics than in soils from heterospecifics, regardless of grazing history of these soils. Thus, plant-soil feedback effects were generally negative and not modified by the vertebrate grazing regime (chapter 4). Such negative species-specific effects of soil organisms on competitive abilities of plants can result in small-scale heterogeneity in the plant community in both space and time (van der Putten and van der Stoel 1998, Olff et al. 2000, van der Putten 2003, Bever 2003). However, I found that spatiotemporal plant community patterns in the field were relatively stable under vertebrate grazing, even if plant species experienced strong negative plant-soil feedback (chapter 4). Probably vertebrate herbivores can exert such a strong control on the plant community that soil organisms have little opportunity to drive plant species composition (chapter 4 and 5). Particularly when herbivores intensively feed on dominant, grazing-intolerant plant species, these plant species will not get the opportunity to reach high abundances in the plant community or may even be removed when they can not recover from being grazed (Fig 8.2B). In such situations only plants that tolerate grazing well will be able to maintain themselves in the community, regardless of plant-soil feedback effects (chapters 4 and 5, Fig 8.2B). In the absence of large aboveground vertebrates soil organisms may take over the control on plant community composition and dynamics, at least partly. In that situation, strong negative plant-soil feedback may reduce the competitive ability of specific plant species, so that other species can outcompete them (Fig 8.2A, van der Putten et al. 1993, van der Putten and Peters 1997), which can promote small-scale spatiotemporal plant community patterns (chapter 4, Fig 8.2A, van der Putten and van der Stoel 1998, van der Putten 2003, Bever 2003). Not only in the absence of vertebrate herbivores, but also under more extensive grazing conditions or in the presence of very selective vertebrates that specifically respond to plant community changes induced by plant-soil feedback effects, soil organisms can become important drivers of plant community composition and dynamics (chapters 5 and 6, Dean et al. 1997, Lenoir



**Figure 8.2** Different scenarios showing how plant-soil feedback may drive the dynamics of two plant species in the absence (A) and presence (B, C and D) of aboveground vertebrate herbivores. Boxes represent the organisms involved in the particular scenarios. Boxes are connected by arrows showing effects of one organism on another. The thickness of the arrows indicate the strength of effects and “+” and “-” the direction of the effects. Plant A represents a certain plant species A, associated with its species-specific soil community Soil A. Similarly, Plant B is species B, associated with its species-specific soil community Soil B. (A) Cyclic dynamics. Both plants experience negative plant-soil feedback. B has the opportunity to replace A when competitive abilities of A decline due to deleterious effects of its soil organisms, and vice versa. This will lead to cyclic dynamics in the absence of aboveground vertebrate herbivores. When herbivores are present they may overrule (B) or modify plant-soil feedback effects (C, D) thereby potentially altering the potential of plant-soil feedback to drive plant community dynamics. (B) Herbivores prefer to forage on one of the plant species, for example species A. When plant species A does not tolerate grazing well, species B will be the only one that can maintain itself in the community, regardless of plant-soil feedback. In this situation, herbivores can overrule plant-soil feedback effects. (C) Herbivores can stimulate soil nutrient cycling through deposition of dung and urine, resulting in more positive plant soil feedback effects. Such effects may be profitable for all plant species and either the plant species that competes best for the nutrients or the one that tolerates grazing best will become dominant. (D) Herbivores may alter the abundance of soil-borne plant pathogens by changing plant species composition and resource allocation roots and shoots. This may lead to species-specific changes in strength or direction of plant-soil feedback effects. For example, negative plant-soil feedback for plant species B could be increased, while A is still preferred by aboveground herbivores. In such a situation, plant species A and B could coexist through cyclic dynamics or either A or B could dominate depending on their competitive interactions and the strength of the negative effects they experience from aboveground and belowground herbivores respectively. (Adapted from: van der Putten and van der Stoep 1998, van der Putten 2003, Bever 2003).

2009). I will explain this in more detail in the paragraph on “the scale of operation and herbivore selectivity’.

My study revealed that net plant-soil feedback effects, as measured in the greenhouse experiment, were generally not modified by vertebrate herbivores (chapter 4). This confirms other recent studies that were testing effects of homogeneous aboveground removal of biomass, i.e. a simulation of vertebrate grazing, and did not find modified plant-soil feedbacks (Sørensen et al. 2008, Ilmarinen and Mikola 2009). However, for one plant species in my experiment, *Agrostis capillaris*, plant-soil feedback was stronger (more negative) in soils from cattle-grazed situations than in soils from ungrazed situations (chapter 4). These results indicate that vertebrate herbivores could modify the strength of the feedback, by changing biotic or abiotic soil properties.

Since most plant species experienced negative plant-soil feedback in the greenhouse, I would have expected that plants performed worse in soils where their conspecifics had been growing in the field. However, I could not establish a direct link between greenhouse performance and the abundance of plant species in the field. On the one hand, in a natural situation these plant-soil feedback interactions may be overruled by the presence of aboveground vertebrate herbivores, as discussed above (chapters 4, 5 and 6). On the other hand, it may be difficult to link greenhouse effects to plant community dynamics in the field, because of differences in the spatiotemporal scale of measurement. Plant-soil feedback effects will be highly local, because individual plant species in a mixed community build up their own specific soil community (Bezemer et al. 2010), while most data on plant community dynamics are collected over larger scales (e.g. as in chapter 4). Also, plant-soil feedback effects that run via arbuscular mycorrhizal fungi and nutrient cycling may play out over relatively long time scales and hence can become underestimated in short-term greenhouse experiments (Bardgett and Wardle 2010). Consequently, plant community dynamics in the field may be regulated more strongly by positive effects of soil nutrient cycling on plant growth than expected based on plant-soil feedback experiments in the greenhouse (Fig 8.2C, Bardgett and Wardle 2010). Alternatively, plant-soil feedback effects in the field could be directly modified by grazing as a result of altered resource availability for root herbivores and pathogens, due to changes in resource allocation within plants or changes in plant community composition (Fig 8.2D). Such direct effects of herbivores on plant-soil feedback effects may not play out in a greenhouse experiment that only takes the grazing history of a soil into account, and not the actual presence of herbivores (e.g. as in chapter 4). Summarizing, net plant-soil feedbacks that plants experience in the field may differ from net plant-soil feedback effects that are measured in a short-term greenhouse experiment. Moreover, in the field plant species can escape highly local, deleterious effects of soil organisms by shifting to a neighboring patch where their own soil-borne pathogens are less abundant (Olff et al. 2000). As a

result, even in plant communities that appear to be relatively stable on the plot scale (chapter 4) plant-soil feedback effects may drive micro-scale dynamics at the scale of individual plants.

In systems that contain few plant species, such as agricultural systems (Bullock 1992), as well as natural, species-poor ecosystems (van der Putten et al. 1993) it may be more straightforward to determine the role of plant-soil feedback in field situations. In these systems it has been clearly shown that soil-borne plant pathogens directly reduce performance and competitive abilities of plant species, which can lead to the decline of entire plant populations and speeding up of vegetation succession (van der Putten et al. 1993). In such assemblages of one or a few species, specific deleterious soil organisms become abundant across a large area and species cannot escape negative plant-soil feedback effects in space. To better understand the role of plant-soil feedback for entire plant communities in species-rich grasslands it will be necessary to explore local effects of plant-soil feedback in a community context.

### **Scale of operation and herbivore selectivity**

Large vertebrate grazers can overrule plant-soil feedback effects (chapter 4). They may exert a strong control on plant species composition over relatively large scales, thereby homogeneously promoting grazing-tolerant species. In such a situation highly local plant-soil feedback interactions may not be important anymore for structuring the plant community (chapter 4). These results indicate that differences in the temporal and spatial scales of operation between aboveground vertebrate herbivores and soil organisms could be important for explaining why large vertebrate herbivores can overrule effects of soil organisms on plant community composition.

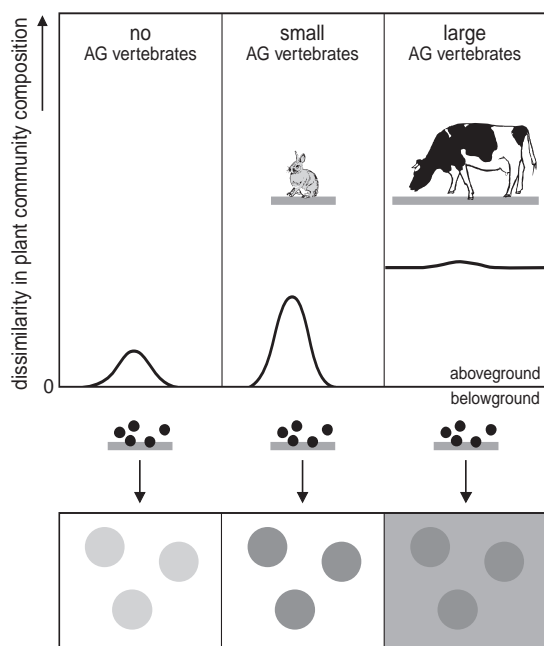
Soil organisms live in a micro world of soil particles and pores filled with water or air, which limits their mobility. Many invertebrate and microbial soil organisms are restricted to a home range of a few centimeters or meters in their whole lifetime and generally only explore the vicinity of an individual plant (Mortimer et al. 1999). In contrast, aboveground organisms live in an open world consisting of air with little resistance to movement. They are more mobile and may explore areas of several square kilometers within a day. As a result, individual soil organisms interact mostly with one or a few plants, while vertebrate herbivores can interact with multiple plants and different plant species. This has important consequences for the spatial scales at which soil organisms and vertebrate herbivores affect plant species distribution and thus for the influence of aboveground-belowground interactions on plant community structure and ecosystem functioning (De Deyn and van der Putten 2005, van der Putten et al. 2009).

The distribution of soil organisms is determined by a hierarchy of factors. At large spatial scales soil biota are aggregated according to landscape gradients in soil

physical conditions, while at more local scales patchiness in plant species composition and differences in growth rate between individual plants are key drivers (Ettema and Wardle 2002). The patterning of soil organisms drives the spatial distribution of soil nutrient supply, and of soil-borne plant mutualists and pathogens. That in turn determines the scale at which soil biota affect ecosystem processes, plant species distribution and plant community composition (Ettema and Wardle 2002). For example, social subterranean invertebrates such as termites and ants show a clear spatial aggregation across the landscape. They create physical structures, and the spatial distribution of these structures drives spatial patterning in the plant community (Spain and McIvor 1988, Blomqvist et al. 2000).

The scale at which aboveground vertebrate herbivores affect the plant community is generally associated to their body size. Large vertebrate herbivores need high quantities of food, but require less energy per unit body mass than small ones (Demment and Van Soest 1985). Therefore, large herbivores can forage less selectively (Olf et al. 2002, Hopcraft et al. 2010), are expected to have large home ranges (Haskell et al. 2002) and will neglect detail in their environment (Ritchie and Olf 1999, Laca et al. 2010). They forage at a coarser grain and may avoid fine-scale plant patches (Cromsigt and Olf 2006). As a result, effects of large, unselective vertebrate herbivores on the plant community will appear on a larger scale than effects of small, selective vertebrate herbivores, which can be highly localized (Bakker 2003).

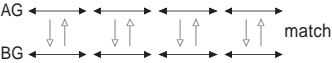


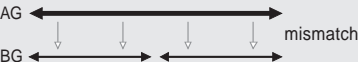


The spatial scale at which aboveground and belowground organisms perceive and affect plant patterns will determine how they respond to each other changes in plant growth and plant species composition. Large, less selective vertebrate herbivores that forage on a coarse grain may not perceive and respond to local effects of soil organisms on the plant community. As a result, they are not particularly attracted to small, high-quality patches (Cromsigt and Olf 2006), such as plant patches on ant mounds (chapter 6) and exert a relatively constant grazing pressure across large spatial scales (chapter 6). As a result, large vertebrates homogenize small-scale plant community patterns (chapter 6, Bakker 2003), thereby overruling highly local plant-soil feedback effects (chapters 4, 5, and 6, Fig 8.3, Fig 8.4). In contrast, vertebrate herbivores are not expected to overrule interactions between plants and soil organisms when they operate across scales more similar to the scales at which soil organisms affect the plant community (chapter 6, Fig 8.3, Fig 8.4). For example, small selective vertebrate herbivores can specifically select nutritious patches created by soil-dwelling ants (chapter 6). As a result, small aboveground vertebrates and ants interact through a positive feedback loop where both affect plant community composition in the same patch, leading to very pronounced small-scale plant community patterns (chapter 6, Fig 8.3). Similarly, in African savannas foraging patterns of large aboveground vertebrate herbivores were determined by large-scale changes in decomposition rates, food plant quality and plant



**Figure 8.3** Effects of aboveground and belowground organisms in plant community composition. The top panel shows how aboveground-belowground interactions can affect dissimilarity in plant community composition. The y-axis in depicts the relative change in plant species composition, the x-axis represents space. The three compartments represent three different combinations of herbivores: left = only soil organisms, no aboveground vertebrates, middle = soil organisms + small aboveground vertebrates, and right = soil organisms + large vertebrates. Soil organisms are represented by the black dots, small vertebrates are represented by the picture of a rabbit, and large vertebrates by the picture of a cow. For each of these groups of organisms the grey bar indicates the scale at which that group of organisms generally has an impact on the plant community. The black line shows how the plant community changes in the three different scenarios. The bottom panel gives an overview of the formation of plant patterns resulting from the different interactions between aboveground vertebrates and belowground organisms.

species composition induced by termites (Holdo and McDowell 2004, Freymann et al. 2010). Moreover, in many grassland ecosystems large-scale changes in soil community activity and nutrient cycling, for example induced by herbivore grazing, can feed back to create high-quality foraging patches (i.e. grazing lawns) which in turn attract vertebrate herbivores (chapter 7, McNaughton 1984, McNaughton et al. 1997a, Cromsigt and Olff 2008). These examples show that when aboveground vertebrates and soil organisms operate across relatively similar scales, i.e., across scales at which they can respond to each others impacts on plant growth and community composition, interactions between vertebrate herbivores and soil organisms may drive the formation of spatial patterns in plant community composition (chapter 6, Fig 8.4).



Scale	Scale match vs. mismatch	Organisms	Examples
< 10 mm < 10 mm	AG 	solitary AG herbivorous insects solitary BG herbivorous insects, plant feeding nematodes	e.g. Gange and Brown 1989, Masters and Brown 1992, Gange and Nice 1997, Bezemer <i>et al.</i> 2003, Soler <i>et al.</i> 2007
1 cm – 1 m < 10 mm	AG 	small AG vertebrate herbivorous (e.g. voles rabbits, hares) herbivorous BG insects, plant feeding nematodes	no studies available
1 cm – 1 m 1 cm – 1 m	AG 	small AG vertebrate herbivorous (e.g. voles rabbits, hares) small BG vertebrate herbivorous (e.g. moles, pocket gophers) small-scale social BG insects (e.g. ants)	(chapter 6)
1 m – 1 km 1 cm – 1 m	AG 	large AG vertebrate grazers (e.g. cattle, wildebeest, bison) small BG vertebrate herbivorous (e.g. moles, pocket gophers) small-scale social BG insects (e.g. ants)	e.g. Steuter <i>et al.</i> 1995, (chapter 6)
1 m – 1 km 1 m – 1 km	AG 	large AG vertebrate grazers (e.g. cattle, wildebeest, bison) large scale social BG organisms	e.g. Coppock <i>et al.</i> 1983, Ruggiero and Fay 1994, Holdo and McDowell 2004
1 m – 1 km < 10 mm	AG 	large AG vertebrate grazers (e.g. cattle, buffalo, bison) herbivorous BG insects, plant feeding nematodes	e.g. Wardle <i>et al.</i> 2001, Veen <i>et al.</i> 2010

**Figure 8.4** A conceptual overview showing how aboveground (AG) belowground (BG) interactions may depend on aboveground and belowground scales of operation. When aboveground organisms operate across much larger scales than belowground organisms (scale mismatch) they may overrule belowground effects. When aboveground and belowground organisms act on a more similar scale (scale match) they may affect each other and the plant community, without effects in one direction being dominant over the other. For different spatial scales of operation (left column) I show how aboveground and belowground organisms may affect each other (middle-left column). I give examples of organisms that may be involved in the interactions (middle-right column) and support these examples with references from the literature when available (right column).

The scale at which plant community patterns are created by aboveground-belowground interactions will depend on the scale of operation of aboveground and belowground organisms (Ettema and Wardle 2002). Differences or similarities in the scale of operation between aboveground and belowground organisms may affect the outcome of aboveground-belowground interactions at many different levels of organization from individual plants to large-scale plant community patches (Fig 8.4). Along all these levels of organization aboveground and belowground organisms that operate across more similar scales are likely to be able to respond to each others changes in plant growth and plant species composition (chapter 6, Fig 8.4), which may result in more pronounced effects of aboveground-belowground interactions on plant community structure and composition. In contrast, aboveground herbivores that operate across much larger scales than soil organisms may not

perceive changes in plant growth and community composition induced by soil organisms. They will graze homogeneously on and off plants or plant patches affected by soil organisms, thereby potentially overruling plant-soil feedback interactions (chapter 4 and 6, Fig 8.4).

## **Future challenges**

### **Plant-soil feedback in the field**

The framework of plant-soil feedback has been mainly developed and tested in greenhouse experiments using individual plant species. Such experiments usually consist of two growing phases, where first a plant is grown to develop a species-specific soil community, and then the soil is used to test effects on subsequent plant growth (Brinkman et al. 2010). Such experiments reveal overall effects of plant-soil interactions for individual plant species, i.e. a positive effect when mutualistic interactions outbalance parasitic ones and a negative effect in the opposite case. These feedback effects often are extrapolated to predict consequences for plant community composition and diversity in field situations. However, it is not always straightforward to translate greenhouse results to a field situation (chapter 4). This may be because, for example, interactions between plant species (Kardol et al. 2007, Casper and Castelli 2007), soil nitrogen availability (Manning et al. 2008) and soil type (Bezemer et al. 2006) can modify plant-soil feedback effects. For example, Kardol et al (2007) showed that negative feedback effects experienced by early successional plant species were enhanced when these plants were grown in mixed plant communities with later succession species. Another study found that negative feedback effects experienced by plant species in monoculture can be eliminated by interspecific competition (Casper and Castelli 2007). Moreover, as discussed previously, it may be difficult to link greenhouse plant-soil feedback effects to plant community dynamics in the field, because of differences in the spatiotemporal scale of measurement. In field experiments highly local effects on plant community dynamics may not be registered, while in short-term greenhouse experiments long-term effects of soil nutrient cycling on plant growth may be underestimated (Bardgett and Wardle 2010). Therefore, it is necessary to consider the context in which plants are growing in the field to be able to conclude whether plant-soil feedback effects are important determinants of the composition and dynamics of whole plant communities (Harrison and Bardgett 2010).

In order to gain knowledge on context-dependent effects, future studies should consider how the environment modifies plant-soil feedback effects. Ideally plant-soil feedback experiments should be carried out in field situations, although this may technically be very difficult since it is hard to control and measure soil communities. A first step may be to transplant species in the field and to compare

plant performance on locations where conspecifics had been growing with performance on locations where heterospecifics had been growing. Such transplant experiments can include manipulation of environmental variables as well. For example, nutrient availability can be modified via application of fertilizer, competition may be altered by removing or adding or plant species, and the presence of aboveground herbivores may be manipulated using exclosures. Including such environmental manipulations will help us to understand the consequences of plant-soil feedback relative to soil physical conditions, plant competition, and aboveground grazing in a community context.

### **Modeling aboveground-belowground interactions**

Although field experiments will be necessary to advance our knowledge of aboveground-belowground interactions in natural communities, it is hard to measure and manipulate soil communities. Therefore, it remains a major challenge to quantify effects of soil organisms on plant community structure and composition in natural ecosystems. This is where mathematical models become very useful to increase our understanding of aboveground-belowground interactions and their consequences for the plant community. Such models can help us to single out crucial parameters that affect the strength and direction of interactions between aboveground and belowground organisms. That will help to generate focused questions for new empirical, preferably field, experiments.

Recently different models that study plant-soil interactions have been developed (Bever et al. 1997, Bever 2003, Umbanhowar and McCann 2005, Eppinga et al. 2006, Eppstein et al. 2006, Eppstein and Molofsky 2007), but very few models considered the influence of aboveground herbivores (or higher trophic organisms) at the same time. Only Meyer et al (2009) use an aboveground-belowground multitrophic approach. They show that third-trophic levels and decomposers can have a considerable influence on plant and herbivore performance. These results reveal that is important to include high-order multitrophic interactions to understand possible consequences of aboveground-belowground interactions for species composition in natural communities. This example illustrates that models may improve our understanding and a combined theoretical-empirical approach may help us to take the next step in aboveground-belowground empirical research.

Key issues in aboveground-belowground research that can be addressed using a theoretical approach are (1) how does the complexity of possible interactions affect aboveground-belowground interactions, (2) how do abiotic environmental conditions modify biotic plant-soil feedback effects, and (3) how do differences in the scale of operation between aboveground and belowground organisms play out (van der Putten et al. 2009). Knowledge on these important issues will help us to better understand how aboveground-belowground interactions shape plant communities.

## Conclusion and synthesis

Aboveground-belowground interactions can be mediated via direct associations between plants and herbivores (van der Putten et al. 2001, Wardle et al. 2004) or via indirect effects of plants and herbivores on soil nutrient cycling and soil physical conditions (chapter 7, Bardgett and Wardle 2003, Wardle et al. 2004). Aboveground effects on the soil community are predominantly determined by species-specific interactions between plant species and soil organisms (chapter 2, De Deyn et al. 2004b, Vikeftoft et al. 2005, 2009, Bezemer et al. 2010). However, over larger spatial or temporal scales indirect pathways including soil physical conditions can become important as well (Ettema and Wardle 2002, Fierer et al. 2009). Changes in the soil community may feed back to alter plant community composition via species-specific effects of root herbivores on the competitive ability of plants or via modifications of soil nutrient cycling (Bever et al. 1997).

Plant-soil feedback effects have been suggested to drive plant species coexistence, plant community composition, diversity and dynamics (van der Putten and Peters 1997, Klironomos 2002, De Deyn et al. 2003, Wardle et al. 2004). In general, negative plant-soil feedback will enhance local replacement of plant species and may increase plant diversity and small-scale spatiotemporal plant community patches (chapters 3 and 4, van der Putten et al. 1993, Olff et al. 2000, Bever 2003, Kardol et al. 2006). However, effects of plant-soil feedback on plant community structure and composition may play out differently under vertebrate grazing. In grazed grasslands localized effects of soil organisms on the vegetation can be overruled by large vertebrate herbivores (chapter 4, 5 and 6), because large herbivores forage on a coarse grain and may not be able to respond to belowground-driven patches specifically (chapter 6). Moreover, when the control on the plant community is strong, grazing-tolerant plants maintain in the community, regardless of the strength and direction of plant-soil feedback effects (chapter 4). When grazing pressure is lower or absent, plant-soil feedback may become an important factor determining plant community structure and dynamics (chapter 4 and 5).

Vertebrate herbivores will not always overrule plant-soil feedback effects; they can also modify the strength and direction of plant-soil interactions. Whether vertebrate herbivores will overrule or modify interactions between plants and soil organisms is dependent on the scale at which vertebrate herbivores and soil organisms operate (chapter 6). Soil biota can affect the plant community across a range of spatial scales, depending on their spatial distribution. Large-scale patterns in the aggregation of soil biota are determined by landscape gradients in soil physical conditions, while at local scales patchiness in plant species composition becomes more important (chapter 2, Ettema and Wardle 2002). Vertebrate herbivores that choose their forage more selectively may be able to respond to small-scale changes in plant quality and community composition (Ritchie and Olff 1999, Olff et al. 2002,

Laca et al. 2010, Hopcraft et al. 2010) driven by local aggregations of soil organisms (chapter 6). Such interactions between aboveground vertebrates and soil organisms that operate across relatively similar scales, i.e., across scales at which they can respond to each others impacts on plant growth and community composition, may drive spatial patterning and species composition in the plant community (chapter 6 and 7, Coppock et al. 1983b, McNaughton 1984, Bardgett and Wardle 2003, Holdo and McDowell 2004, Cromsigt and Olff 2008, Freymann et al. 2010). In contrast, when vertebrate herbivores are less selective and forage across coarse grains, they may not perceive small-scale differences in the plant community and exert a relatively homogeneous grazing pressure across large scales (chapter 6, Ritchie and Olff 1999, Olff et al. 2002, Laca et al. 2010, Hopcraft et al. 2010). When aboveground vertebrates operate across much larger scales than belowground organisms; they can overrule local plant-soil feedback effects (chapter 4, 5 and 6).

In conclusion, my research shows that aboveground vertebrate herbivores can be an important driver of soil community composition by altering plant community composition. Such changes in the soil community may in turn feed back to drive plant species composition. As a result, plant-soil feedback can be important shaping plant communities, because it may enhance temporal dynamics and spatial patterning in plant species composition. In grazed grasslands however, aboveground vertebrate herbivores alter the effect of plant-soil feedback on plant community composition. On the one hand, herbivores enhance spatial patterns in the plant community created by plant-soil interactions when they selectively respond to changes in plant quality or community composition induced by soil organisms. On the other hand, vertebrate herbivores overrule effects of soil organisms when they operate across much larger scales and graze intensively and homogeneously on and off plant patches affected by plant-soil feedback effects. Therefore, the potential of soil organisms to drive plant community structure and composition in natural grasslands is to a large extent dependent on the presence, identity, and grazing intensity of aboveground vertebrate herbivores.





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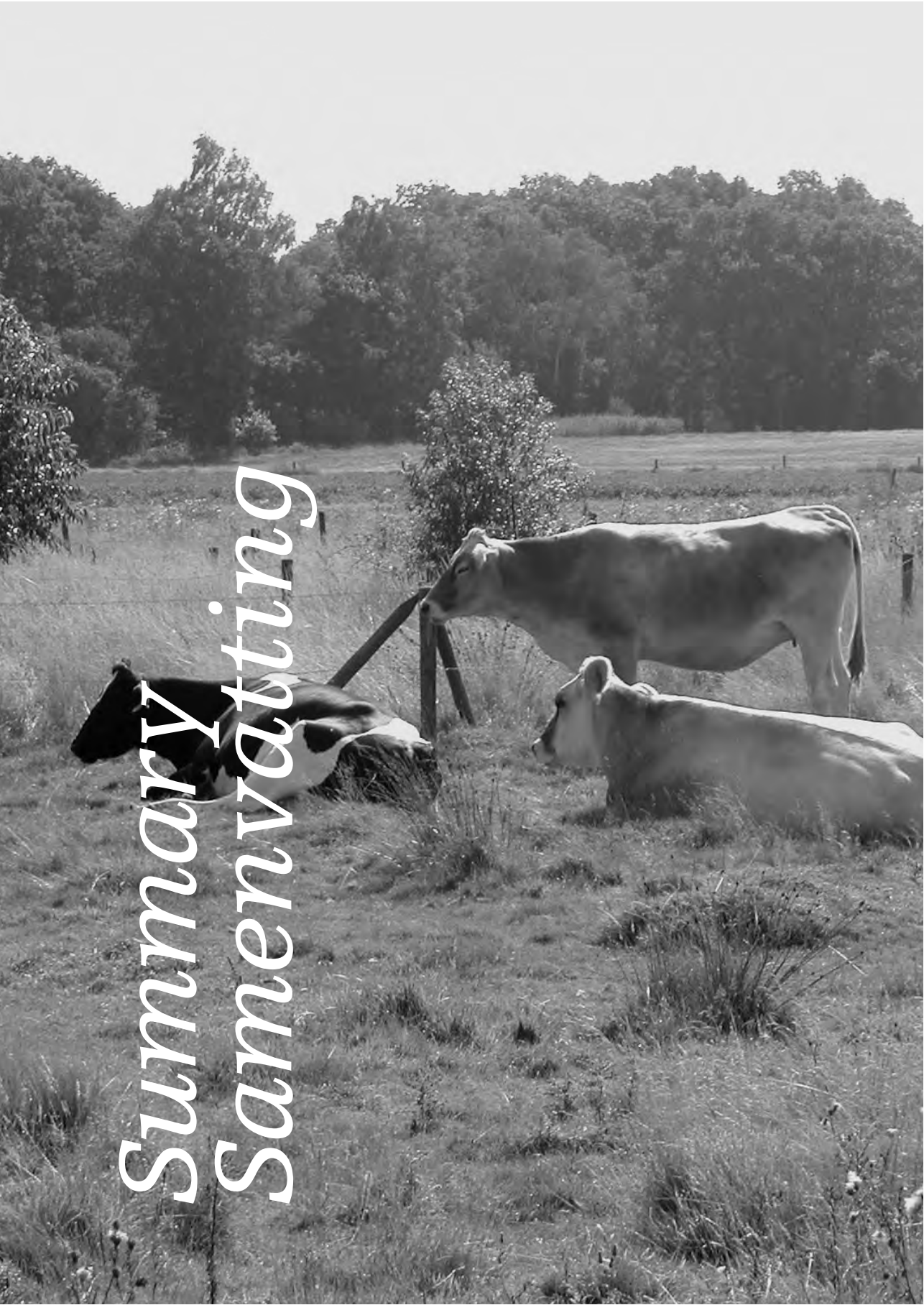
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# *Summary Samenvatting*





Summary  
Samenvatting

## Summary

Aboveground and belowground organisms interact with each other using plants as intermediates. Plants provide organic carbon for decomposer organisms, as well as resources for root and shoot herbivores, pathogens and symbiotic mutualists. In turn, these aboveground and belowground organisms influence plant performance and plant community composition via direct removal of plant material, by enhancing access to limiting resources, and by altering soil nutrient cycling. As a result, interactions between aboveground and belowground subsystems will have major consequences for community structure and ecosystem functioning in terrestrial ecosystems.

Most studies have been focusing on aboveground-belowground interactions involving single plant species, or on the interplay between aboveground and belowground invertebrates. However, how vertebrate aboveground grazers and soil organisms interact to drive plant community structure and ecosystem processes is much less well investigated. Aboveground vertebrate herbivores and soil organisms differ largely in their mobility and scale of operation, particularly as a result of vast differences in body size and the physical structure between the aboveground and belowground subsystems. This may have important consequences for effects of aboveground-belowground interactions on plant community structure and composition.

In this thesis I study how the interplay between aboveground vertebrate herbivores and soil organisms determines plant community structure and composition. The main aim of my thesis is to explore the potential of soil organisms to drive plant community structure and composition under different vertebrate grazing regimes. I am particularly interested in finding out how differences in the scale of operation between organisms influence the outcome of above-belowground interactions. I use a combination of field studies in a floodplain grassland (Junner Koeland, the Netherlands) and a salt marsh (Schiermonnikoog, the Netherlands), greenhouse experiments and mathematical modeling to investigate effects of vertebrate herbivores on the soil community, how soil organisms in turn feed back to alter plant community composition and dynamics, and how interactions between vertebrate herbivores and soil organisms drive plant community composition and structure.

Grazing by vertebrate herbivores is a key determinant of plant community composition and ecosystem processes in grasslands worldwide. On the one hand, vertebrate herbivores alter plant community composition by affecting competitive interactions between plant species through the removal of aboveground plant biomass. On the other hand, they alter soil physical conditions and soil nutrient cycling as a result of trampling, local deposition of dung and urine, changes in root exudation patterns and altered plant litter quality and quantity. Such changes in the soil physical environment, as well as in the plant species composition, can have

important consequences for the community composition and dynamics of soil organisms. Soil organisms often prefer or avoid specific soil physical conditions, but are also known to be specifically associated with certain plant species. In an enclosure experiment in the Junner Koeland nature area I investigated which of these factors was most important in driving effects of combined cattle and rabbit grazing on the local taxonomic composition in the soil nematode community (chapter 2). I found that vertebrate grazing affected soil physical conditions, as well as plant species composition, while the soil nematode composition was determined only by herbivore-induced changes in plant community composition. This indicates that species-specific associations between plants and soil organisms were more important driving the local composition in the belowground community in our experiment, than was variation soil physical conditions.

Changes in the soil community, as well as in soil abiotic conditions, mediated by the plant community, may in turn feed back to influence plant growth and the competitive ability of plant species. This process is referred to as plant-soil feedback. The biotic component of plant-soil feedback is mediated by direct and indirect interactions between plant roots and soil organisms. Root herbivores directly remove plant tissue, pathogens and mutualists may drain or provide resources, while decomposer organisms indirectly affect plant growth by promoting soil nutrient cycling. Plant-soil feedback effects can be highly species-specific as a result of specific associations between plants and soil organisms. They may range from positive to negative, depending on the balance between beneficial and antagonistic interactions. In general, plants that experience strong positive plant-soil feedback will outcompete other species, which reduces plant community diversity and may enhance community stability (chapter 3). In contrast, when the plant-soil feedback effect is negative, the competitive ability of a plant species is reduced. In this situation replacement of plant species in space and time is stimulated, resulting in increased coexistence, diversity, and temporal variability in the plant community (chapter 3). However, the mathematical model in chapter 3 revealed that plant species can still coexist under positive plant-soil feedback, when interspecific plant competition is very low. Moreover, negative plant-soil feedback increased plant coexistence by increasing the parameter range in which oscillations between plant species occurred. This means that coexistence was possible under conditions that would otherwise lead to competitive exclusion, but also that oscillations occurred in situations that would otherwise lead to stable coexistence between plant species. Such oscillations induced by negative plant-soil feedback enhanced coexistence through replacement of species in time and hence increased plant diversity, but could easily drive plant species to extinction in natural situations resulting in a decline in diversity.

In natural ecosystems the effects of vertebrate herbivores and plant-soil feedback interactions often operate simultaneously. Aboveground and belowground

organisms can alter the strength and direction of each others effects on plant community structure and composition. As a result, the sum of their separate effects can be different from the effect they have in combination. I used a greenhouse and field experiment (in the Junner Koeland nature reserve) to investigate the potential of plant-soil interactions to control plant species composition in the absence and presence of vertebrate herbivores. I grew four dominant plant species from the Junner Koeland floodplain grassland in soils originating from plots grazed by cattle and rabbits and in soils originating from ungrazed plots. Half of the soils were incubated with conspecifics plant species, the other half with heterospecifics plant species. All plant species performed worse in soils incubated by conspecifics, regardless of vertebrate grazing history in the field where the soil originated from. In other words, all plant species experienced a negative plant-soil feedback in previously grazed and ungrazed soils (chapter 4). Such negative plant-soil feedback effects may stimulate spatiotemporal dynamics in plant communities in the field, because deleterious soil organisms will reduce the competitive abilities of plant species, leading to replacement by other species. In ungrazed situations I found such dynamics, however, in grazed situations species composition in the plant community was relatively stable in time and space. This indicates that large vertebrate herbivores can overrule negative plant-soil feedback interactions (chapter 4). Probably the effect of herbivores on the plant community was so strong that only grazing-tolerant plant species can become dominant in the plant community, regardless of the plant-soil feedback effects they experience.

To date, it is still very difficult to quantify effects of soil organisms on plant community composition under field conditions, because soil organisms are hard to measure and manipulate. Moreover, the vast majority of soil organisms has not been identified yet. Effects of subterranean, mound building, social insects, such as termites in African savannas and yellow meadow ants (*Lasius flavus*) in Northwest European grasslands, are easier to recognize and hence easier to study. They affect plant community structure and composition on a larger scale than most soil organisms and they create visible small-scale plant patterns. Therefore, to get more insight in the potential of soil organisms to drive plant community structure in grazed grasslands, I investigated how yellow meadow ants and vertebrate grazers interactively influenced plant community composition and heterogeneity (chapter 5 and 6). Yellow meadow ants are subterranean ecosystem engineers that strongly modify their biotic and abiotic environment. They dig up fresh sand for building nest mounds, thereby creating competition-free space for plant species. Moreover, digging activities were previously found to alter abundances of root pathogens and soil physical conditions. Finally, ants tend root aphids for honey dew production, thereby potentially actively affecting belowground grazing pressure. In chapter 5 I studied in a greenhouse experiment how simulated grazing (clipping), the presence of yellow meadow ant mounds and the presence of yellow meadow ants themselves

affected the structure and species composition of the plant community. Short-term plant biomass production was altered by an interaction between simulated grazing and ant mounds. In the presence of grazing production on and off mounds was similar, while in the absence of grazing production was enhanced off mounds. In the long run, both biomass production and species composition were affected by simulated grazing only. The actual presence of ants did not have any effects. This experiment shows that ants can potentially drive local variation in plant biomass production, but when grazing is too intensive or absent for longer periods of time, these effects can disappear. Again, these results imply that grazing by aboveground vertebrates has the potential to overrule plant-soil feedback effects.

As explained before, aboveground and belowground organisms operate at very different spatial and temporal scales. Many soil organisms are limited in their mobility and spend their whole life in the vicinity of one or a few individual plants, while large vertebrate herbivores can explore areas of a few hectares in one day. As a result, soil organisms will affect plants and the plant community locally, whereas effects of large herbivores can play out across much larger scales. In chapter 6 I investigated whether such differences in scale of operation can explain why vertebrate herbivores can overrule plant-soil feedback interactions. On the salt marsh of Schiermonnikoog I studied plant community changes on mounds of yellow ants under hare grazing (*Lepus europaeus*) and under combined grazing by hares and cattle. Small vertebrate herbivores, such as hares, preferentially select higher quality food. They will therefore select their forage very specifically, distinguishing between fine-grained differences in local plant quality. In contrast, large herbivores, such as cattle, are less selective because they mainly depend on high quantities of food and hence choose their food on a much coarser grain. Also they can be less selective due to their larger muzzle size. As a result, hares and cattle may respond differently to local changes in the plant community induced by ants. In chapter 6 I showed that plant species composition on and off mounds of yellow meadow ants was strongly altered under hare grazing. On mounds the cover of *Elytrigia atherica* decreased, while the relative cover of *Festuca rubra* increased. Hares grazed more intensively on the mounds than off the mounds, probably due to their response to small-scale differences in food quality. The combination of hare and cattle grazing resulted in a more homogeneous plant species composition on and off mounds, with a general increase in *Festuca rubra* cover. Grazing intensity was also similar between mounds and the surroundings. These results also show that large vertebrate grazers can overrule effects of soil organisms, because they operate across large scales and graze homogeneously on and off patches created by soil organisms. However, when vertebrate herbivores operate across a scale more similar to the one of belowground organisms, they can selectively respond to local heterogeneity in the plant community induced by soil organisms. In such a situation the feedback between aboveground and belowground organisms can enhance the formation of spatial plant community patterns.

Large vertebrate grazers do not always overrule effects of soil organisms. They may respond to plant-soil feedback interactions when effects of these interactions play out on a large spatial scale. An example is the formation of grazing lawns in African savannas. Large vertebrates have been suggested to increase soil microbial activity and nutrient cycling in large patches. This enhances plant quality and hence the foraging patterns of the herbivores. In contrast, in other ecosystems it has been shown that large herbivores can reduce soil nutrient cycling. Existing theories explain these contrasting results by the net effect of herbivores on the quantity and quality of organic matter input into the soil food web. Herbivores enhance nutrient cycling when they increase the quality of organic matter by excretion of dung and urine, by stimulation of root exudation and by enhancing plant litter quality. They decrease the quality of organic matter when selectively removing nutrient rich plant species, thereby decreasing the quality of remaining plant litter. These theories do not take effects of herbivores on soil physical conditions into account, while such effects can be of major importance driving nutrient cycling in many terrestrial ecosystems (chapter 7). For example, in wet, fine-textured soils trampling by grazers can reduce soil aeration and increase soil moisture levels. This may reduce the abundance and activity of soil microbes and hence has a negative on nutrient cycling. Therefore, it is very important to include effects of herbivores on soil physical conditions into current theories on soil nutrient cycling in grazed grassland. This will advance our understanding of the impacts of herbivores on soil organisms and the processes that these organisms drive.

## Conclusion

In my thesis I have shown that aboveground vertebrate herbivores can be an important driver of soil community composition by altering plant community composition. Such changes in the soil community may in turn feed back to drive plant species composition. As a result, plant-soil feedback can be important shaping plant communities, because it may enhance temporal dynamics and spatial patterning in plant species composition. In grazed grasslands however, aboveground vertebrate herbivores alter the effect of plant-soil feedback on plant community composition. On the one hand, herbivores enhance spatial patterns in the plant community created by plant-soil interactions when they selectively respond to changes in plant quality or community composition induced by soil organisms. On the other hand, vertebrate herbivores overrule effects of soil organisms when they operate across much larger scales and graze intensively and homogeneously on and off plant patches affected by plant-soil feedback effects. Therefore, the potential of soil organisms to drive plant community structure and composition in natural grasslands is to a large extent dependent on the presence, identity, and grazing intensity of aboveground vertebrate herbivores.

## Samenvatting

Organismen die onder- en bovengronds leven beïnvloeden elkaar doordat ze via planten met elkaar in verbinding staan. Planten leveren voedingsstoffen voor organismen die organisch materiaal verteren en voor onder- en bovengrondse plantenetters (herbivoren), plantpathogenen en mutualisten. Op hun beurt hebben onder- en bovengrondse organismen een invloed op de groei van planten en de samenstelling van de plantengemeenschap, omdat ze plantenmateriaal eten, zorgen dat planten een betere toegang hebben tot voedingsstoffen of omdat ze de nutriëntenkringloop in de bodem kunnen veranderen. Dit samenspel tussen onder- en bovengrondse organismen bepaalt hoe goed planten het doen in een gemeenschap en dat heeft belangrijke gevolgen voor de soortensamenstelling in de plantengemeenschap en voor het functioneren van terrestrische ecosystemen.

In de meeste studies zijn de effecten van het samenspel tussen ondergrondse en bovengrondse organismen op één specifieke plantensoort onderzocht, of zijn interacties tussen onder- en bovengrondse ongewervelde (invertebrate) organismen onderzocht, zoals plantenetende insecten of organismen die organisch materiaal verteren. Er is veel minder bekend over de gecombineerde invloed van gewervelde (vertebrate) herbivoren en bodemorganismen op de soortensamenstelling en structuur van een plantengemeenschap. Een belangrijk verschil tussen bovengrondse vertebraten en bodemorganismen is de schaal waarop ze planten en plantengemeenschappen beïnvloeden. Bodemorganismen zijn meestal kleiner en leven in een wereld die bestaat uit een netwerk van bodemdeeltjes gevuld met water en lucht. Ze zijn daardoor beperkt in hun mobiliteit en blijven hun hele leven vooral in de buurt van een enkele plant. Vertebrate herbivoren kunnen daarentegen binnen één dag meerdere kilometers afleggen, waardoor hun invloed op de plantengemeenschap zich over veel grotere afstanden uitstrekt. Deze verschillen in mobiliteit en schaal van invloed kunnen in grote mate bepalen hoe bovengrondse vertebrate herbivoren en bodemorganismen reageren op elkaars veranderingen in groei en soortensamenstelling van planten.

In dit proefschrift beschrijf ik hoe het samenspel tussen bovengrondse vertebrate herbivoren en bodemorganismen de structuur en soortensamenstelling van de plantengemeenschap beïnvloedt. Het belangrijkste doel van mijn promotie-onderzoek is te onderzoeken in hoeverre bodemorganismen de soortensamenstelling en structuur in de plantengemeenschap bepalen in verschillende bovengrondse begrazingsregimes en welke rol schaalverschillen tussen onder- en bovengrondse effecten daarbij spelen. Ik gebruik een combinatie van veldstudies in een overstromingsgrasland (Junner Koeland, Nederland) en een kwelder (Schiermonnikoog, Nederland), kasexperimenten, en wiskundige modellen om te bestuderen hoe vertebrate herbivoren de bodemgemeenschap beïnvloeden, hoe bodemorganismen op hun beurt de soortensamenstelling en structuur van de plantengemeenschap kunnen



veranderen en hoe effecten van plant-bodem interacties worden beïnvloed door bovengrondse vertebrate herbivoren.

Begrazing door vertebrate herbivoren speelt een sleutelrol bij het vormen van graslanden wereldwijd. Vertebrate herbivoren beïnvloeden de soortensamenstelling in de plantengemeenschap. Aan de ene kant eten ze bovengronds plantenmateriaal en dat verandert de concurrentiepositie van plantensoorten. Aan de andere kant veranderen ze bodemfysische eigenschappen en de kringloop van voedingsstoffen in de bodem doordat ze de bodem vertrappen, mest en urine uitscheiden, wortellexudatiepatronen veranderen en de kwaliteit en hoeveelheid dood plantenmateriaal, die moet worden verteerd, beïnvloeden. Veranderingen in bodemfysische condities en in de soortensamenstelling van planten hebben een groot effect op de soortensamenstelling van bodemorganismen. Bodemorganismen komen voor in een bepaald bodemfysisch milieu, maar het is ook bekend dat ze soortspecifieke associaties vormen met planten. In hoofdstuk 2 beschrijf ik hoe begrazing door koeien en konijnen (vertebrate herbivoren) de lokale soortensamenstelling van nematoden (ook wel aaltjes genoemd) in de bodem verandert door bodemfysische condities te wijzigen of door de soortensamenstelling van planten te beïnvloeden. Het onderzoek liet zien dat begrazing door vertebraten een groot effect had op zowel bodemfysische factoren als de soortensamenstelling in de plantengemeenschap. Echter, alleen de veranderingen in de plantengemeenschap bepaalden de soortensamenstelling in de nematodengemeenschap, terwijl veranderingen in bodemfysische eigenschappen geen effect hadden. Daarom lijken soortspecifieke associaties tussen planten en bodemorganismen belangrijker voor het bepalen van de lokale soortensamenstelling in de bodemgemeenschap in ons veldexperiment dan de fysische eigenschappen van de bodem.

Veranderingen in de soortensamenstelling in de bodemgemeenschap (biotisch), maar ook in de bodemfysische eigenschappen (abiotisch), hebben op hun beurt een invloed op de groei en de concurrentiepositie van planten. Dit proces wordt ook wel *plant-soil feedback* (plant-bodem terugkoppeling) genoemd. De biotische component van plant-bodem terugkoppeling bestaat uit directe en indirecte interacties tussen plantenwortels en bodemorganismen. Aan de ene kant beïnvloeden ondergrondse herbivoren, pathogenen en mutualisten planten direct door het verwijderen van plantenmateriaal of door het aanleveren van limiterende voedingsstoffen. Aan de andere kant hebben organismen die plantenmateriaal verteren een indirect effect op plantengroei. Zij sturen de nutriëntenkringloop aan en beïnvloeden daarbij de beschikbaarheid van voedingsstoffen in de bodem. Effecten van plant-bodem terugkoppeling kunnen zeer soortspecifiek zijn, doordat bepaalde bodemorganismen en plantensoorten specifieke associaties vormen met elkaar. De effecten kunnen variëren van positief tot negatief, afhankelijk van de balans tussen voordelige en schadelijke organismen in de bodem. Als het netto effect van bodemorganismen op een plant

positief is, kan zo'n plant andere plantensoorten vaak wegconcurreren omdat haar concurrentiepositie beter is. Dit leidt tot een lagere soortenrijkdom en een hogere stabiliteit in de plantengemeenschap (hoofdstuk 3). Als daarentegen het netto effect negatief is, wordt de concurrentiepositie van een plantensoort verminderd. Andere plantensoorten in de gemeenschap die minder gevoelig zijn voor de soortspecifieke schadelijke bodemorganismen kunnen deze plant wegconcurreren en vervangen. Als de nieuwe plant vervolgens ook haar eigen schadelijke bodemorganismen stimuleert, zal zij uiteindelijk weer worden vervangen door een andere plant. Daardoor neemt de dynamiek in een plantengemeenschap toe en is er niet één plant die heel dominant wordt. Plantensoorten kunnen naast elkaar in een gemeenschap voortbestaan, waardoor soortenrijkdom en diversiteit wordt verhoogd (hoofdstuk 3). Toch kunnen onder positieve plant-bodem terugkoppelingen plantensoorten ook samen in een gemeenschap voortbestaan, als ze weinig met elkaar concurreren. Bovendien kunnen negatieve plant-bodem terugkoppelingen ook leiden tot een lagere diversiteit, als schadelijke bodemorganismen zorgen dat plantensoorten uit een plantengemeenschap verdwijnen. De verhoogde dynamiek die wordt veroorzaakt door de aanwezigheid van schadelijke organismen kan er namelijk toe leiden dat sommige plantensoorten zo slecht gaan presteren dat ze zich niet meer kunnen herstellen in een gemeenschap.

In natuurlijke ecosystemen werken de invloeden van vertebrate herbivoren en plant-bodem terugkoppelingen vaak gelijktijdig. Onder- en bovengrondse organismen kunnen de sterkte en richting van elkaars effect op de plantengemeenschap veranderen. Daardoor kan de optelsom van de afzonderlijke effecten van herbivoren en bodemorganismen op de plantengemeenschap verschillen van het effect dat ze hebben in combinatie. Om dit te onderzoeken heb ik in een gecombineerd kas- en veldexperiment (Junner Koeland) gekeken hoe plant-bodem terugkoppelingen de dynamiek in de soortensamenstelling van planten controleren in de aan- en afwezigheid van bovengrondse vertebrate herbivoren (hoofdstuk 4). In de kas heb ik vier dominante plantensoorten uit het Junner Koeland laten groeien in bodems afkomstig uit proefvlakken begraasd door koeien en konijnen en uit proefvlakken die niet werden begraasd door deze herbivoren. De helft van deze bodems was eerder begroeid door soortgenoten, terwijl de andere helft was begroeid door andere plantensoorten. Onafhankelijk van de begrazingshistorie van de bodem groeiden alle planten slechter in bodems waarop soortgenoten hadden gegroeid, dan in bodems waarop andere plantensoorten hadden gegroeid. Dit betekent dat alle plantensoorten beïnvloed werden door negatieve plant-bodem terugkoppelingen en dat deze terugkoppelingen over het algemeen niet werden veranderd door de historie van bovengrondse begrazing (hoofdstuk 4). Zulke negatieve plant-bodem terugkoppelingen stimuleren dynamiek in de plantensamenstelling omdat soortspecifieke schadelijke bodemorganismen de concurrentiepositie van een plant zodanig kunnen verminderen dat die afneemt in aantal en wordt vervangen door andere

plantensoorten. In het veld vond ik een dergelijke dynamiek, waar verschillende soorten elkaar afwisselen in de tijd en ruimte, in onbegraasde situaties, terwijl begrazing de soortensamenstelling van de vegetatie stabiliseerde. Hieruit blijkt dat vertebrate herbivoren de negatieve effecten van plant-bodem terugkoppelingen teniet kunnen doen (hoofdstuk 4). Waarschijnlijk is de invloed van vertebrate herbivoren op de plantengemeenschap in het veld zo sterk dat bodemorganismen weinig kansen krijgen om de plantensamenstelling te sturen. Herbivoren aten bij voorkeur de smakelijke planten die slecht bestand zijn tegen begrazing. Daardoor stimuleerden ze de toename van plantensoorten die begrazing goed kunnen weerstaan, zelfs als deze begrazingstolerante soorten negatieve effecten van hun eigen bodemgemeenschap ondervonden.

Het is moeilijk om effecten van bodemorganismen op de plantengemeenschap te kwantificeren in het veld, omdat er heel veel soorten bodemorganismen zijn, omdat een groot deel van die bodemorganismen nog niet is geïdentificeerd en omdat het lastig is om ondergrondse gemeenschappen experimenteel te manipuleren. Effecten van ondergrondse sociale insecten, zoals termieten in Afrikaanse savannes en gele weidemieren (*Lasius flavus*) in noordwest Europese graslanden, op de plantengemeenschap zijn makkelijker te bestuderen in het veld, omdat die zich vaak afspelen op een grotere schaal en een grote invloed hebben op de ruimtelijke variatie in plantensoortensamenstelling. Daarom heb ik het samenspel tussen vertebrate herbivoren en gele weidemieren onderzocht (hoofdstuk 5 en 6) om de effecten van plant-bodem terugkoppelingen op soortensamenstelling in de plantengemeenschap beter te begrijpen. Gele weidemieren zijn *ecosystem engineers* (ecosysteembouwers: organismen die hun omgeving zelf creëren of sterk veranderen). Ze leven ondergronds en hebben een grote invloed op de biotische en abiotische eigenschappen van hun omgeving. Om nestbulten te bouwen graven ze zand op. Dit zorgt voor open plekken in de plantengemeenschap waar nieuwe soorten zich kunnen vestigen. Daarnaast veranderen graafwerkzaamheden de doorluchting van de bodem en de hoeveelheid voedingsstoffen, vocht, organisch materiaal en wortelpathogenen in de bodem. Verder houden de mieren actief wortelluizen, ondergrondse planteneters, in hun nestbulten, zoals mensen koeien kunnen houden. Ze “melken” de luizen voor hun honingdauw, een belangrijke voedselbron voor de mieren, en in ruil beschermen ze de luizen in hun nest. Mieren beïnvloeden de plantengemeenschap daarom zowel via de fysieke structuur van de nestbulten als via het effect van hun aanwezigheid op het welzijn van de wortelluizen. In hoofdstuk 5 beschrijf ik een kasexperiment waarmee ik heb bestudeerd hoe nagebootste begrazing (door middel van wegnippen van bovengronds plantenmateriaal), de aanwezigheid van nestbulten van gele weidemieren (*Lasius flavus*) en de aanwezigheid van gele weidemieren zelf de structuur en soortensamenstelling in de plantengemeenschap beïnvloeden. Op de korte termijn (na 3 maanden) werd de biomassa-productie van planten bepaald door de combinatie van nagebootste begrazing en de

aanwezigheid van mierenbulten. Als de planten niet werden geknipt was de biomassaproductie hoger op dan naast mierenbulten, terwijl er geen verschil in productie was als planten wel werden geknipt. Daarentegen, werden de soortenrijkdom en de soortensamenstelling in de plantengemeenschap op de lange termijn (na 9 maanden) alleen beïnvloed door nagebootste begrazing en niet door de aanwezigheid van mierenbulten. De aan- of afwezigheid van de mieren zelf had geen effect op biomassa productie, soortenrijkdom en soortensamenstelling. Dit experiment laat zien dat mieren lokale variatie in biomassaproductie kunnen aansturen, maar dat begrazing deze effecten teniet kan doen. Als begrazing intensief is, of voor lange tijd afwezig is, verdwijnen effecten van bodemorganismen op de plantengemeenschap. Deze resultaten bevestigen dat grote, bovengrondse, vertebrate herbivoren effecten van bodemorganismen op de plantengemeenschap teniet kunnen doen.

De schalen waarop onder- en bovengrondse organismen opereren, verschillen sterk. Veel bodemorganismen brengen hun hele leven door in de nabijheid van één of een paar planten, terwijl grote vertebrate herbivoren gebieden van een paar hectaren binnen één dag kunnen gebruiken. Daardoor zullen de meeste bodemorganismen de plantengemeenschap lokaal beïnvloeden, terwijl effecten van grote herbivoren zich op een grotere schaal uiteten. In hoofdstuk 6 beschrijf ik hoe dergelijke schaalverschillen kunnen verklaren waarom grote, minder selectieve grazers plant-bodem terugkoppelingen teniet kunnen doen. Op de kwelder van Schiermonnikoog heb ik de plantengemeenschap op en naast mierenbulten van gele weidemieren (*Lasius flavus*) onderzocht onder begrazing door hazen (*Lepus europaeus*) en onder gecombineerde begrazing door hazen en koeien. Kleine herbivoren, zoals hazen, zijn afhankelijk van een hoge voedselkwaliteit. Ze selecteren hun voedsel heel precies en maken keuzes op een kleine schaal. Koeien daarentegen zijn afhankelijk van grote hoeveelheden voedsel. Ze zijn minder selectief en maken hun voedselkeuze op een grotere schaal. Bovendien zijn ze minder selectief omdat ze een grotere bek hebben. Hazen en koeien kunnen daarom verschillend reageren op vegetatiepatronen die worden gemaakt door gele weidemieren. Ik heb laten zien dat de soortensamenstelling van de plantengemeenschap op en naast mierenbulten sterk verschilde wanneer alleen hazen voorkwamen. Op de bulten was de bedekking van zeekweek (*Elytrigia atherica*) sterk afgenomen, terwijl de bedekking van rood zwenkgras (*Festuca rubra*) was toegenomen. Hazenbegrazing was intensiever op mierenbulten dan ernaast, waarschijnlijk doordat de voedselkwaliteit op de bulten hoger was. Wanneer hazen en koeien samen voorkwamen, was de soortensamenstelling van planten op en naast mierenbulten vergelijkbaar en was er overal een hoge bedekking van rood zwenkgras. De begrazingsintensiteit op en naast mierenbulten was even hoog. De resultaten van dit veldexperiment laten zien dat selectieve vertebrate herbivoren lokale heterogeniteit in de plantengemeenschap kunnen stimuleren door selectief te reageren op effecten van plant-bodem terug-

koppelingen. Grote grazers daarentegen maken hun voedselkeuze op een veel grotere schaal en doen de effecten van bodemorganismen teniet, waardoor de lokale plantengemeenschap homogener wordt.

Grote, minder selectieve, vertebrate herbivoren doen het effect van bodemorganismen niet altijd teniet. Wanneer plant-bodem terugkoppelingen plaatsvinden op een grote schaal, veranderen ze de plantengemeenschap ook op een grote schaal en kunnen grote herbivoren eventueel specifiek reageren op effecten van bodemorganismen. Zo kunnen grote vertebrate herbivoren bijvoorbeeld een sterke invloed hebben op de nutriëntenkringloop in de bodem. Dit verandert de kwaliteit en soortensamenstelling van planten op een grote schaal en bepaalt vervolgens de voedselkeuze van de grote herbivoren. Een goed voorbeeld hiervan is de vorming van zogenaamde *grazing lawns* in Afrikaanse savannes. In deze systemen versnellen grote grazers de nutriëntenkringloop waardoor ze de kwaliteit van de vegetatie, en dus hun eigen voedsel, verhogen. Echter, in andere ecosystemen is aangetoond dat grote grazers de nutriëntencyclus in de bodem juist afremmen. Bestaande theorieën verklaren deze tegenovergestelde effecten van grazers op nutriëntencycli in de bodem voornamelijk door het netto effect van herbivoren op de kwaliteit en kwantiteit van het organisch materiaal dat de basis vormt van het bodemvoedselweb. Wanneer de netto kwaliteit van het organisch materiaal verhoogd is doordat een deel van het organische materiaal in de vorm van mest en urine, of door de stimulatie van wortellexudatie in de bodem terecht komt, evenals doordat de kwaliteit van het dode plantenmateriaal hoger is, wordt de activiteit van bodemorganismen gestimuleerd en de nutriëntencyclus versneld. Echter, wanneer de netto kwaliteit van het organisch materiaal negatief wordt beïnvloedt, bijvoorbeeld doordat herbivoren planten van hoge kwaliteit selectief weggrazen waardoor de kwaliteit van het overige plantenmateriaal omlaag gaat, kan de nutriëntencyclus worden afgeremd. Deze theorieën houden geen rekening met de mogelijke effecten van herbivoren op bodemfysische condities, terwijl in veel ecosystemen zulke effecten van groot belang zijn voor het sturen van de nutriëntenkringloop (hoofdstuk 7). In natte, kleiige bodems kan vertrapping door grote herbivoren bijvoorbeeld zorgen voor een toename van vocht en een afname van doorluchting in de bodem. Hierdoor wordt de activiteit van veel bodemorganismen geremd en dus ook de kringloop van nutriënten. Hoofdstuk 7 laat daarom zien dat het belangrijk is om de effecten van herbivoren op bodemfysische parameters op te nemen in bestaande theorieën over effecten van herbivoren op nutriëntencycli. We leren dan beter begrijpen hoe begrazing de bodemorganismen en de processen beïnvloeden, die zij uitvoeren in ecosystemen.

## Conclusie

In dit proefschrift heb ik laten zien dat bovengrondse vertebrate herbivoren de soortensamenstelling in de bodemgemeenschap beïnvloeden door de soortensamen-

stelling in de plantengemeenschap te veranderen. Zulke veranderingen in de bodemgemeenschap kunnen op hun beurt de groei en soortensamenstelling van planten veranderen. Plant-bodem terugkoppelingen hebben daardoor een belangrijke invloed op de dynamiek en ruimtelijke patronen in de soortensamenstelling van de plantengemeenschap. In begraasde graslanden kunnen vertebrate herbivoren het effect van plant-bodem terugkoppelingen op de plantengemeenschap echter veranderen. Aan de ene kant kunnen ze ruimtelijke patronen die bodemorganismen creëren versterken, als ze selectief reageren op lokale veranderingen in de plantenkwiteit of de soortensamenstelling die worden veroorzaakt door bodemorganismen. Aan de andere kant kunnen vertebrate herbivoren effecten van bodemorganismen teniet doen als ze op een grotere schaal opereren dan bodemorganismen en de vegetatie intensief en homogeen begrazen. De potentie van bodemorganismen om de structuur en soortensamenstelling van een plantengemeenschap in een grasland te bepalen is daarom voor een groot deel afhankelijk van de aanwezigheid, selectiviteit en begrazingsintensiteit van bovengrondse vertebrate herbivoren.







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# List of publications

## Publications in peer-reviewed journals

- **Veen, G.F.**, H. Olff, H. Duyts, W.H. van der Putten (2010) Vertebrate herbivores influence soil nematodes by modifying plant communities. *Ecology* **91**: 828-835
- **Veen, G.F.**, J.M. Blair, M.D. Smith & S.L. Collins (2008) Influence of grazing and fire frequency on small-scale plant community structure and resource variability in tallgrass prairie. *Oikos* **117**: 859-866
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- Mouissie, A.M., C.E.J. van der Veen, **G.F. Veen**, R. van Diggelen (2005) Ecological correlates of seed survival after ingestion by Fallow Deer. *Functional ecology* **19**: 284-290.

## Other publications

- Mousissie, A.M., **G.F. Veen** & R. van Diggelen (2005) Seed dispersal by roe deer, red deer and fallow deer: a survey of pellet groups and the digestive tract of shot deer. In dissertation: A.M. Mouissie. '*Seed dispersal by large herbivores: implications for the restoration of plant biodiversity*'. University of Groningen , Groningen

## In the media

- VPRO Noorderlicht webdocument: "Behoud de mier"  
<http://noorderlicht.vpro.nl/dossiers/35813434/hoofdstuk/35813435/>



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# Curriculum vitae

Geertje Franciska (Ciska) Veen was born on the 20<sup>th</sup> of March 1981 in Apeldoorn, the Netherlands. She attended high school (VWO) at “De Heemgaard” in Apeldoorn. In 1999 she started to study biology at the University of Groningen. For her Masters she performed three main research projects focusing on interactions between plants and animals. She first studied seed dispersal by deer in the Netherlands. Then she investigated the foraging ecology of Barnacle geese (*Branta leucopsis*) and spent two months on Gotland in Sweden to perform field work. Her last project was carried out at Kansas State University, (Manhattan, USA) under the supervision of Prof. Dr. John M. Blair. She studied the interactive effects of fire and bison grazing on plant community heterogeneity in the tallgrass prairie. During her Masters, she followed the course tropical biology in Kibale Forest, Uganda, which was organized by the Tropical Biology Association (Cambridge, UK). In February 2005 she received her Masters degree and graduated *cum laude*.

From April 2005 – September 2010 Ciska worked on her PhD project with Prof. Dr. Han Olff (University of Groningen) and Prof. Dr. Wim H. van der Putten (Netherlands Institute of Ecology) on the interactions between aboveground vertebrate herbivores and soil organisms which led to this thesis. Besides that, she has been joining an Arctic expedition in 2007 to work for one month as a field assistant to catch, color-ring and collect observations on breeding Barnacle geese at the Russian tundra.

From September – December 2010 Ciska has been working as a post-doc researcher together with Prof. Dr. Wim H. van der Putten and Dr. T. Martijn Bezemer (Netherlands Institute of Ecology). She investigated long-term temporal variation in functional diversity in a biodiversity field experiment (CLUE).

