Herbivores as mediators of their environment: the impact of large and small species on vegetation dynamics

Herbivoren in interactie met hun omgeving:
de invloed van grote en kleine soorten op de vegetatie

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Abstract

Regarding the scale at which grasslands are grazed and the use of large grazers as a tool in nature conservation, it is important to understand the impact that herbivores may have on grasslands. Over the last decades evidence has accumulated that herbivores can have a strong impact on plant communities and vegetation dynamics, but the direction and strength of herbivore effects differs much between studies. One reason could be that different types or sizes of herbivores have different effects. This study compares the effects of large and small herbivores on plant species diversity, vegetation and nutrient dynamics and grassland-woodland transitions. The data presented result from field experiments on a moderately nutrient rich riverine floodplain grassland, grazed by cattle, European rabbits and common voles.

The herbivores studied showed strong interactions: cattle facilitated for rabbits, i.e. most rabbits grazed where cattle also grazed, whereas voles preferred vegetation where cattle were excluded. Cattle grazing created a short sward where colonization rates of plant species were enhanced, resulting in more species per square meter than in ungrazed vegetation. However, most species within this short sward were found where rabbits created bare soil patches through digging, thus providing colonizing species with a suitable regeneration site. Grazing by cattle and rabbits resulted in lower nitrogen availability for plants compared to treatments where only voles grazed. This can be due to a different scale of returning nutrients through faeces: cattle create a few very rich patches and remove nutrients from most of the vegetation, whereas voles redistribute nutrients at a very fine scale, returning small amounts to many plants. The grazing pressure of voles was calculated to be roughly similar to that of cattle and rabbits together. However, grazing by cattle and rabbits caused a short sward and a very stable plant composition with regard to the dominant species, whereas vole grazing caused the dominant plants to show large year-to-year fluctuations in abundance and a high average vegetation height. These differences are probably induced by the size of the herbivores and thus whether they graze the vegetation from above or from below.
In the transition zone of grassland to woodland, palatable trees (Oaks) could invade grazed grassland through the association with unpalatable thorny shrubs (Blackthorn), a process called associational resistance. Associational resistance did work effectively against cattle, but not against rabbits, that both consumed Blackthorn sprouts and young Oaks by going under the shrubs to consume the tree seedlings. Therefore rabbits inhibited tree regeneration, whereas under cattle grazing a mosaic of shrubs, trees and grassland could develop.

Concluding, herbivores can strongly affect their environment, but not in a standard way, i.e. different herbivores have different potentials that are explained both by differences in herbivore size and in habits, as burrowing or consumption of woody plants.

Keywords: *cattle, grassland, herbivory, plant-animal interactions, rabbit, vole*

Contents

Chapter 1

General introduction

Introduction

Grasslands are found on all continents and occupy a broad climatic range, with large differences in temperature, moisture and nutrients (Coupland 1992). All these grassland systems support an assemblage of native vertebrate herbivores, ranging from a single goose species on the tundra vegetation in Churchill in Canada (Jefferies 1999) to more than 28 species of ungulates alone in the Serengeti in Tanzania (Sinclair 1995). Many native large grazers are worldwide endangered and their occurrence is more and more limited to nature reserves, where their existence continues to be threatened by expanding human exploitation (Campbell and Borner 1995; Prins and Olff 1998). Outside the reserves native large grazers have mostly been replaced by livestock (Prins 1992; Voeten 1999; Mishra et al. 2001). Therefore grasslands continue to be grazed, from a vegetation perspective, although the spatio-temporal dynamics and intensity of grazing pressure are now regulated by man. In areas where native large grazers went extinct, as in western Europe, livestock is introduced in nature reserves to maintain or restore grassland biodiversity (Bakker 1989; WallisDeVries et al. 1998; Vera 2000). Regarding the scale at which grasslands are grazed and the use of grazers as a tool in nature conservation, it is important to understand the impact that herbivores may have on grasslands. Over the last decades evidence has accumulated that herbivores can exert a strong influence on vegetation development. Herbivores have been shown to affect vegetation composition (Huntly 1987; Milchunas et al. 1988; Augustine and McNaughton 1998), vegetation structure (Gough and Grace 1998), species diversity (Coppock et al. 1983; Pacala and Crawley 1992; Olff and Ritchie 1998), stability of the vegetation (Sankaran and McNaughton 1999), vegetation succession (Davidson 1993; Van der Wal et al. 2000), resource availability for plants as light (Bakker and De Vries 1992) or mineral nitrogen (Pastor and Dewey 1993; McNaughton et al. 1997; Ritchie et al. 1998), up to the total destruction of native salt marshes along the Hudson bay due to goose grubbing (Srivastava and Jefferies 1996; Jefferies 1999) and demolition of trees by African elephants that opens up savannas (Owen-Smith 1989; Dublin 1995). Because of this diverse array of effects, including the ability to change physical soil properties, herbivores have been proposed as keystone species (American bison, (Knapp et al. 1999)) or ecosystem engineers in grasslands (pikas (Aho et al. 1998), prairie dogs (Bangert and Slobodchikoff 2000) and pocket gophers (Reichman and Seabloom 2002)). Even for the late-Pleistocene a new hypothesis has been forwarded that proposes that the shift from steppe to moss dominated tundra vegetation in northern regions could have been substantially enhanced by the extinction of most of the herbivores at that time, that as an assemblage might have been able to modify their habitat to retain it in the steppe-phase (Zimov et al. 1995).

However, the studies of herbivore effects on the vegetation community composition and ecosystem properties have yielded conflicting results. Herbivores have been found to increase as well as decrease plant species richness (Coppock et al. 1983; Belsky 1992; McNaughton 1993; Milchunas and Lauenroth 1993; Collins et al. 1998;

Proulx and Mazumder 1998; Ritchie and Olff 1999a). They can increase (Holland and Detling 1990; McNaughton et al. 1997; Frank and Groffman 1998; Tracy and Frank 1998; Sirotnak and Huntly 2000) and decrease (Pastor et al. 1993; Ritchie et al. 1998; Van Wijnen et al. 1999) nitrogen mineralization rates. Also the conditions under which herbivores might optimize their food source through repeated grazing (the herbivore optimisation model, (McNaughton 1979; Ydenberg and Prins 1981; Frank and McNaughton 1993; Drent and Van der Wal 1999)) remain under discussion (Hik and Jefferies 1990; Belsky et al. 1993; Bakker and Loonen 1998; De Mazancourt et al. 1998).

Therefore more insight in the underlying mechanisms is needed. Herbivore density may be one source of variation that can cause different effects of the same herbivore species at the same fields (Hik et al. 1990; Shariff et al. 1994; Hart 2001). Secondly, recent work suggests that the impact of herbivores may be understood along environmental gradients (Milchunas et al. 1993; Olff et al. 1998; Proulx et al. 1998; Osem et al. 2002). Thirdly, there is a growing recognition that herbivore size may amplify their effects on the vegetation (Olff et al. 1998). In this thesis I explore the impact of different-sized herbivores on their environment at different levels ranging from plant species richness, vegetation dynamics and nutrient dynamics to grassland-woodland transitions. By studying these diverse aspects of herbivory in one location I aim to find out through which mechanisms herbivores affect their environment and how these different effects are related. I studied an assemblage of cattle, European rabbits (*Oryctolagus caniculus*) and common voles (*Microtus arvalis*) grazing in a moderately nutrient rich floodplain grassland in The Netherlands.

The role of herbivore size

Body size is a critical parameter in the scaling of animal physiology, life history and behavior (Peters 1983; Brown 1995), including metabolic rate and digestive capacity (Demment and Van Soest 1985), home range size (Mysterud et al. 2001; Haskell et al. 2002) and herbivore foraging parameters (Peters 1983; Belovsky 1997; Wilmshurst et al. 2000). Consequently, the impact that herbivores have on the vegetation may vary with herbivore body size. Despite accumulating evidence of herbivore effects on the vegetation (Huntly 1991; McNaughton 1993; Crawley 1997), the role of herbivore size is usually not addressed (Olff et al. 1998). Obviously herbivores differ in more aspects than size alone, as digestive system (ruminants or non-ruminants), social system (solitary or colonial) or being residential or migratory species. However, body size might be a key axis along which general patterns in herbivore grazing on community composition may be found (Prins et al. 1998; Ritchie and Olff 1999b; Olff et al. 2002). Herbivores of different sizes may differ in the scale of their effect, their vulnerability for predators during foraging and their selectivity while foraging (Olff et al. 1998).
Larger species can store more reserves to overcome periods of scarcity (Mysterud et al. 2001), have a longer lifespan (Peters 1983) and larger home ranges (Mysterud et al. 2001; Haskell et al. 2002), but at the expense of lower per capita metabolic and

reproductive rates (Peters 1983). Populations of large versus small herbivores therefore show less changes in numbers over years, whereas populations of small herbivores can show large changes within a few years and even within a year (Krebs et al. 1995; Hanski et al. 2001). Therefore effects of small mammals are also expected to be pulsed in time, coinciding with peak abundance (Hamback et al. 1998; Norrdahl et al. 2002). Temporal grazing pressure by large herbivores on the other hand is expected to be more stable between years.

The largest herbivores are unaffected by predation and limited by food availability (Owen-Smith 1988), whereas populations of most other herbivores may be limited by food and predation (Krebs et al. 1999). Consequently, small herbivores as rodents and small burrowing mammals are often restricted to cover (Cassini 1991; Kotler et al. 1991; Jacob and Brown 2000) and forage in a gradient of decreasing grazing pressure further from cover (Huntly et al. 1986). This spatial variation in grazing pressure could enhance spatial variation in vegetation composition (Huntly 1987).

Size differences between herbivores determine their foraging selectivity and food quality requirements. Food selectivity is limited by the size width of the upper arc (Gordon et al. 1996) through which large herbivores can only graze on multiple plants as their lowest level of selection whereas small herbivores can feed on individual plants or even select plant parts (Ritchie et al. 1999b). Small herbivores have a higher energy demand per gram body mass and a shorter digestive tract (Demment et al. 1985), therefore they need a higher plant nutrient content than bulk feeders to meet their food requirements (Wilmshurst et al. 2000). Therefore palatability of plants may be different to large and small herbivores (Ritchie et al. 1999b). As a consequence large or small herbivores may have different effects on the vegetation composition.

Herbivore assemblages

Most natural grasslands are grazed by more than one species of herbivore. Herbivore species within a grazer assemblage can compete or facilitate for each other (Van der Wal et al. 2000; Arsenault and Owen-Smith 2002; Olff et al. 2002). Facilitation can occur when herbivores show enough difference in body sizes to select different diets (Prins et al. 1998; Olff et al. 2002): small herbivores may profit from grazing by large herbivores through improved quality or accessibility of their food source (Huisman and Olff 1998; Arsenault et al. 2002). When herbivores select similar diets competition can occur, resulting in compensatory effects within an assemblage: the decline of one species of herbivore may be compensated by an increase in other species (Prins and Douglas-Hamilton 1990). The effects of combinations of different-sized herbivores on the vegetation can be additive or compensatory (Ritchie et al. 1999a). Therefore, the interactions between herbivores can have important consequences for the net-effect of the assemblage on the vegetation composition and nutrient cycling. The effects of a single herbivore species on the vegetation should thus be evaluated in a community context. In analogy, when working with an experimental design it is important to take into account behavioural and population responses of the remaining species to the exclusion of others.

Study site and experimental set-up

The study was performed in the Junner Koeland area (52° 32'N, 6° 36'E), a 100 ha nature reserve including 50 ha floodplain grassland along the river Overijsselse Vecht in the Northeast of The Netherlands (fig. 1.1). The site is managed by the National Forest Service (Staatsbosbeheer) as a nature reserve since 1967. The area used to be common grazing land for the farmers from the village Junne, and has probably been grazed by livestock since medieval times (Bokdam 1987). Currently, cattle graze in the area from April till October at a stocking rate of 1 heifer per 2.5 ha. Most common naturally occurring vertebrate grazers are European rabbits (*Oryctolagus caniculus*) and common voles (*Microtis arvalis*). During the study the following other herbivores were observed: roe deer (*Capriolus capriolus*), brown hares (*Lepus europaeus*), bank voles (*Clethrionomys glareolus*), harvest mice (*Micromys minutus*), wood mice (*Apodemus sylvaticus*) and water voles (*Arvicola terrestris*). As predators foxes (*Vulpes vulpes*) and polecat (*Mustela putorius*) inhabit the area and several pairs of raptors nest in the surroundings and hunt on Junner Koeland including buzzards (*Buteo buteo*), goshawk (*Accipiter gentilis*), kestrel (*Falco tinnunculus*) and hobby (*Falco subbuteo*). The vegetation is dominated by the graminoids *Agrostis capillaris*, *Festuca rubra*, *Holcus lanatus* and *Luzula campestris*. Frequently occurring herbs are *Rumex acetosa* and *Stellaria graminea* and the area is known for the occurrence of *Dianthus deltoides* in relatively large numbers for The Netherlands. The nomenclature follows Van der Meijden (1990). The area used to be regularly flooded, but since the canalization of the Vecht (at about 1910) and regulation of the water level, the frequency of flooding strongly decreased over the last century. During the study, the area was flooded in October 1998 for two weeks. Soil fertility varies from rather nutrient poor on the riverdunes to more fertile on the loamier soils closer to the river. Earlier work showed that the nutrient limiting plant growth is generally nitrogen (Boekhoff 2000).

I wanted to study herbivore impact under natural conditions, i.e. in the field with the aid of experiments. Unfortunately it is very hard to study the effects of cattle, rabbits and voles separately in the field. Therefore I used a Russian doll approach meaning
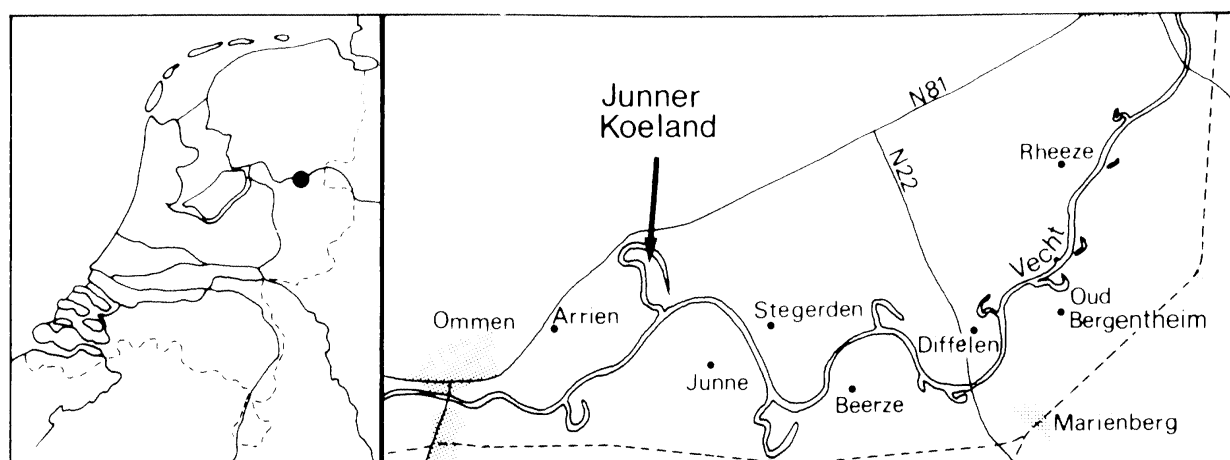

Figure 1.1 Location of Junner Koeland in The Netherlands. The floodplain grassland of Junner Koeland lies within an old oxbow of the river Overijsselse Vecht. From Bokdam (1987).

that I excluded vertebrate herbivores stepwise from large to small (fig. 1.2). Outside the exclosure cattle, rabbits and voles can graze, then cattle are excluded by a barbed wire, leaving access to rabbits and voles. Then rabbits are excluded using chicken mesh, leaving only voles to graze the vegetation. Five of these exclosures (blocks) were built in May 1994 (fig. 1.2a). Later five new blocks of exclosures were built in May 1998 where within the chicken mesh an extra fence was installed where voles could also not enter, leaving no vertebrate herbivores to graze the vegetation (fig. 1.2b). In chapter 2 through 5 data from these exclosures are used, data for the other two chapters were collected elsewhere in the area.

Outline of the thesis

First I examined the variation in plant species richness in the vegetation and the frequency distribution of the species through combining seven years of vegetation recordings in the grassland grazed by cattle, rabbits and voles. Subsequently, I tested the success of seedling establishment of four subordinate herb species by sowing them in different experimentally created regeneration niches, including several that represented disturbances created by cattle and rabbits (Ch. 2). I tested the interactive effects of plant biomass, litter formation and grazing on species colonization and extinction rates in an experiment where I manipulated the amount of litter as well as herbivore grazing through excluding the herbivores stepwise from large to small (Ch. 3). The effect of grazing and nutrient availability on plant community dynamics was tested in another exclosure experiment. Here artificial grazer assemblages were created through stepwise exclusion of herbivores from large to small, although no exclosure was available where all herbivores were excluded. Within each grazing treatment a treatment where nutrients were added was applied (Ch. 4). Dynamics of the dominant species as well as species richness were monitored over seven years. In the same set of exclosures the impact of the different herbivore assemblages on nutrient dynamics was studied through sampling the vegetation and soil and measuring nitrogen mineralization over a whole year (Ch. 5). The importance of plant nutrient content for herbivore patch selection and subsequent grazing pressure was tested in an experiment with rabbits, where food quality and perceived predation risk were manipulated along a natural rabbit grazing gradient (Ch. 6). Finally I investigated the impact of herbivores on the dynamics of a grass-shrub-tree mosaic and especially the establishment of light demanding trees and thorny shrubs under grazing (Ch. 7). For this study tree regeneration and spatial association between trees and thorny shrubs was compared in four riverine floodplains with different grazing intensities.
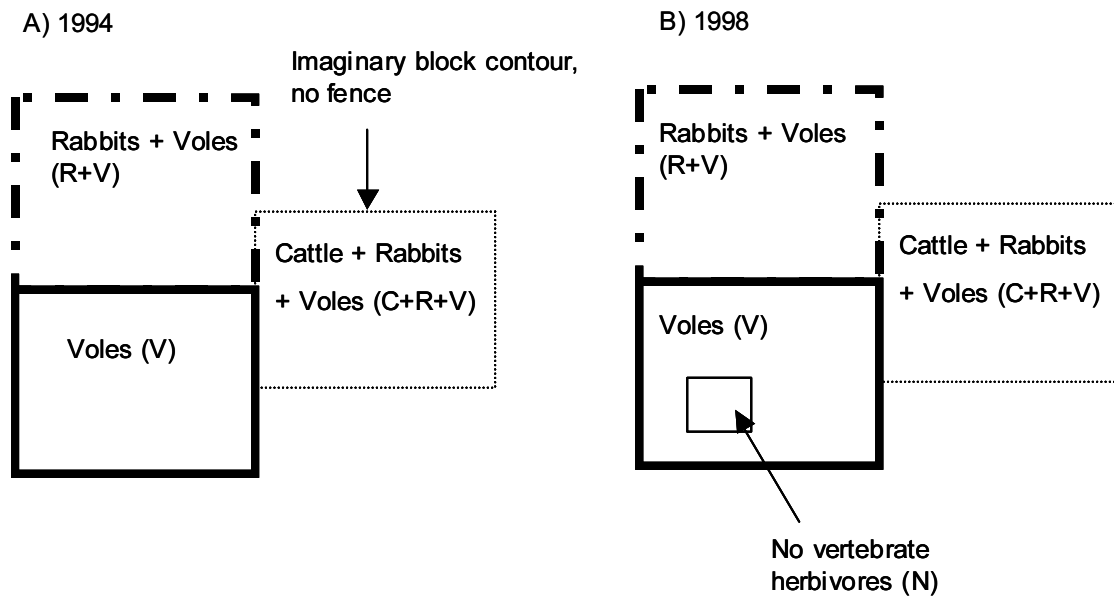
A) 1994

Imaginary block contour, no fence

Rabbits + Voles (R+V)

Cattle + Rabbits + Voles (C+R+V)

Voles (V)

B) 1998

Rabbits + Voles (R+V)

Cattle + Rabbits + Voles (C+R+V)

Voles (V)

No vertebrate herbivores (N)

Fig. 1.2. Design of the exclosures where herbivores are stepwise excluded from large to small. Outside the exclosure cattle, rabbits and voles could graze the vegetation. Two barbed wires at 0.5 and 1.0 m excluded cattle, leaving access for rabbits and voles. With chicken mesh rabbits were excluded, leaving only voles to graze the vegetation. Within each grazing treatment 2 x 2 m plots were established where experimental manipulations were performed and the vegetation was monitored. This exclosure set-up represents one block, five blocks in total were available. The different grazing treatments are referred to as C+R+V: grazed by cattle + rabbits + voles, R+V: grazed by rabbits + voles, V: grazed by voles, N: no vertebrate herbivores

A. Design of the exclosure built in May 1994. Each grazing treatment measured 12 x 12 m.

B. Design of the exclosure built in May 1998 following the same design, but with a 3 x 3 m vole exclosure placed within the treatment that was grazed by voles only. The vole exclosure consisted of transparent plastic sheet at a height of 0.5 m, that extended to 1 m belowground. The size of the other grazing treatments was 15 x 15 m.

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Chapter 2

The impact of different-sized herbivores on recruitment opportunities for subordinate herbs in grasslands

E. S. Bakker & H. Olff

Abstract

Potential effects of herbivores on plant species diversity depend on herbivore size, species and density. In this study we examine the effect of different-sized herbivores (cattle and rabbits) on recruitment of subordinate herbs in grasslands. We show that in a grazed floodplain, grassland plant species richness is mainly determined by the presence of many species of subordinate herbs. These herbs experience high colonization and extinction rates. We conclude that the creation of colonization opportunities for subordinate herbs plays a crucial role in maintaining plant species richness in productive grasslands. We found that cattle disperse large amounts of seeds via their dung, over ten times more than rabbits. Rabbits create more and on average larger bare soil patches than cattle. In a field experiment artificial disturbances improved germination success tremendously for four tested herb species. We found that bare soil is the best regeneration site, while cattle dung gave a too strong nutrient stimulus, resulting in a tall vegetation and therefore light limitation. These results can be confirmed with results from field monitoring plots where plant species richness was positively related to the occurrence of bare soil patches. Therefore both large and small herbivores have a major impact on dispersal and colonization, but for different reasons. Cattle are identified as most important for seed dispersal whereas rabbits have a main effect as creators of disturbances. These results emphasize the importance of distinguishing between herbivore species in assessing their (potential) effects.

Introduction

Herbivores can have a large impact on the plant species richness of grasslands (Huntly 1991; Pacala & Crawley 1992; Collins et al. 1998; Ritchie & Olff 1999). However, the magnitude and direction of their effects vary strongly between habitat and herbivore types. Herbivores have been found to reduce species richness in low-productive systems, whereas they can increase species richness in more productive systems (Milchunas & Lauenroth 1993; Olff & Ritchie 1998; Proulx & Mazumder 1998). Size differences between herbivores determine their foraging selectivity and food quality requirements (Demment & Van Soest 1985). Bulk feeders grazing on dominant plants have potentially different effects on plant species diversity than small grazers, feeding selectively on high quality, subordinate herbs (Ritchie & Olff 1999). The conflicting predictions on how herbivory affects plant species diversity call for more insight into underlying mechanisms.

Herbivores can promote or reduce diversity through several possible mechanisms (Olff & Ritchie 1998). Generally, local plant diversity is determined by the balance between local colonization and extinction. Diversity is promoted when colonization is enhanced and/or extinction is reduced. Extinction is reduced when grazing balances competitive interactions between plants (Berendse 1985). Colonization is enhanced when dispersal and recruitment are promoted by herbivores.

Competition, dispersal and recruitment affect different aspects of community composition. Community structure is determined by plant abundance and plant species richness. Competition affects plant species abundance, especially in dominant species (Tilman 1982). Dispersal and recruitment are particularly important for plant species diversity, since they affect the presence and abundance of subordinate, rare species (Grubb 1977). The effects of herbivores on plant species diversity are mainly determined by the response of subordinate species.

Furthermore, the impact of herbivores on dispersal and recruitment may depend on herbivore size. Large herbivores are expected to have the strongest effect on plant dispersal. They consume large amounts of seeds (Malo & Suarez 1995b) and move larger distances, due to their larger homeranges (Haskell et al. 2002). Also seeds may survive better when passing through larger herbivores compared to smaller sized herbivores (less physical damage), although chemical damage can occur due to a longer retention time in the rumen of large herbivores (Ocumpaugh & Swakon 1993; Olson & Wallander 2002). Large herbivores can create regeneration niches by trampling and via their dung. Smaller herbivores, such as rabbits and large rodents, commonly burrow (Huntly & Reichman 1994) creating other kinds of recruitment opportunities for plant species (Platt 1985). Since the effects of large and small herbivores on plant diversity potentially differ, they should be studied separately.

In this study we compared the effects of different-sized herbivores on both dispersal and recruitment of subordinate grassland plants. In a nutrient-rich wet floodplain we first examined whether local plant diversity is mostly set by subordinate species. For this, we graphed the dominance-diversity relationship and calculated local colonization and extinction rates of each plant abundance class. We then quantified

seed dispersal through faeces by cattle and rabbits. Because consumption of seeds by large herbivores results in both dispersal and creation of recruitment opportunities (gaps created by dung), we separated these effects experimentally. In a grazed grassland we sowed subordinate herb species to test recruitment success in and outside artificial gaps, and in natural and artificial dung.

Methods

Study site

The study was performed on Junner Koeland, a 100 ha nature reserve including 50 ha floodplain grassland along the river Overijsselse Vecht in the north-east of The Netherlands. The site is owned by the National Forestry Service (Staatsbosbeheer) and managed as a nature reserve. The area used to be common grazing land for the villagers from Junne, and has probably been grazed by livestock for several centuries. Currently, cattle graze in the area from April till October with a density of 1 heifer per 2.5 ha. Naturally occurring grazers are rabbits which are present at a density of 14 rabbits ha^{-1} (calculated from pellet counts, E.S. Bakker unpubl. data). Dominant graminoids in the floodplain meadows are *Festuca rubra*, *Agrostis capillaris* and to a lesser extent *Holcus lanatus* and *Luzula campestris*. Most abundant herbs are *Rumex acetosa* and *Stellaria graminea*.

Plant species richness and turnover in the field

In Junner Koeland 5 blocks of 2 plots of 2 x 2 m were established following a randomized block design. Every year from 1996 until 2001 the vegetation in the plots was recorded during peak standing crop, following the decimal Londo scale. With these data colonization and extinction rates per species per plot were calculated as the proportion of new colonized plots compared to total number of occupied plots in a year and extinction as proportion of plots where a species disappeared compared to the number of plots it occupied in the previous year.

Occurrence of gaps in the field

In the 10 monitoring plots the occurrence of bare soil and cattle dung pats was recorded from January 1998 until December 2001, approximately every month (51 times in total). The surface occupied by bare soil or a cattle dung pat is expressed as percentage of the plot surface i.e. 1% = 20 x 20 cm. Bare soil was subdivided in categories according to the cause of the bare soil. Categories were: moles, ants (sand deposited by digging *Lasius flavus* or *L. niger*), cattle (trampling), rabbits (digging). Bare soil covering < 0.5% of the plot was not included in the observations. Bare soil could be created simultaneously by more than one of the above mentioned agents and when the cause was uncertain it was registered as unknown.

Seed dispersal via dung

We collected dung pats in the field to determine the potential of the herbivores as endozoochorous seed dispersers. 15 Separate dung pats of cattle and rabbits were collected on Junner Koeland in spring (June 2000) and in autumn (September 2000). For cattle fresh pats (less than 1 day old) were collected. When collecting the dung pats care was taken to leave the lower part in the field to avoid collection of seeds which were sticking on the bottom. For rabbits, fresh droppings were collected within a few square meters per sample (distance between samples at least 10 m). The dung samples were weighed and stored for two weeks at 5°C to improve germination of the seeds (Olff et al. 1994). Afterwards, the dung samples were spread out in a tray in the greenhouse on sterilised potting soil topped with white river sand. Two trays containing only potting soil and sand were kept as a control. The cattle dung was spread in a layer of less than 0.5 cm deep. Average sample weight (g dry weight) was 141 gram (± 11 se) in June and 223 gram (± 19 se) in September. Rabbit droppings were crumbled before they were distributed over a tray. Average sample weight was 19 g (± 1 se) resp. 31 g (± 2 se) for June and September. Temperature was 20°C at daytime, 15°C during the night (12/12 hours day-night light regime). Dung samples were left for 3 months to germinate. After 2 months the cattle dung patches were broken in small pieces by hand to stimulate germination of the seeds in a deeper layer of the dung. Seedlings were removed as soon as they could be identified to species.

Plant germination and seedling establishment experiment

On the basis of the abundance of species in the grassland, four subordinate herb species (< 1% cover) were selected for a field germination experiment: *Ranunculus acris, Plantago lanceolata, Trifolium pratense* and *Prunella vulgaris.* From here we will refer to these species by their genus name. Seeds from these species were collected at Junner Koeland in late summer 2000.
The field experiment was designed to compare germination success of the selected herb species in grassland, open soil and a cattle dung pat. A cattle dung pat acts as a nutrient source and a bare patch. To separate the effects of nutrients and open space a treatment was included with a dung pat made from paper and water ("paper dung pat") to mimic the bare patch and a paper dung pat with nutrients to compare its performance with a real cattle dung pat. A separate treatment was included in which seeds were sown in grassland with extra nutrients added. To check for natural germination of the sown species in the undisturbed grassland a control plot was established. In total seeds were added to 6 treatments: closed grassland, closed grassland with nutrients, cattle dung pat, paper 'dung' with nutrients, paper 'dung' and bare soil (fig. 2.1).

A)

B)

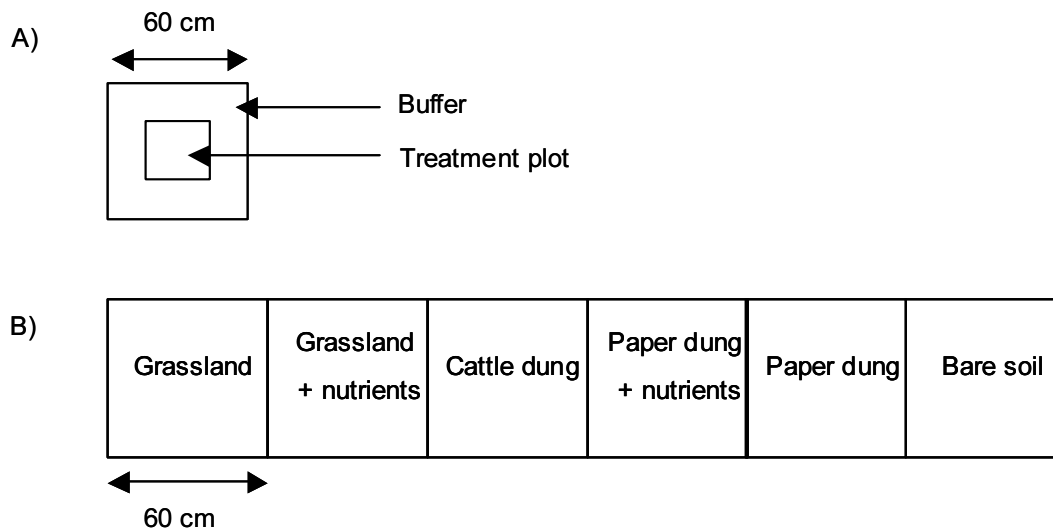| Grassland	Grassland + nutrients	Cattle dung	Paper dung + nutrients	Paper dung	Bare soil

Figure 2.1 Design of the experimental field plots.
A. A plot of 60x 60 cm is marked, containing the inner experimental plot of 20 x 20 cm.
B. The buffer plots are adjacent to each other and form a row. The treatments are written inside each plot (the inner plot is not depicted here for clarity). There are 5 replicate rows, each row contains all treatments once, randomly distributed over the plots.

Treatments were applied to a plot of 20 x 20 cm nested within a 60 x 60 cm plot, which served as a buffer. The larger plots were marked with sticks and positioned in a row with plots adjacent to each other. The treatments were replicated 5 times, yielding 5 rows. Each row contained all treatments once, randomly distributed over the row. Distance between neighbouring rows was 15-25 m. For each of the four selected herb species 200 seeds were sown in the inner 20 x 20 cm plot, resulting in a seed mixture of 4 species x 200 seeds = 800 herb seeds added per plot.

The concentration of added nutrients was based on the nutrient content of a cattle dung pat, which was determined from 5 cattle dung pats collected in September 2000. A standard dung pat weight of 2 kg wet weight was used (= 275 g dry weight). Nutrient addition consisted of 6 g N, 1.3 g P and 0.6 g K per plot and was added as NH_4NO_3, NaH_2PO_4 and KCl respectively. The nutrients were dissolved in 1 litre of tap water and watered over the plots. The extra water added this way was presumed to have no additional effect, because it was not warm (9 °C) with occasional rain. Fresh cattle dung (less than 1 day old) was collected on the day of the start of the experiment. The dung was stirred and portions of 2 kg wet weight were made. The seeds were mixed through the dung sample, which was then applied within the 20 x 20 cm plot. Paper dung was composed of grey toilet paper (9 % of total weight) and water mixed in a blender, resulting in a paper dung patch of 2 kg fresh weight. In the field the seed mixture was stirred through the paper dung and applied in a 20 x 20 cm plot. The bare soil patch was created by cutting a sod of 20 x 20 cm wide and approximately 3 cm deep from the vegetation. Seeds were sown on the bare soil.

Three samples of 2 kg wet weight cattle dung were taken to the lab to check whether the four sown herb species were already present as seeds in the dung applied in the experiment.

The experiment was established and started on 25 October 2000 and every month the number of seedlings in each plot was counted. The numbers recorded represented the number of germinated seedlings minus the seedlings that died within the period in between counts. The last count was on 1 August 2001. In the months March and April it was not possible to count the seedlings because the study area was closed due to foot and mouth disease. In June vegetation height was measured using a stick with a scale and a light polystyrene disc of 10 cm diameter. Vegetation height was recorded in a regular grid of 8 points around the inner plot of 20 x 20 cm. Percentage vegetation cover in the inner plot was recorded in June as well.

Data analysis

We used a logarithmic regression to analyse the relation between plant species abundance (% cover) and the proportion of plots that a species colonized or where it went extinct. The relation between gap occurrence and plant species richness was tested using Pearson correlation. The relation between abundance of a plant species and the amount of seeds of that species in dung was tested with regression analysis after log-log transformation. Total number of seeds in dung, germination of seeds in the experiment and vegetation height in the experiment were tested with Anova after sqrt- or ln-transformation (see results for details). When differences were significant, the Anova was followed by a posthoc Tukey test.

Results

Plant species diversity in the field

The dominance-diversity relationship of plant species in Junner Koeland in 2000 showed a left-skewed distribution (fig. 2.2a). Two dominant grass species were found in all plots. Of the 36 species, 29 were low in abundance (< 1% cover), especially herbs. On average 2.1 (± 0.2) species colonized and 1.6 (± 0.1) went locally extinct per year on an average of 12.5 (± 0.9) species per plot. We found a negative logarithmic relation between species abundance and proportion of plots that a species colonized or where it went extinct (logarithmic regression analysis using all species, colonization r^2=0.52, df = 34, p<0.001, extinction r^2=0.48, df = 34, p<0.001). The low-abundant species had a higher turnover than the higher abundance class (fig. 2.2b). The three grass species with more than 5% cover occurred in all plots over all years, therefore colonization rates could not be calculated by this method, while extinction rates were zero.
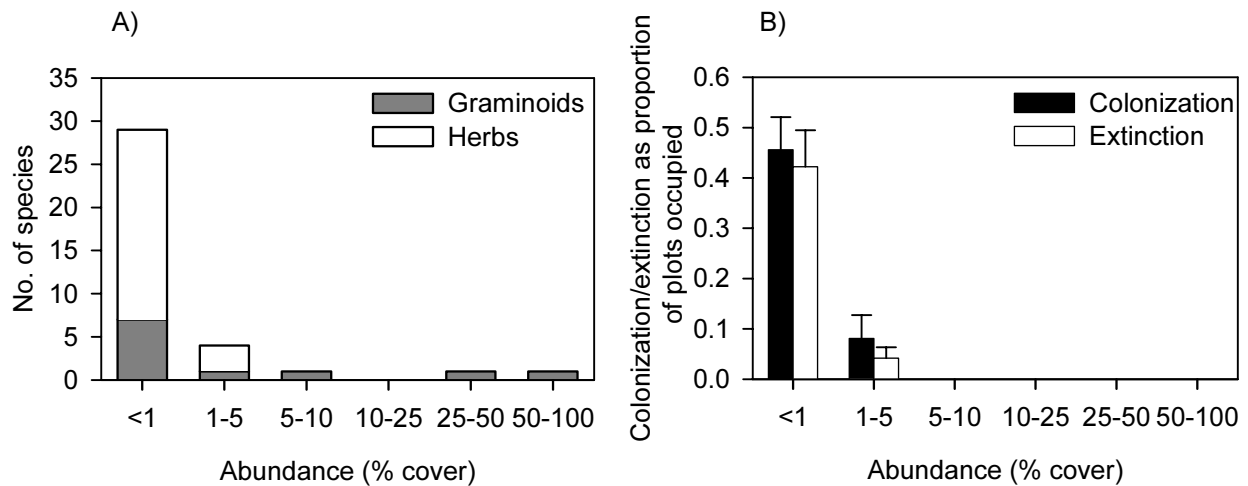
Figure 2.2 A. Distribution of plant species over abundance classes in the field. Abundance is % cover averaged over 6 years (n=10).
B. Colonization and extinction in relation to species abundance. Colonization and extinction are expressed as proportion of plots where species appeared or disappeared, compared to the number of plots occupied this year for colonization and previous year for extinction. The proportion of colonization and extinction is averaged over 5 years for each species. Per abundance class the average of colonization and extinction is calculated over all species in that class. The three grass species with more than 5% cover occur in all plots over all years, therefore colonization rate was not calculated and extinction rate was zero.

Table 2.1 Gap size and gap frequency in monitoring plots (January 1998 – December 2001). Relative frequency gives the contribution to disturbance per agent as proportion of all disturbances caused by all agents. The size of a disturbed patch is averaged over all disturbances (excluding zeros). The percentage of plots containing a disturbance is averaged over sampling dates (n=51 for bare soil, n= 33 for cattle dung pats). All values are means ± se.

Bare soil	Relative frequency (%)	Size of disturbance per plot (dm^2)	% Of plots with disturbance over time
Actor:			
Mole	21%	15 ± 2	7 ± 1
Rabbit	43%	12 ± 1	15 ± 2
Ant	13%	4 ± 1	5 ± 1
Cow	0.5%	2 ± 0	1 ± 1
Multiple actors	14%	21 ± 2	5 ± 1
Actor unknown	9%	5 ± 1	3 ± 1
		mean: 13 ± 1	total: 36 ± 3
Cattle dung		6 ± 0.4	24 ± 4

Occurrence of gaps in the field

On average 36% of all plots contained some kind of soil disturbance at any one time (tab. 2.1). The average size of a bare soil patch was 13 (± 1) dm^2, resulting in an average of 1.2% (± 0.4) cover of bare soil of the total plot area (4 m^2) when all plots were considered. Almost half of the bare soil patches were created by rabbits, a little less than a quarter by moles. Anthills and cattle trampling contributed least to the occurrence of bare soil and also resulted in the smallest gaps (tab. 2.1). Cattle dung pats were present in 24% of all plots (measured when cattle were present from April until late October). The average size of a dung pat was 5.7 dm^2 (± 0.4), resulting in an average of 0.3% (± 0.1) dung cover per plot.

Occurrence of gaps and plant species richness

To examine the relationship between occurrence of gaps and plant species richness, the occurrence of bare soil in each plot during a year preceding the vegetation recording in August 2000 was compared with the number of species recorded in each plot. This was done on a presence/absence basis, we recorded whether a plot contained gaps, but not how many. Gap occurrence was added over the year, i.e. when a plot contained a gap during the whole year, which had been observed on 13 counting dates, the plot received the number 13. This number therefore represented the persistence of gaps during the year, 0 when no gaps occurred and 13 when a plot contained gaps during the whole year. The same was done for the occurrence of dung pats. In this case only the counts when cattle were present in the field (i.e. from mid April until late October) were considered. There was a significant positive correlation between the occurrence of bare soil and plant species richness (Pearson correlation, r=0.663, p=0.037, n=10) while that between gap size and species richness was almost significant (r=0.593, p=0.071). Occurrence of bare soil and gap size were strongly correlated (r=0.944, p<0.001). Dung occurrence and dung pat size showed no relationships with species richness (occurrence r=-0.421, p=0.226, n=10, pat size r=-0.187, p=0.605).

Seed dispersal via dung

From the collected cattle dung pats 6124 plants representing 35 species emerged in the greenhouse (tab. 2.2). No seedlings emerged from the control trays containing only potting soil. The dung pats collected in June contained much less seeds compared to the September pats (487 and 5637 respectively), although the number of species in the pats was almost equal for June and September (18 and 20 respectively, tab. 2.2). The proportion of the various plant species in the dung pats resembled that in the field monitoring plots, i.e. a few species appeared with numerous seedlings, and from most species only a few seedlings were found (fig. 2.3). The abundance of the species in the dung pats was related to the abundance of plants in the field (regression after log-log transformation r^2=0.49, p=0.002, n=17). In total 17 (56%) of the 30 plant species that occurred in the field plots in the year 2000 also occurred in cattle dung. From the rabbit dung 63 seedlings emerged from 12 different species. Only one seedling belonging to a *Spergularia* spec. was unique in

the rabbit pellets, the other species were a subset of the species recovered from cattle dung. For rabbits there was no significant relationship between species abundance in dung compared to the field, which was probably due to the low number of species recovered from rabbit dung, since the r^2-value was equally large (regression after log-log transformation $r^2=0.51$, $p=0.109$, $n=6$). We did collect less dung from rabbits than from cattle. We weighted the amounts we collected, but did not use portions of dung of standardized weight for both species in the greenhouse. We corrected the total amount of seedlings for the amount of dung that plants germinated from (tab. 2.2). However, we could not correct the number of plant species that germinated from the dung for weight of dung used, because the relationship between number of species and the amount of dung that was used is non-linear. Therefore we could only compare the amount of seeds in cattle and rabbit dung, but not the number of plant species in it. There was a significant interaction

Table 2.2 Species found in dung samples of cattle and rabbit dung, collected in spring and autumn 2000. Number of seedlings in dung is the sum of 15 dung samples per sampling date in 2000. Number of seedlings per 100 g dung (dry weight) is calculated per dung pat and was significantly different for cattle and rabbits (One-way Anova after sqrt-transformation on categories herbivore * season, $F_{3,56} = 94.08$, $p< 0.001$, $n=15$). Different letters indicate statistically different groups (Tukey test, $p<0.05$).

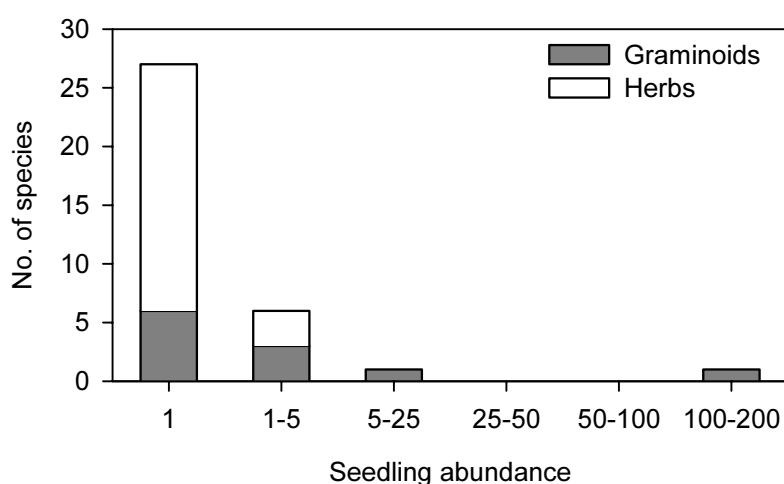|	Cattle		Rabbit	
	June	September	June	September
Total number of species	18	20	6	11
Total number of seedlings	487	5637	17	46
Number of seedlings / 100 g dung	$26.7^{b} \pm 5.8$	$183.1^{c} \pm 21.1$	$6.1^{a} \pm 1.6$	$9.8^{a} \pm 2.1$

Figure 2.3 Distribution of plant species numbers over seedling abundance classes as found in the cattle dung pats.

between herbivore species and season for number of seeds per 100 g dung ($F_{1,56}$ = 51.674, p<0.001). Therefore each herbivore and season were taken as separate categories (cattle in June, cattle in September, rabbits in June and rabbits in September). Rabbit droppings contained fewer seeds per 100 g than cattle dung, which contained most seeds in autumn (One-way Anova, $F_{3,56}$ = 47.74, p< 0.001, tab. 2.2).

Germination of herbs in the field experiment

In the field the four herb species had a different timing of germination. *Trifolium* germinated immediately after sowing and was most abundant in mid November. The other species remained dormant over winter and did not germinate until late February in the experiment. All species except *Trifolium* were most abundant in early May. From early May the number of seedlings declined during spring and levelled off during summer. Germination of seeds was significantly different between treatments ($F_{5,7.05}$ = 13.047, p=0.002, tab. 2.3). There was no significant effect of species or block on the number of seedlings ($F_{1,1.02}$ =2.547, p=0.221 resp. $F_{4, 7.92}$ =1.159, p=0.397, tab. 2.3). Significantly more seedlings were present at the date when most seedlings were found than at the end of the experiment ($F_{1,4.58}$ =7.92, p=0.041, tab. 2.3). Because species had no overall effect and the number of seedlings per species may depend on the success of the other species (seedling competition), numbers of seedlings per species were added per plot and the effect of treatment was tested on the sum of all seedlings together (fig. 2.4, tab. 2.4). For the maximum number of seedlings the maximum number for each species was taken. Because there was a significant effect of season, seedling numbers were tested for both dates separately (tab. 2.4).

Table 2.3 Analysis of germination of the four sown herb species in the experiment after sqrt-transformation. Anova with fixed factors species and treatment, random factors season and block. Season is the period of maximum germination or the end of the experiment in August.

| Factor	Df	F	P
Species	1, 1.02	2.547	0.221
Treatment	5, 7.05	13.047	0.002
Season	1, 4.58	7.92	0.041
Block	4, 7.92	1.159	0.397
Species*Treatment	15, 29.11	3.727	0.001
Species*Season	3, 18.41	14.483	<0.001
Treatment*Season	5, 22.51	3.589	0.016
Species*Plot*Season	15, 60	3.716	<0.001
Species*Block	12, 28.98	1.243	0.303
Treatment*Block	20, 40.39	1.473	0.146
Species*Treatment*Block	60, 60	2.879	<0.001
Season*Block	4, 19.49	1.971	0.139
Species*Season*Block	12, 60	2.370	0.014
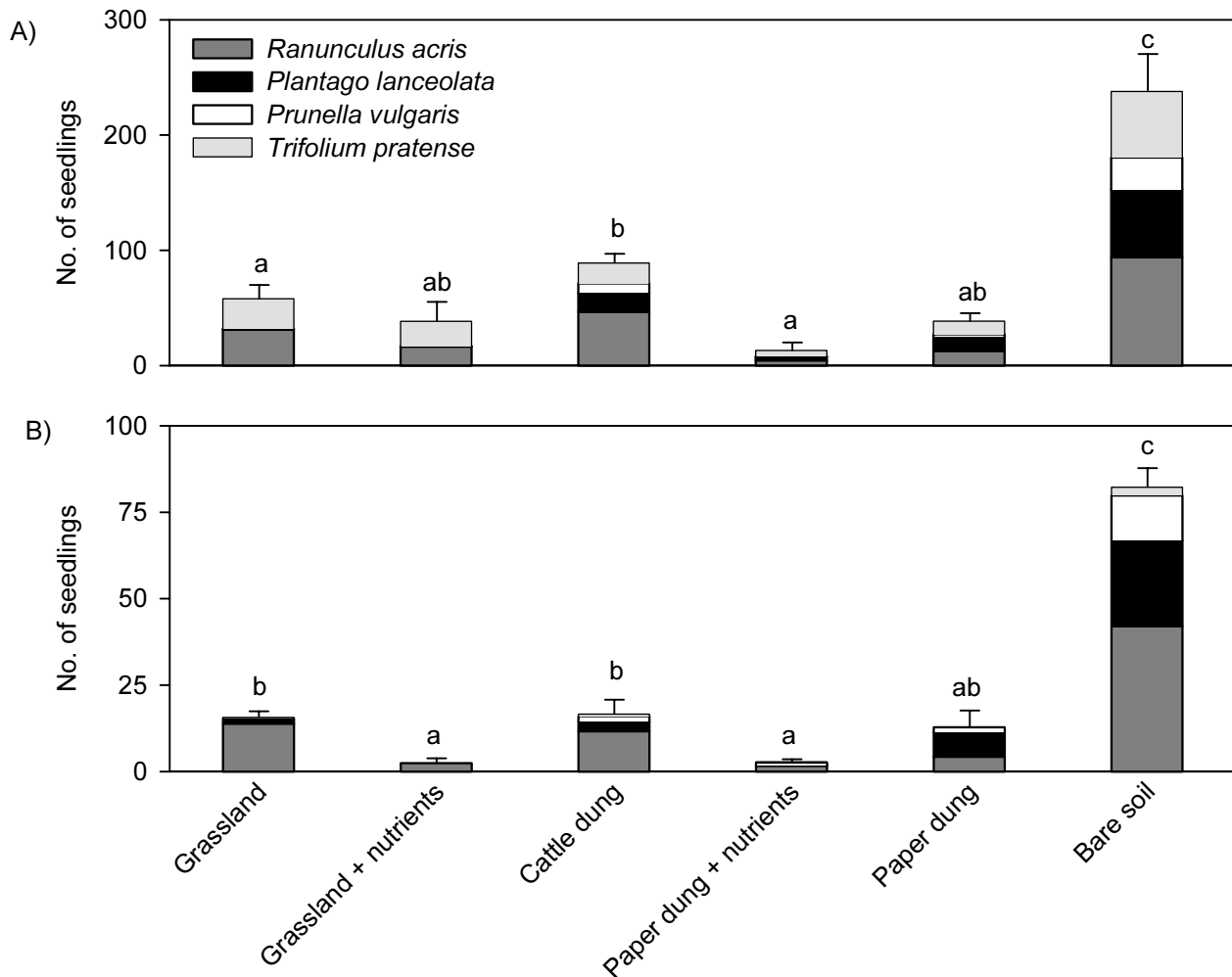
Figure 2.4 Germination success of herbs in the field experiment. Different letters indicate statistically significant differences between treatments (total number of seedlings tested, One-way Anova, after sqrt-transformation, p<0.05). Error bars represent standard errors.
A. Maximum number of seedlings found at one date. This is mid November for *Trifolium* and early May for all other species.
B. The number of seedlings present at the end of the experiment in August 2001.

At the end of the experiment in August only a few of the seedlings that had appeared during the experiment were still present. However, the response to the experimental treatments at that time was the same as at maximum seedling abundance (fig. 2.4). Approximately four times as many seeds germinated in the bare soil treatment compared to the grassland control (maximum number of seedlings: $F_{5,20}$ = 32.380, p<0.001, number of seedlings in August: $F_{5,20}$ = 27.259, p<0.001, tab. 2.4). Seeds germinated second best in grassland and cattle dung and least well in paper dung. Both treatments with artificial nutrient addition resulted in the lowest seedling numbers, especially in August (fig. 2.4).

All species had their maximum number of seedlings in the bare soil patch (fig. 2.4). *Plantago* and *Prunella* hardly germinated in closed grassland (with or without

nutrients) whereas *Ranunculus* and *Trifolium* germinated successfully in closed grassland. For *Trifolium* hardly any seedlings survived until August.

In the grassland control plots without seed addition no seedlings from the test species emerged during the experiment. From the three dung pats in the greenhouse, collected from the dung used in the experiment, two of the herbs used in the experiment germinated, *Plantago lanceolata* and *Prunella vulgaris* with 3 and 2 seedlings, respectively.

Vegetation height in the experiment

The vegetation immediately surrounding the treatments was significantly higher in June for the fertilized treatments (cattle dung, paper dung with nutrients and the nutrient addition in closed grassland) compared to all other treatments (Two-way Anova after ln-transformation, fixed effect treatment $F_{5,20} = 7.808$, $p < 0.001$, random factor block $F_{4,20} = 9.855$, $p < 0.001$, treatment*block $F_{20,210} = 1.627$, $p = 0.049$, Tukey posthoc test, $p < 0.05$). In June most treatments still contained non-vegetated parts, the mean percentage bare was 6% (± 2 se) for cattle dung, 27% (± 12) for paper dung with nutrients, 68% (± 17) for paper dung only and 46% (± 11) for the bare soil patch. However, these differences were not significant (Two-way Anova after ln-transformation, fixed effect treatment $F_{3,12} = 1.614$, $p = 0.238$, random factor block $F_{4,12} = 0.075$, $p = 0.989$).

Table 2.4 Analysis of germination in the experiment at maximum number of seedlings and in August (end of the experiment), after sqrt-transformation. Number of seedlings is the sum of seedlings of all four sown species. Fixed factor is treatment, random factor block. A Tukey post-hoc test is performed; the results are depicted in fig. 2.4.

Factor	Df	F	P
Maximum germination			
Treatment	5, 20	32.380	<0.001
Block	4, 20	3.640	0.022
Germination August			
Treatment	5, 20	27.259	<0.001
Block	4, 20	1.066	0.399

Discussion

We conclude that most variation in species richness in Junner Koeland can be attributed to subordinate herb species. Although species richness of the field plots remained relatively stable over the 6-year study period, the subordinate herbs experienced high colonization and extinction rates.

In our field experiment germination success was strongly improved when seeds were sown in a bare soil patch, compared to germination in undisturbed vegetation. The species *Prunella vulgaris* and *Plantago lanceolata* were not able to germinate in grassland at all (*Prunella*) or only with very few individuals (*Plantago*). Many herb

species are reported to germinate better in gaps than in the matrix vegetation (Gross & Werner 1982; Klinkhamer & De Jong 1988; Silvertown & Smith 1989). The number of established seedlings on the paper dung pat with nutrients was much less than on the cattle dung pat. This could be due to a different rate of release of nutrients from the paper dung pat compared to the cattle dung pat or to a difference in texture that influenced abiotic conditions. Seedling establishment on paper and cattle dung pats was not significantly better than in undisturbed grassland. The paper and cattle dung pats did provide competitor free space that seemed to be the main advantage of a bare soil patch. However, with the onset of the growing season this initial advantage of germination on the competitor free dung pat disappeared because the vegetation surrounding it started to grow due to the nutrient pulse. The bare soil patch remained unvegetated for a longer period, thereby offering the best site to germinate for all species. Slower re-growth of the vegetation results also in increased light availability, which often has a positive effect on germination success of grassland plants (Van Tooren & Pons 1988; Pons & Van der Toorn 1988).

In the dense sward of perennial grasses at our study site, gaps were almost exclusively created by animals (tab. 2.1). Bare soil patches only covered 1.2% of the area when averaged over all plots (including plots without disturbance) but they did occur frequently, approximately one third of the plots contained a bare soil patch, mainly caused by rabbit and mole burrowing activity. Cattle dung pats were found in almost a quarter of the plots. The increased germination success of herbs on bare soil compared to dung pats in the experiment corresponded with the data from the field monitoring plots: plant species richness was positively related to the occurrence of bare soil gaps and not to the presence of cattle dung pats. The creation of bare soil patches by herbivores may increase regeneration possibilities for herb species and thereby increase local plant species richness. Several herbivore species are reported to increase colonization rates and local plant species diversity through gap creation, e.g. wallows of bison (Collins & Barber 1985), uprooting wild boar (Milton et al. 1997) and burrowing prairie dogs (Coppock et al. 1983; Platt 1985). Gap creation may therefore be an important mechanism through which herbivores can increase plant diversity.

The impact of herbivores on plant dispersal depends on herbivore type. Cattle graze on green plant material and thereby consume seeds that are mixed with the foliage (Janzen 1984). As a consequence abundant species are also frequently distributed. Although low abundant species are also infrequently encountered by cows, a sample of only 30 cattle dung pats collected at two days in spring and fall already contained 56% of the plant species which occurred in the field monitoring plots. Because bulk feeders quantitatively consume a lot of seeds, they have more chance to include subordinate species compared to smaller herbivores, hence promoting their dispersal (Malo & Suarez 1995b). The survival probability of seeds after consumption is related to seed size (Pakeman et al. 2002). Large seeds are more sensitive to physical damage by chewing than small seeds, the effect being stronger in smaller herbivores (Pakeman et al. 1999). On the contrary, chemical damage to seeds may be less in small herbivores, since the seeds spend a shorter time in the digestive tract (Ocumpaugh & Swakon 1993; Olson & Wallander 2002). Herbivore size has a strong

impact on dispersal distance of seeds when considering mammalian herbivores: large herbivores have a larger digestive tract resulting in a slower passage rate for seeds (Demment & Van Soest 1985) and thus more time to travel and large herbivores have larger homeranges (Haskell et al. 2002).

The relative importance of colonization and dispersal for diversity depends on habitat structure and productivity. In a heterogeneous habitat herbivores can disperse seeds between suitable patches. In this way they increase local diversity, but in the same time reduce between-patch differences in species composition (Welch 1985; Malo & Suarez 1995a). Habitat productivity plays a key role in the discussion about the importance of dispersal versus regeneration opportunities. In unproductive habitats regeneration niches seem not to be limiting (Van der Maarel & Sykes 1993; Zobel et al. 2000). These systems tend to have a high number of annuals and therefore an enormous seed production (Malo & Suarez 1995a). Herbivores will then mainly contribute to seed dispersal. In productive systems regeneration niches can become limiting and herbivores are expected to contribute to colonization opportunities via soil disturbance whereas seed dispersal remains also important.

In our study site regeneration niches seem to be limiting, at least for some species, regarding the increased germination success of the subordinate herbs in the bare soil plots. However, seed dispersal seems to be a limiting factor as well since the establishment of seedlings took place in the grassland plots where seeds were added while seedlings of these species were absent in the control plots. Since plant species richness in the field monitoring plots is related to gap occurrence and not to cattle dung occurrence, seeds seem to be able to reach the bare soil gaps somehow. This could be via rabbit droppings (for example on a latrine) or exozoochorous dispersal by cattle and rabbits, some other dispersal mechanism (barochorie or wind dispersal) or seeds were present in the local seed bank that became suddenly exposed at the surface. Though seedling establishment in dung pats is less successful than on bare soil, still many species may colonize a new dung spot, even though with few individuals, since dung pats are numerous.

We conclude that the creation of establishment opportunities for subordinate herbs plays a crucial role in maintaining plant species richness in productive grasslands. Cattle are identified as most important for seed dispersal whereas rabbits appear to have a main effect as creators of disturbances. Therefore both large and small herbivores have a major impact on dispersal and colonization, but for different reasons. These results emphasize the importance of distinguishing between herbivore species in assessing their (potential) effects.

Acknowledgements

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Chapter 3

The interactive effects of litter formation and grazing on grassland plant diversity

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Abstract

Herbivores have a strong impact on plant species diversity in grasslands, which may be understood from their impact on local colonization and extinction rates of plants. Through grazing herbivores remove biomass, but also prevent that litter accumulates. Both the amount of standing crop and litter formation are known to affect plant species richness. We studied the effect of herbivore grazing on standing crop and litter formation and the interaction of both on plant diversity. We performed a 3-year exclosure experiment in a floodplain grassland where we excluded herbivores (cattle, European rabbit and common vole) stepwise from large to small. Within these different grazing treatments we performed a litter removal experiment and a biomass removal (i.e. clipping) experiment. After three years litter removal had no effect on plant species richness, which slightly increased under joint grazing by cattle, rabbits and voles compared to the other grazing treatments. Clipping had a strong positive effect on plant species richness, mainly in the treatment where all herbivores were present.

Litter removal and clipping had no effect on extinction rates of plant species, litter removal also had no effect on colonization rates, but clipping strongly enhanced colonization rates. Light measurements revealed that clipping strongly enhanced light availability at the soil, whereas litter removal had only moderate effects on light availability. Plant species richness was significantly positively correlated with light availability and negatively with the amount of litter, although this last relationship was much weaker. Living and total biomass showed no significant relationship with species richness. We conclude that recruitment, and hence species richness was light limited even where all herbivores grazed. Although litter strongly accumulated when cattle were excluded, it had no additional negative effect on plant diversity since recruitment was already light limited. Light limitation had little effect on extinction rates but caused severely reduced colonization rates and therefore reduced species richness. We thus conclude that large herbivores can enhance plant diversity in light limited vegetation through grazing a short turf that leads to increased species colonization rates.

Introduction

Herbivores have a profound impact on plant species diversity in grassland ecosystems (Milchunas et al. 1988; Huntly 1991; Pacala and Crawley 1992). However, the magnitude and direction of the change in species richness under grazing varies strongly between studies (Milchunas and Lauenroth 1993; Olff and Ritchie 1998; Proulx and Mazumder 1998). Possible explanations for these contrasting results are differences in habitat productivity (Proulx and Mazumder 1998; Osem et al. 2002), herbivore density (Bullock et al. 2001; Hart 2001; Taddese et al. 2002) or herbivore type (Ritchie and Olff 1999), that are all shown to affect the impact of herbivores on plant species richness.

 However, the mechanisms through which herbivores affect plant species diversity under different conditions are still unclear. Each herbivore species can have multiple effects (Huntly 1991; Olff and Ritchie 1998). Herbivore effects are commonly studied through comparison of grazed vegetation with vegetation in an exclosure where the herbivores are excluded. Often changes in plant species richness are observed, but it is usually difficult to identify the mechanism causing these changes. Multiple factors change when herbivores are excluded, for example plant standing crop, litter, light interception or nutrient availability (Bakker 1989; Hik and Jefferies 1990; Pastor et al. 1993; Singer and Harter 1996; Ritchie et al. 1998; Gough and Grace 1998; Van Wijnen et al. 1999; Sirotnak and Huntly 2000). To clarify the confounding effects of herbivores we need to disentangle these covarying factors. Experimental manipulation of grazers, and direct manipulation of their effects may allow the discrimination between the causes of vegetation change. Furthermore, most grasslands are grazed by an assemblage of herbivore species. To understand herbivore effects on the vegetation it is necessary to separate the effects of the herbivore species (Ritchie and Olff 1999).

The mechanisms regulating plant diversity are mostly studied under ungrazed conditions. Plant species richness in ungrazed grasslands is shown to depend strongly on the amount of biomass at peak standing crop, often also taken as a surrogate for productivity (Grime 1973; Al-Mufti et al. 1977; Waide et al. 1999; Mittelbach et al. 2001). A shift in intensity of resource competition and type of limiting resource along a productivity gradient is thought to determine the coexistence opportunities between plant species (Tilman 1985). At high productivity competition for light between established plants leading to competitive displacement is considered the main mechanism for the decrease of species richness (Newman 1973; Tilman 1982; Tilman 1985; Goldberg and Miller 1990).

However, island biogeography theory pointed out that local species richness is the result of the balance between local extinction and colonization (MacArthur and Wilson 1967). Therefore a decrease in species richness may not necessarily be only explained from competitive exclusion, but could be the result of increased extinction, reduced colonization or both. Indeed, Tilman (1993) found a decrease in colonization rate to coincide with increased vegetation production, thereby negatively affecting species richness.

The relation between productivity and species richness may not operate through interactions between living plants alone, but can be strongly influenced by interactions after death (Facelli and Facelli 1993; Foster and Gross 1998; Berendse 1999). With increasing plant production also the amount of litter increases (Al-Mufti et al. 1977). A litter layer may reduce colonization through inhibition of seedling establishment both through shading and physical obstruction (Fowler 1988; Bergelson 1990). Litter can also enhance extinction through outshading of small plants or through nutrient release from the litter layer that can stimulate fast-growing, tall plants (Berendse et al. 1994). A negative effect of litter on plant species richness is found in several studies where productivity or the amount of litter was manipulated (Carson and Peterson 1990; Foster and Gross 1998). However, it is not clear whether litter has an additive or interactive effect relative to increased living biomass production (Tilman 1993).

These conclusions on main determinants of plant diversity in grassland may however not hold under grazed conditions. In grazed systems a higher local productivity leads to a higher herbivore grazing pressure through attracting grazers, less standing crop, and therefore less light competition. Herbivores remove biomass, but at the same time they prevent litter formation (Bakker 1989; Hik and Jefferies 1990; Pastor et al. 1993; Singer and Harter 1996; Gough and Grace 1998; Ritchie et al. 1998; Van Wijnen et al. 1999; Sirotnak and Huntly 2000). Herbivores could therefore directly affect plant species richness through biomass removal, but also indirectly through preventing negative effects of litter accumulation on species richness. Through the direct effect of biomass removal herbivores may balance the competitive abilities among dominants that can lead to reduced extinction rates. They improve regeneration opportunities through creating small disturbances (Bakker and Olff 2003) and higher light intensities at the soil surface due to a more open canopy (Bakker and De Vries 1992) and enhance seed input (Malo and Suarez 1995; Fischer et al. 1996; Pakeman et al. 1998), leading to increased local colonization rates. Surprisingly, despite the agreement on the importance of colonization and extinction rates as determinants of plant species richness, this balance has hardly been investigated under grazed conditions (but see Glenn & Collins (1992)).

Until now, experiments that investigated the relationship between standing crop, litter accumulation and plant species richness were conducted in grasslands or old fields that were ungrazed. However, most grasslands are, or have been, grazed by herbivores. Since herbivores reduce the standing crop and prevent the accumulation of plant litter and both litter and standing biomass are shown to have important effects on plant species richness, we want to test whether herbivores affect plant species richness through biomass removal and prevention of litter formation. To do this we performed an experiment where we excluded grazers and removed litter and biomass separately in a factorial design. In a floodplain grassland grazed by cattle, European rabbits and common voles we excluded the herbivores stepwise from large to small to create different herbivore assemblages. We monitored species richness and colonization and extinction rates in the different treatments during three years.

Methods

Study site

The study was performed on Junner Koeland, a 100 ha nature reserve in the Northeast of The Netherlands. The site is owned by the National Forest Service (Staatsbosbeheer) and managed as a nature reserve. The area is grazed by cattle at a density of 1 heifer at 2.5 ha. Naturally occurring vertebrate herbivores are European rabbits (*Oryctolagus caniculus*) and common voles (*Microtus arvalis*). The reserve includes 50 ha of floodplain grassland, where the experiment was performed.

Exclosure experiment

At Junner Koeland 5 exclosures (blocks) were built in May 1998 that excluded herbivores stepwise from large to small. Outside the exclosure cattle, rabbits and voles could graze. Two barbed wires at 0.5 and 1.0 m excluded cattle. Rabbits were excluded by 1.0 m high chicken mesh with a mesh width of 2.5 cm. The fences were 15 x 15 m wide. Inside the chicken mesh fence in each block a plot of 3 x 3 m was surrounded by transparent plastic sheet of 0.6 m tall and 0.4 m deep in the soil to exclude voles. Voles that were trapped inside the fence by coincidence were caught with snap traps and removed. The plots were regularly (approximately every month) checked for signs of vole activity. Vole burrows or runways were easy to detect in the rapidly increasing litter layer. During the first winter a few voles entered one exclosure. In spring 1999 an extra sheet was buried in each exclosure, reaching to a depth of 0.8 m. The voles were removed with snap traps. Since then no signs of vole activity were found. The grazing treatments will be referred to as C+R+V for cattle + rabbits + voles, R+V for rabbits + voles, V for voles only and N for the treatment where voles were exclosed and therefore no vertebrate herbivores were present.

Litter and biomass removal experiments

Within the exclosures two experiments were established: the first to study the effect of litter removal on plant species richness, the second to study the effect of biomass removal through clipping (artificial grazing) on plant diversity. In this study we refer to litter as the dead material that lays on the soil and could be accumulated during several years, standing dead is mostly current-year dead material that is still standing upright and attached to a tiller or tuft. Total biomass comprises standing living + dead biomass + litter.
Experiment I: litter removal. Within each exclosure fence two plots were installed. One plot of 1 x 1 m served as a control and in the other plot litter was removed during winter. Litter was removed every winter for three years, starting in the winter of 1998-1999. Litter was removed using small hand rakes that removed the litter layer and as much of the standing dead plants as was loosened through raking. The litter was collected, dried at 70ºC for 48 hours and weighed.
Experiment II: biomass removal through clipping. Within each grazing fence two plots of 2 x 2 m were established, one control plot and one 2 x 2 m plot where above ground biomass was removed through clipping. The grazing treatment where voles

were excluded did not receive this clipping treatment due to limited space in the exclosure. The first clipping was done in February 1999; later clippings were done when the vegetation in one of the plots grew over 10 cm tall (excluding flower stems). Then all plots were clipped to a height of 5 cm. Consecutive clipping was done in June 1999, February 2000 and July 2000.

Biomass of both experimental plots and the control plot of the litter removal experiment was harvested in July 2001 by clipping a strip of 10 x 100 cm to soil level. The old litter layer was harvested separately. Biomass samples were sorted to graminoids and herbs and life and dead, dried at 70°C for 24 hours and weighed.

Vegetation measurements

Experiment I: litter removal. Each year, starting in 1998, the vegetation composition was recorded by visually estimating aerial cover following the decimal Londo scale (Schaminée et al. 1995). Cover of each species was estimated separately; due to overlapping leaves total cover could be more than 100%. Each August the vegetation height was recorded using a stick with a polystyrene disc (10 cm diameter) that was dropped on the vegetation. Vegetation height was measured at 9 points equally distributed over a plot in a 3 x 3 grid. In May 1999 and 2000 seedlings were counted in the plots. Seedling density was not very high, therefore it was possible to count the entire 1m^2 plot. Only dicot seedlings were counted, since grasses proved to be too hard to recognize as a seedling. Presumed dicot seedlings were tested by uprooting when there was doubt whether it was a seedling.

Experiment II: biomass removal. The vegetation composition of the plots in the clipping experiment was recorded in 2000 and 2001. In 2001 the vegetation was also recorded in the inner 1m^2 of the clipping plots to make a comparison of species richness with the litter removal experiment possible, which had plots of 1m^2 size. Vegetation height in the plots was recorded in August 2000.

After the final harvest in August 2001 plant density was determined in all raked, clipped and control plots through counting stems in a randomly placed square of 6 x 6 cm in the strip where the vegetation was just harvested.

Light measurements

In August 2001 light was measured in all plots. Light was measured using a 1 m tall probe with a light sensitive surface of 1 cm wide (SunScan Canopy Analysis System, Delta-T Devices Ltd, U.K.). In each plot two measurements were done at soil level.

Herbivore presence

Cattle were present at a density of 0.4 cows per ha in the entire reserve. Cattle presence in the plots was not separately recorded. Rabbit presence was measured by pellet counts from 1998 till 2001 in the vegetation plots outside the exclosures where cattle grazed and in the fence where rabbits had access. Pellets were counted every month and removed. Rabbit density was calculated by assuming a dropping rate of 400 droppings per rabbit per day (Lockley 1962). Vole density was measured in fall 1999 and 2000 by live trapping. Longworth live-traps were used baited with

peanut butter, oats, carrot and apple and stocked with some hay. Traps were pre-baited during two days followed by a five-day trapping session. Traps were checked every 8 hours, resulting in 11 checks per session in total. Voles were individually marked by clipping a small piece of the fur at various places on the back and then released. After 4 days still new individuals were caught. This made it unreliable to calculate potential vole density from the mark-recapture data. Therefore the number of individuals caught was interpreted as a minimum vole density. In total 90 traps were used distributed equally over the grazing treatments with voles. Each fence contained 6 traps with in between distances of 4-5 m. Vole trapping was approved by the committee for animal experiments of Wageningen University (DEC no. 99112a).

Data analysis

Data were ln-transformed prior to analysis to improve equality of variances. In most cases an Anova is performed with grazing treatment and litter removal or clipping as fixed factors and block as random factor. When one of the fixed factors was significant and block was not, a posthoc Tukey test was performed where block was left out of the analysis.

Results

Plant species richness

Litter removal had no effect on species richness in any of the grazing treatments in 2001, after three years of experimental treatment (fig. 3.1a, tab. 3.1). Grazing by cattle, rabbits and voles resulted in higher species richness only in 2000; in the other years the effect of grazing was not significant (tab. 3.1). Litter removal had no effect on species turnover in all years (effect on colonization rates $F_{1,4}=0.217$, $p=0.666$, block effect: $F_{4,8.5}=0.510$, $p=0.731$, interaction grazing*litter removal: $F_{3,12}=4.635$, $p=0.022$, effect on extinction rates: $F_{1,4}=0.532$, $p=0.506$, grazing*litter removal: $F_{3,12}=0.436$, $p=0.731$), but grazing by cattle, rabbits and voles increased local extinction rates relative to the ungrazed (N) treatment in 2001 (grazing effect: $F_{3,12}=10.654$, $p=0.001$), but not in the other years (fig. 3.1c, e for 2001, data for 1999 and 2000 not presented). Grazing by cattle, rabbits and voles increased local colonization rates in 2000 ($F_{3,12} = 4.127$, $p=0.032$), in 1999 and 2001 grazing had no effect on local colonization (fig. 3.1e for 2001, data for 1999 and 2000 not given).

Clipping increased the number of species in 2001 in all grazing treatments (fig. 3.1b, tab. 3.1). In 2000 the effect of clipping was not significant, but grazing by cattle, rabbits and voles lead to higher species richness in this year (tab. 3.1). Clipping increased local colonization rates in all grazing treatments (effect of clipping: $F_{1,4}=34.224$, $p=0.004$, block effect: $F_{4,6.0}=0.153$, $p=0.955$, grazing*clipping: $F_{2,8}=2.254$, $p=0.167$), but had no effect on local extinction (fig. 3.1d, f, effect of clipping: $F_{1,4}=0.375$, $p=0.573$, block effect: $F_{1,4}=0.516$, $p=0.732$, grazing*clipping: $F_{2,8}=1.190$, $p=0.353$). Grazing had no effect on species turnover in the clipping experiment (effect on colonization: $F_{2,8}=0.173$, $p=0.844$, effect on extinction: $F_{2,8}=0.029$, $p=0.972$).

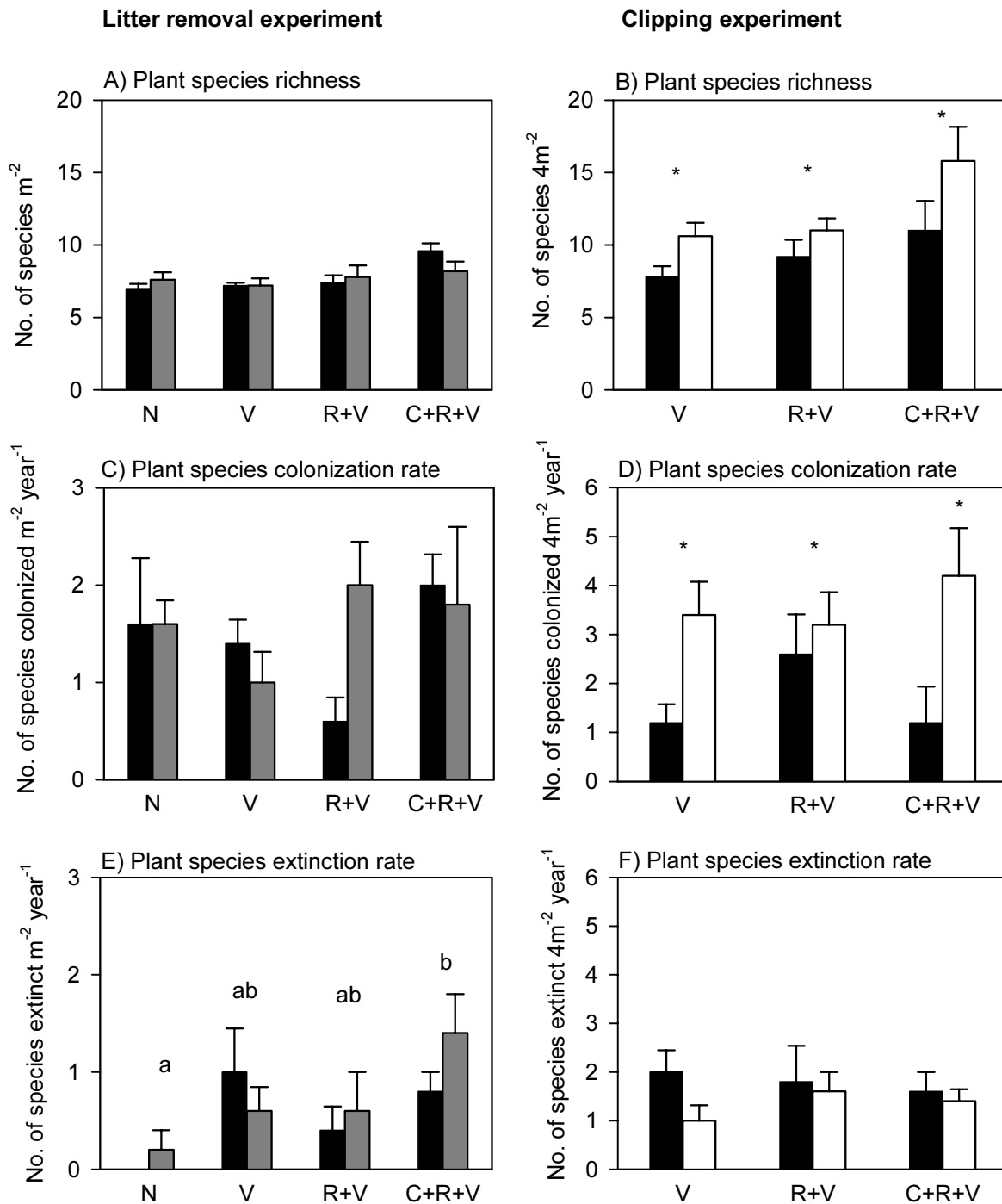**Litter removal experiment**   **Clipping experiment**

Figure 3.1 Number of plant species in 2001 and species turnover from 2000 to 2001 in the litter removal (A, C, E) and clipping (B, D, F) experiment. Note that the plots were 1 m^2 and 4 m^2 for the litter and clipping experiment, respectively. Significant effects of litter removal or clipping are indicated with an *, significant effects of grazing treatment are indicated with different letters. No letters or asterisk means that there were no statistical differences between the treatments. The bars represent the litter removal treatment (hatched), control plots (black) and clipping treatment (white). N: no herbivores, V: voles, R+V: rabbits + voles, C+R+V: cattle + rabbits + voles.

A. Number of plant species m^{-2} in the litter removal experiment. Both grazing and litter removal had no effect on species richness (see tab. 3.1 for statistics).

B. Number of plant species 4 m^{-2} in the clipping experiment. Grazing had no effect on the number of species, but clipping did significantly increase species richness (tab. 3.1).

C. Local colonization of plant species in 2001 in the litter removal experiment.

D. Local colonization of plant species in 2001 in the clipping experiment.

E. Local extinction of plant species from 2000-2001 in the litter experiment.

F. Local extinction of plant species from 2000-2001 in the clipping experiment.

Table 3.1 Effect of grazing, litter removal and clipping on plant species richness per plot per year. There was a significant effect of year of experiment in the litter experiment ($F_{3,8.3}$ =13.098, p=0.002), therefore data were tested per year. Data were tested separately for the litter removal and clipping experiment due to different plot sizes (1 m^2 and 4 m^2 respectively). The number of species was tested in an Anova with grazing and litter removal or clipping as fixed factors and block as random factor. F-values are given with level of significance indicated; a posthoc Tukey test was performed when the effect of grazing was significant. [NS] p>0.05, * p<0.05. ND means that no data were available.

	Litter removal experiment				Clipping experiment			
Year	Effect of grazing $F_{3,12}$	Effect of litter removal $F_{1,4}$	Interaction $F_{3,12}$	Block $F_{4,10.8}$	Effect of grazing $F_{2,8}$	Effect of clipping $F_{1,4}$	Interaction $F_{2,8}$	Block $F_{4,8.1}$
1998	0.459 [NS]	0.692 [NS]	0.684 [NS]	2.260 [NS]	ND	ND	ND	ND
1999	3.139 [NS]	1.941 [NS]	0.518 [NS]	0.843 [NS]	ND	ND	ND	ND
2000	3.701 *	0.056 [NS]	0.756 [NS]	2.046 [NS]	5.364 *	0.636 [NS]	2.022 [NS]	0.279 [NS]
2001	2.829 [NS]	0.089 [NS]	0.362 [NS]	1.442 [NS]	1.445 [NS]	14.960 *	3.376 [NS]	0.333 [NS]

Seedling emergence

The first year of litter raking resulted in a 3 to 25-fold increase in number of seedlings that emerged in the vegetation (fig. 3.2a, litter removal: $F_{1,4}$=91.847, p=0.001, interaction litter*grazing: $F_{3,12}$=2.461, p=0.113, block: $F_{4,2.3}$=4.442, p=0.164). The total number of seedlings that appeared was much less in the second year, but still significantly higher seedling numbers were found in the raked plots (fig. 3.2b, litter removal: $F_{1,4}$=14.833, p=0.018, interaction: $F_{3,12}$= 1.529, p=0.258, block: $F_{4,2.3}$=1.598, p=0.266). In the first year, also more species were present with seedlings in the raked plots (fig. 3.2c, litter removal: $F_{1,4}$=48.761, p=0.002, interaction: $F_{3,12}$=1.886, p=0.186, block: $F_{4,2.3}$= 6.066, p= 0.142), whereas this effect disappeared in the second year (fig. 3.2d, litter removal: $F_{1,4}$= 0.828, p=0.414, interaction: $F_{3,12}$=0.771, p=0.532, block: $F_{4,2.3}$=1.505, p=0.401). Grazing by more species of herbivores increased the number of seedlings (1999: $F_{3,12}$ = 4.057, p=0.033, 2000: $F_{3,12}$ = 5.140, p=0.016) as well as the number of species with seedlings (1999: $F_{3,12}$ = 5.398, p=0.014, 2000: $F_{3,12}$ = 11.613, p=0.001), with slight differences between years.
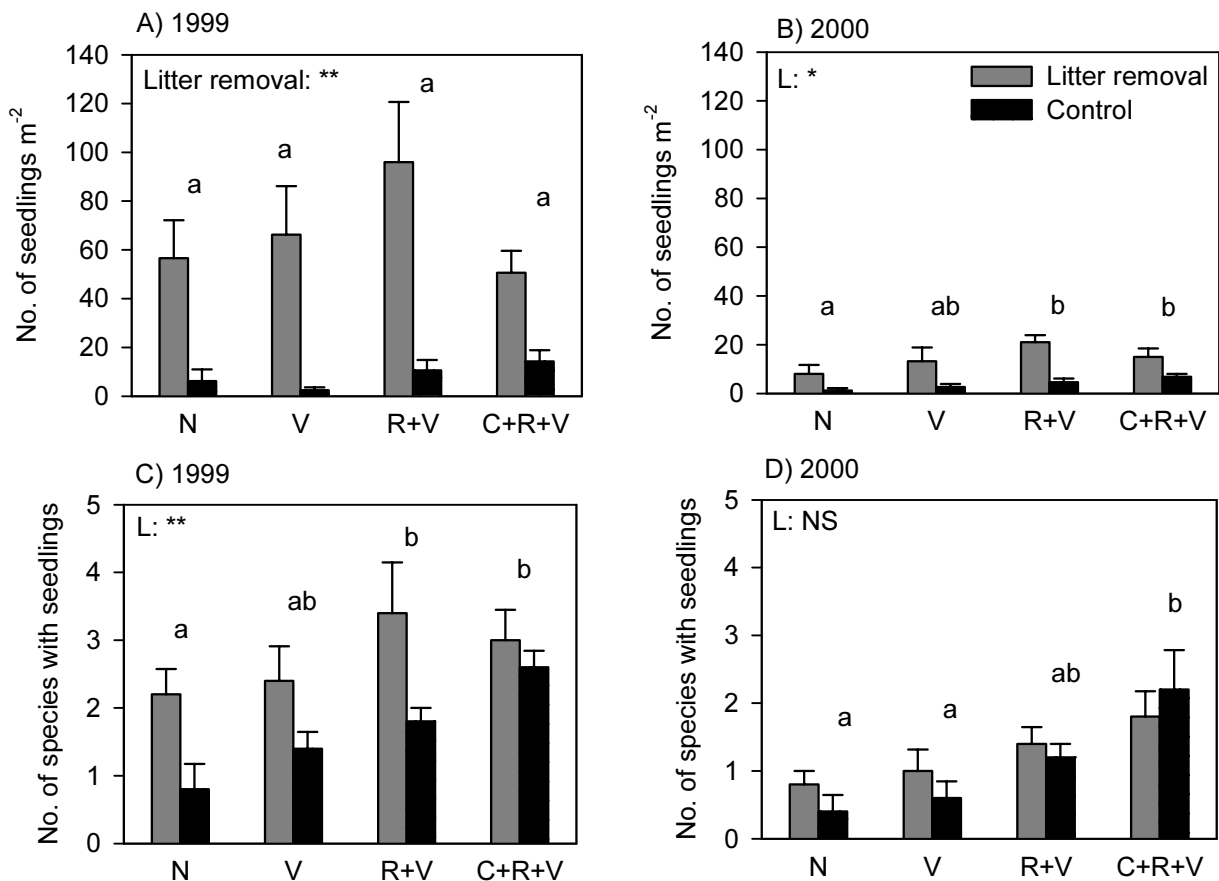
Figure 3.2 Number of seedlings and number of species with seedlings in the vegetation plots of the litter removal experiment in 1999 and 2000. Data were tested for grazing and treatment effect in a two-way Anova. Significant grazing effects are indicated with different letters, the effect of litter removal (L) is separately indicated with an asterisk where NS $p > 0.05$, * $p < 0.05$, ** $p < 0.01$. There were no significant interactions between grazing and litter removal treatment. N: no herbivores, V: voles, R+V: rabbits + voles, C+R+V: cattle + rabbits + voles.
A. No. of seedlings m^{-2} in 1999. In a post hoc test on grazing, the differences between grazing treatments were just not significant.
B. No. of seedlings m^{-2} in 2000.
C. No. of species with seedlings in 1999.
D. No. of species with seedlings in 2000.

Vegetation height and light availability

Grazing and experimental treatment both had a significant effect on vegetation height (grazing effect: $F_{2,8}=23.665$, p<0.001, treatment: $F_{2,8}=11.422$, p=0.005, block effect: $F_{4,10.9}=0.229$, p=0.916), but these factors showed a significant interaction (fig. 3.3a, interaction: $F_{4,16}=3.343$ p=0.036). Because of the significant interaction, categories were grouped and all data were tested with a one-way Anova ($F_{10,44} = 22.018$, p<0.001). Litter raking had no effect on vegetation height and clipping only significantly reduced the height of the vegetation in the treatment grazed by cattle, rabbits and voles that also had a lower vegetation in the control plots compared to the ungrazed treatment (fig. 3.3a). Light availability at soil level was significantly different between the treatments (fig. 3.3b, Kruskall-Wallis test, Chi-square=39.7, df=10, p<0.001, each pair of plots compared with Wilcoxon sign rank test). Clipping increased light availability as did grazing by cattle, rabbits and voles, although this last effect was not significant due to the heterogeneity in light levels in the grazed plots.
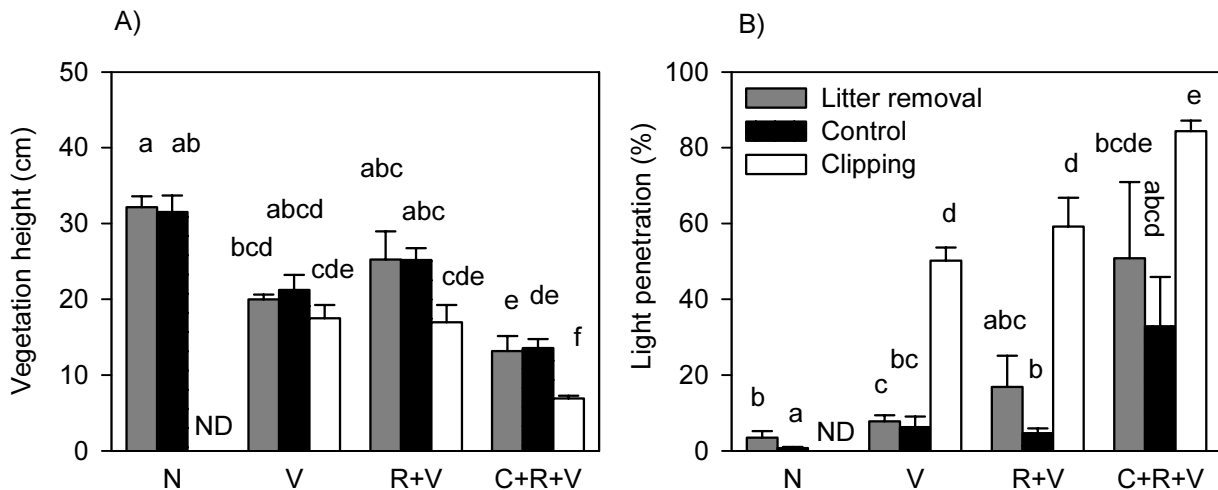

Figure 3.3 A. Vegetation height in the different treatments in 2001. Different letters indicate statistically different categories. N: no herbivores, V: voles, R+V: rabbits + voles, C+R+V: cattle + rabbits + voles. B. Light penetration to the soil as percentage of ambient light levels, measured in 2001. ND = no data

Stem density

Grazing and experimental treatment both had a significant effect on the density of stems in the vegetation without showing an interaction (fig. 3.4, interaction: $F_{4,16} = 1.421$, p=0.272.). Grazing by cattle, rabbits and voles resulted in a denser vegetation than the other grazing treatments, about 12.800 versus 7200 stems per square meter in the ungrazed treatment ($F_{2,8} = 18.892$, p=0.001). Litter removal significantly increased stem density about 1.5 times compared to the control, but not as much as the clipping treatment that increased stem density more than twice up to 27.222

stems per square meter in the C+R+V treatment (fig. 3.4, treatment effect: $F_{2,8}$ = 48.294, p<0.001, block $F_{4,8.6}$=69.586, p=0.995).
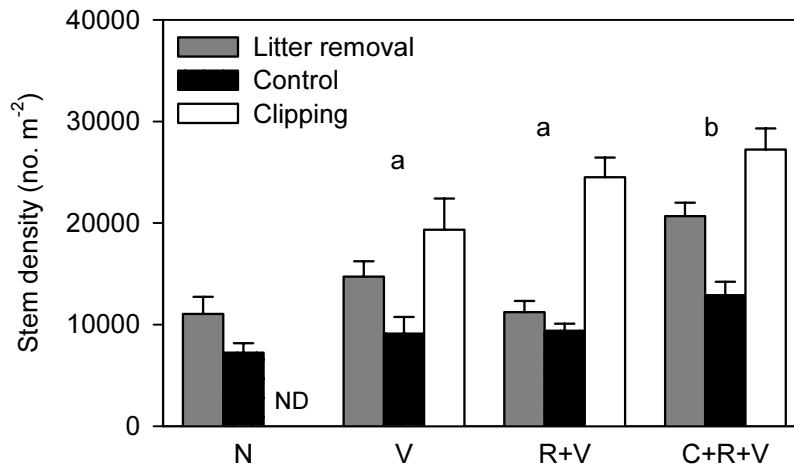

Figure 3.4 Stem density in 2001 as number of stems m^{-2} in the different treatments. N: no herbivores, V: voles, R+V: rabbits + voles, C+R+V: cattle + rabbits + voles. Different letters indicate statistically different densities of stems. ND = no data.

Biomass of dead and living plant material

After three years the cumulative amount of litter removed was 1297 (± 66 se) g dry weight m^{-2} of litter from the ungrazed N plots, 1136 (± 79) and 1158 (± 78) from the V and R+V treatment and 641 (± 58) from the C+R+V treatment. In the clipping treatments 1195 (± 49), 1120 (± 37) and 593 (± 38) g biomass m^{-2} was removed from the V, R+V and C+R+V treatment respectively. Clipping and raking thus removed similar amounts of biomass from the experimental plots within the grazing treatments (treatment effect on amount biomass or litter removed: $F_{1,4}$ = 0.71, p=0.804). Excluding cattle doubled the amount of litter that could be removed (effect of grazing: $F_{3,12}$ = 32.109, p<0.001, block effect: $F_{4,10.8}$ = 0.183, p=0.942, grazing*removal treatment: $F_{2,8}$ = 1.738, p=0.236).

Litter raking resulted in a significant reduction of litter biomass at an equally low level in all grazing treatments (fig. 3.5, tab. 3.2). Litter removal had no effect on the amount of living biomass of herbs and grasses or standing dead, but significantly reduced the total amount of biomass (including litter). In the clipped plots no accumulated litter was present. Also standing dead material was significantly reduced under clipping (fig. 3.5, tab. 3.2). No effect of clipping on living biomass could be found. Because of the strong impact of clipping on accumulated dead biomass, total biomass was also reduced under clipping. Grazing had little impact on living biomass (tab. 3.3). In the litter experiment standing dead was significantly reduced in all grazing treatments compared to the ungrazed N treatment (tab. 3.2). Grazing had almost a significant effect on the amount of litter (p=0.068). Grazing by voles in the V treatment did not significantly reduce the total biomass, but combined

with rabbits the total biomass was reduced relative to the ungrazed N treatment. The total biomass in the C+R+V was significantly lower than both the N and V treatment. In the clipping treatment herbivores had no effect on living or dead biomass separately, but total biomass was significantly reduced in the C+R+V treatment (fig. 3.5, tab. 3.2).
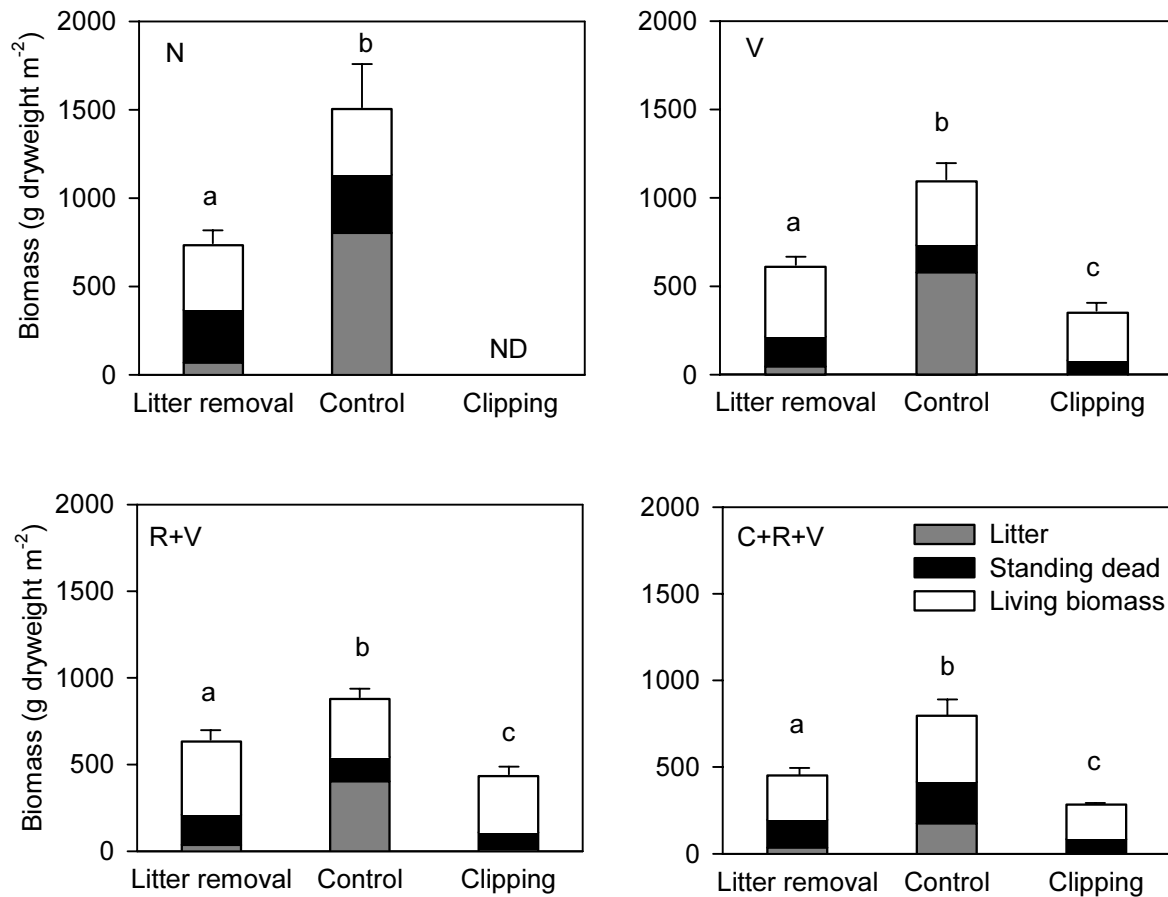

Figure 3.5 Standing aboveground biomass (g dry weight m^{-2}) in the grazing treatments in 2001 after three years of litter removal and clipping. Different letters indicate statistically significant different amounts of total biomass in the plots (see tab. 3.2 for other statistical results). ND: no data, there was no clipping plot in the N treatment. N: no herbivores, V: voles, R+V: rabbits + voles, C+R+V: cattle + rabbits + voles.

Table 3.2 Effects of grazing and litter removal on plant biomass (g dry weight m^{-2}) in August 2001, after three years of experiments. Biomass was harvested in the litter removal plots, in the control plots of the litter removal experiments and in the clipped plots. No data from the control plots of the clipping experiment were available. Because a litter removal plot, but no clipping plot could be established in the N treatment, the design of the multifactorial Anova is unequal. Therefore the data were tested separately for the litter removal experiment and the clipping experiment, although both experimental plots were tested against the same control. Litter is the dead material that lays on the soil and could be accumulated during all three years, standing dead is mostly current-year dead material that is still standing upright. Total biomass comprises standing living + dead biomass + litter. NS: p>0.05; *: P<0.05; **: p<0.01; ***: p<0.001.

	Effect of grazing	Effect of treatment	Block effect	Interaction
Litter removal experiment	$F_{3,12}$	$F_{1,4}$	$F_{4,9.06}$	$F_{3,12}$
Living herb biomass	2.015 NS	0.362 NS	0.126 NS	1.652 NS
Living grass biomass	0.350 NS	0.160 NS	0.613 NS	1.533 NS
Standing dead	6.430 **	0.011 NS	0.299 NS	1.929 NS
Litter	3.078 NS	16.138 *	1.017 NS	0.302 NS
Total biomass	11.096 **	39.843 **	1.326 NS	1.478 NS
Clipping experiment	$F_{2,8}$	$F_{1,4}$	$F_{4,4.3}$	$F_{2,8}$
Living herb biomass	0.103 NS	3.092 NS	0.673 NS	0.208 NS
Living grass biomass	0.491 NS	1.464 NS	0.745 NS	2.100 NS
Standing dead	1.266 NS	24.667 **	0.002 NS	1.366 NS
Litter	2.961 NS	240.261 ***	1.000 NS	2.961 NS
Total biomass	6.434 *	84.214 **	0.308 NS	2.874 NS

Correlations between species richness, light and biomass

Plant species richness was significantly correlated with a set of interrelated variables (tab. 3.3). The tightest relationship was found with vegetation height (tab. 3.3, Pearson correlation: r=-0.52, p<0.001, n=55). Multiple linear regression in a stepwise design revealed that vegetation height significantly explains part of the variation in species richness (R^2=0.27, $F_{1,53}$ = 19.28, p<0.001). After vegetation height was entered in the model, the other variables (see tab. 3.3) did not significantly explain the residual variation. The relation between vegetation height and species richness was not linear, but was best fit with a logarithmic relation (R^2=0.36, $F_{1,53}$ = 30.03, p<0.001).

Table 3.3 Correlations between species richness (per m^2), light penetration to the soil (% of ambient light levels), vegetation height (cm), litter, necromass (standing dead + litter), living biomass and total aboveground biomass (necromass + living biomass) for all plots in August 2001. Pearsons correlation, n=55, NS p>0.05, * p<0.05, ** p<0.01, *** p<0.001.

	No. of species	Vegetation height	Litter	Necromass	Living biomass	Total biomass
Light availability	0.47 ***	-0.72 ***	-0.47 ***	-0.55 ***	-0.49 ***	-0.62 ***
Vegetation height	-0.52 ***	-	0.42 **	0.51 ***	0.50 ***	0.60 ***
Litter	-0.29 *	-	-	0.97 ***	0.21 NS	0.90 ***
Necromass	-0.32 *	-	-	-	0.29 *	0.95 ***
Living biomass	-0.18 NS	-	-	-	-	0.57 ***
Total biomass	-0.33 *	-	-	-	-	-

Herbivore densities

Voles were mainly captured in the R+V and V treatment with 250-350 individuals per hectare (tab. 3.4). In the C+R+V treatment vole numbers were 2-3 times lower. Vole densities were similar between both years. Rabbits were present at low densities varying from 0.5-2.5 per hectare (tab. 3.4). Rabbits showed no preference for the R+V or C+R+V treatment nor for the litter removal plots (grazing effect: $F_{1,4}$=4.289, p=0.107, litter removal: $F_{1,4}$ = 1.684, p=0.264, block: $F_{4,5.6}$=0.594, p=0.681, interaction: $F_{1,4}$ = 2.791, p=0.170). Rabbits did have a strong preference for the clipped plots in the C+R+V treatment (fig. 3.6), where eight times more droppings were found (grazing effect: $F_{1,4}$=5.226, p=0.084, clipping: $F_{1,4}$=20.046, p=0.011, block: $F_{4,3.0}$=0.880, p=0.564, interaction $F_{1,4}$=8.396, p=0.044).

Table 3.4 Densities of herbivores in the artificial grazer assemblages in 1999 and 2000 per ha (means ± se). Rabbit density was measured through year-round pellet counts in the control vegetation plots. Vole density was measured during peak densities in fall, cattle densities are given for the whole study area.

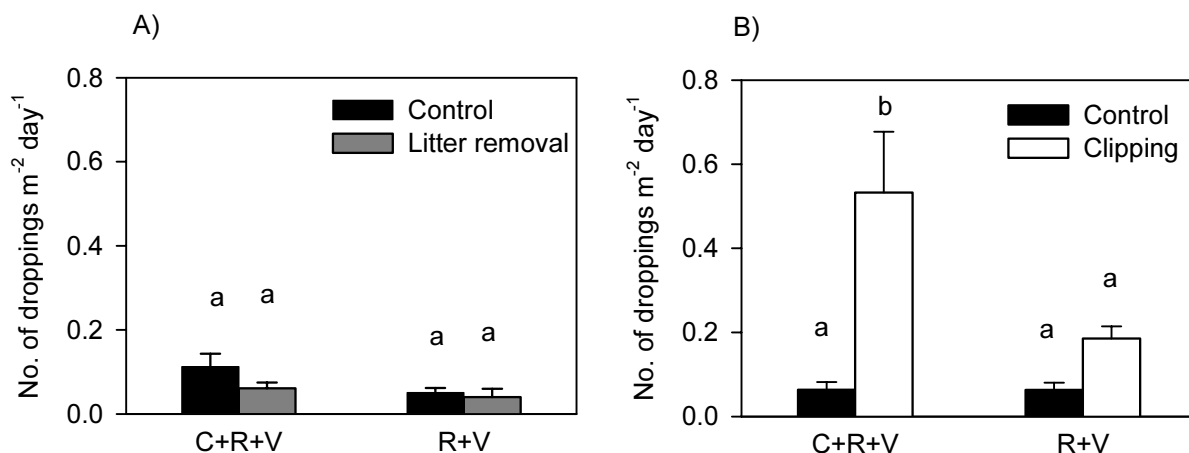| Grazing treatment	Year	Herbivore density (no. of individuals ha^{-1})		
		Cattle	Rabbits	Voles
C+R+V	1999	0.4	1.1 ± 0.4	80 ± 26
	2000	0.4	2.4 ± 0.5	151 ± 50
R+V	1999	0	0.6 ± 0.3	284 ± 61
	2000	0	1.9 ± 0.3	302 ± 22
V	1999	0	0	347 ± 59
	2000	0	0	258 ± 62
N	1999	0	0	0
	2000	0	0	0

Figure 3.6 Rabbit visitation of the plots with the different treatments, expressed as average number of droppings per m^2 per day. R+V: rabbits + voles, C+R+V: cattle + rabbits + voles. Different letters indicate statiscally different dropping rates (One-way Anova; p<0.05).
A. Litter removal experiment.
B. Clipping experiment.

Discussion

With the exclusion of more herbivores progressively more litter had accumulated during the three years of the experiment. However, despite the severe reduction of litter and therefore also total biomass in the litter removal plots, plant species richness was not affected. Also grazing had only a marginal effect on species richness, with slightly higher species numbers in the cattle, rabbit and vole grazed treatment in one year. Clipping on the other hand did result in an increase in species richness in 2001, but not in 2000 when grazing by cattle, rabbits and voles increased species richness. Both clipping and grazing by cattle, rabbits and voles reduced the height of the vegetation at peak standing crop. Vegetation height seemed the most important parameter that was closely related with species richness. Probably vegetation height is a proxy for light availability for plants. The vegetation height measurements may have captured heterogeneity in light better than the direct light measurements because they were taken in a grid over the whole plot whereas light was measured in two narrow (1 cm) bands.

Since both clipping and grazing by all herbivores increased species richness, there must have been either a decrease in local extinction or an increase in colonization rates compared to the other treatments. Litter removal did result in an increase in seedling numbers in all grazing treatments, but this was largely a transient response. Before the establishment of the exclosures litter was already present and the seedlings probably responded to the sudden light availability in late winter of the first year. In the second year this effect was much less and also the increased seedling numbers were not representing more plant species than in the control plots. Grazing by more herbivores resulted in more seedlings and especially a higher number of species found as seedlings. However, the increased species richness of the seedlings in May, especially in the plots grazed by cattle, rabbits and voles did mostly not translate into increased local colonization rates in the established vegetation in August of the same year (only in the year 2000). This was different in the clipped plots, where no seedlings were counted, but where the local species colonization rate was considerably enhanced by clipping. Extinction rates were not affected by litter raking or clipping, and were slightly increased under grazing by grazing of cattle, rabbits and voles in only one year.

We conclude that light limitation negatively affected plant species richness due to reduced local colonization, but that local extinction was not significantly increased. Litter did inhibit seedling appearance, but since establishment of seedlings as adult plants was light limited and the number of species with seedlings was not different after the first year of experiment, the reduction in seedling numbers by the litter layer had no effect on species richness.
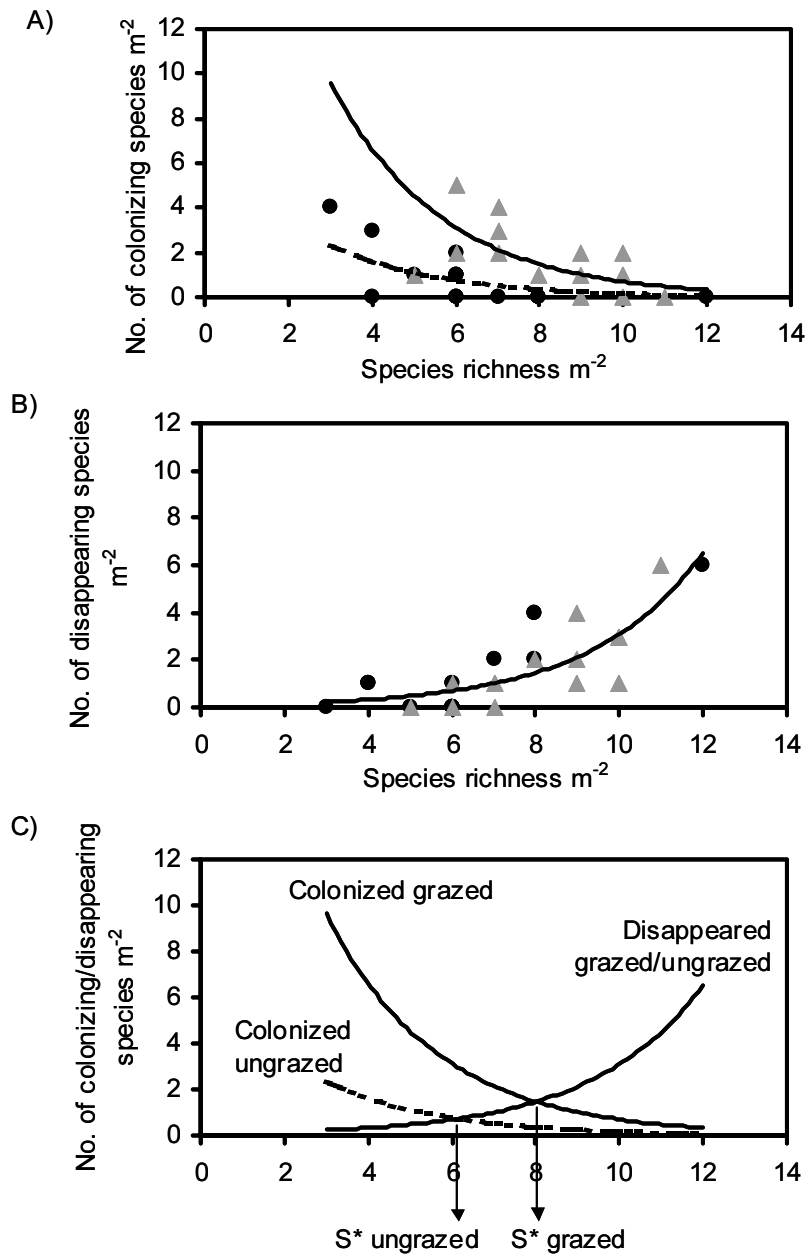
Figure 3.7 Colonization (A) and disappearance (B) rates in 1 m^2 plots in relation to the number of species present. Colonization and disappearance rates are calculated from the control plots (n=5) in the ungrazed (N, circles) and grazed (C+R+V, triangles) treatments by combining the data from all three years of the experiment. The number of species colonized or disappeared were related to the number of species present in the previous year. A Poisson regression (McGullagh and Nelder 1989) revealed significant effects of both grazing and species richness on colonization rate, grazing: $\chi^2_1=$ 13.51, p<0.001, species richness: $\chi^2_1=$ 6.50, p<0.01, grazing*species richness: $\chi^2_1=$ 2.62, p=0.10. Grazing had no significant effect on disappearance rate of species, but disappearance was strongly related to species richness, grazing: $\chi^2_1=$ 0.53, p>0.10, species richness: $\chi^2_1=$ 30.80, p<0.001, grazing*species richness: $\chi^2_1=$ 0.33, p>0.10. Since grazing had no effect on disappearance rates only one regression line is given based on all disappearance values.

At equilibrium, plant species richness is 6.1 m^{-2} in ungrazed and 8.0 m^{-2} in grazed vegetation (C).

Grazing pressure was highest in the plots grazed by cattle, rabbits and voles. Together they reduced the vegetation height considerably, but apparently just not enough to relax light competition. The extra biomass removal through clipping reduced vegetation height to a level where increased colonization was possible. This increased colonization could be due to enhanced seed input, enhanced seedling survival or both. Herbivores, at least rabbits, visited the clipped plots far more often than the control plots. Rabbits generally favour short, clipped or grazed vegetation; either because of enhanced food quality or intake rates or because of reduced apparent predation risk due to a better view on approaching predators (Iason et al. 2002). Rabbits enhanced the effect of clipping through increasing grazing pressure in the clipped plots, thereby reducing vegetation height further and maintaining a short sward for a longer period. In this short sward seedling appearance from the soil seedbank may be enhanced through increased light availability and also seedling survival may have been better (Bakker and De Vries 1992; Jutila and Grace 2002). More bare soil was present in the clipped plots, potentially serving as regeneration niche in the dense vegetation (2.8% ± 1.0 SE bare surface versus 0.2% ± 0.2 bare in the control plots under grazing by cattle, rabbits and voles, paired t-test, p=0.032). Previous work showed that bare soil considerably enhanced establishment of seedlings of subordinate herbs, that make-up most of the total species number (Bakker and Olff 2003). Also more seeds could be dispersed into the clipped plots through exozoochorous or endozoochorous seed dispersal. Rabbits can also disperse considerable numbers of seeds in faeces (Malo et al. 1995; Pakeman et al. 1999; Bakker and Olff 2003), but since rabbit pellets were removed from the plots to avoid double counting, this could not have been the main effect in this study, although some pellets probably had disintegrated before removal.

Interestingly, living plant biomass showed no relationship with plant species richness in our study. When plant litter was included in total biomass the relationship with species richness became strongly significant. It is therefore remarkable that removal of considerable amounts of litter had no effect on species richness. This may be due to a threshold effect. The hump-shaped curve of the relation between plant biomass and species richness reveals no relation between these two factors at the higher biomass values (Grime 1973; Al-Mufti et al. 1977). Biomass in the study of Al-Mufti et al. (1977) consisted of living, standing dead and litter biomass, i.e. total biomass in this study. Therefore the species response is to total biomass values and not to living biomass or even current-year production alone. If one component of this total biomass is removed, as we did with the litter layer, the remaining biomass determines species richness. If this remaining biomass is more than the threshold value, where biomass and species richness are no longer related, litter removal has no effect. We suspect that this is the case in our study. Therefore we conclude that litter had an additive effect on plant species richness relative to living biomass. Litter is important because it forms a large part of the total biomass but not necessarily because it has different effects than standing life or dead plants. We expect that this result is partly dependent on the relatively nutrientrich, moist habitat where we performed our study. In dryer habitats were the production is lower or light is not limiting plant growth, litter may have different effects on species richness, i.e. through microclimate effects or providing nutrients.

If biomass removal through clipping was a suitable surrogate for more intensive grazing, than grazing by herbivores could increase plant species richness if grazing pressure is high enough. Although different-sized herbivores may have different effects on plant species richness through differences in diet selection and food requirements (Demment and Van Soest 1985; Ritchie and Olff 1999) no additional effects of rabbits or voles were found, probably due to the low densities of both herbivore species. Therefore we compare the ungrazed (N) treatment with the treatment grazed by all herbivores (C+R+V). Following the approach of island biogeography theory, grazing apparently lead to a higher equilibrium number of species per square meter than in ungrazed vegetation. In our study this was due to enhanced colonization. However, colonization rate depends on the number of species already present in a plot (MacArthur and Wilson 1967). When more species are present less species can colonize the plots or establish successfully (Tilman 1997). We plotted colonization and extinction rate relative to species richness for the plots grazed by all herbivores and for the ungrazed plots for the three year study period (fig. 3.7, see figure for methods). Both in grazed and ungrazed plots, colonization rate was significantly negative related to species richness and extinction rate positively. Grazing significantly increased colonization rate relative to ungrazed plots, but extinction rate was not different for grazed and ungrazed plots (fig. 3.7). The equilibrium number of species in the ungrazed plots would be 6.1 species m^{-2}, in grazed plots 8.0 m^{-2} (fig. 3.7). There is considerable evidence that herbivores can increase plant species richness, but only few studies take local colonization and extinction rates into account. In western Kansas grasslands bison enhanced diversity through reduced extinction rates, but no effect on colonization was found (Glenn and Collins 1992). In our study we found the opposite, herbivores increased local plant species richness through enhancing colonization rates, but had no effect on local extinction rates. The combination of the study of Glenn & Collins (1992) and ours shows that herbivores can increase plant species richness both through increased colonization or decreased extinction rates as was proposed by Olff & Ritchie (1998). However, the conditions under which herbivores may have a larger effect on colonization or extinction rates are still unclear.

We conclude that plant species richness can be maintained under large herbivore grazing because they prevent severe light limitation for recruitment under nutrient-rich conditions. Litter has a negative effect on plant diversity, but this effect is additive to living plant biomass and not due to specific unique effects associated with litter.

Acknowledgements

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Chapter 4

Compositional constancy in grazed plant communities increases with herbivore size

E.S. Bakker, H. Olff and J.M. Gleichman

Abstract

An important current discussion is how the loss of biological diversity will affect community structure and ecosystem functioning in grasslands. Several recent experimental and theoretical studies have shown important links between plant diversity, the stability in species composition of plant communities and the reliability of grassland ecosystems to perform certain critical functions, e.g., to maintain productivity. In most grassland ecosystems, vertebrate herbivores are key elements without which community structure and ecosystem functioning cannot be understood. However, herbivores have hardly been incorporated in studies on the relation between diversity, compositional stability of plant communities and ecosystem functioning.

We experimentally investigated the effects of different-sized herbivores on the spatio-temporal stability of grassland community composition and biomass by stepwise exclusion of vertebrate herbivores from large to small body size at two nutrient levels. We found that large herbivores had a strong stabilizing effect on plant community composition, whereas excluding them (leaving only small herbivores) induced strong variability in plant species composition, both in space and time. Seven years of nutrient addition did not change the vegetation in the presence of large herbivores, due to selective grazing in the fertilized plots. Small herbivores in contrast, did not counteract the effects of nutrient addition, resulting in strong compositional changes in the vegetation. Furthermore, large herbivores suppressed the expression of small-scale soil differences in the vegetation, resulting in homogeneous vegetation, while the presence of small herbivores enhanced vegetation heterogeneity. The temporal stability of the vegetation composition increased towards higher plant species richness, and large herbivores increased plant species richness more than small herbivores. We conclude that different-sized vertebrate herbivores have important and unique effects on plant diversity, compositional stability and thus possibly ecosystem functioning in grasslands and therefore should be incorporated in studies that link these aspects.

Introduction

The current rapid decline of plant diversity has renewed interest in the relation between diversity and the stability of community processes (McNaughton 1993; Sankaran & McNaughton 1999) and ecosystem functions (Tilman 1997; Hector et al. 1999; Loreau et al. 2001; Tilman et al. 2001). These community processes involve the regulation of relative abundance's of species and functional types (Dodd et al. 1995; Naeem & Li 1997), while ecosystem functions include productivity (Hector et al. 1999; Tilman et al. 2001), decomposition rates (Knops et al. 2001), nutrient cycling (Hooper & Vitousek 1997; Spehn et al. 2002), or resistance to invasions (Tilman 1997; Kennedy et al. 2002). The impact of diversity on ecosystem functioning has been separated in two components: selection effects (consequences of the presence of a particular, e.g., highly productive species) and complementarity effects (e.g., higher productivity due to more complete or efficient use of resources) (Loreau & Hector 2001). Recent grassland biodiversity experiments have provided evidence for the simultaneous operation of both effects (Hooper & Vitousek 1997; Dukes 2001; Loreau & Hector 2001; Tilman et al. 2001). Selection effects form the link between community structure and ecosystem functions. Different dominant species or functional types can have very different effects on primary productivity (Hooper & Vitousek 1997; Hector et al. 1999), decomposition rates (Cornelissen 1996; Berendse 1998), and nutrient cycling (Wedin & Tilman 1990; Hobbie 1992; Van der Krift & Berendse 2001). On the other hand different ecosystem-level conditions (e.g. mineralization rates) may allow different species to attain competitive dominance (Berendse 1990; Tilman & Wedin 1991b). Stability of ecosystem processes will thus be strongly affected by the stability of community composition, which in turn determines the ecosystem-level reliability to perform certain functions or produce services.

Vertebrate herbivores are key components of grassland ecosystems with strong impacts on community structure (Milchunas & Lauenroth 1993; Olff & Ritchie 1998; Bakker & Olff 2003) and ecosystem functioning (Frank & McNaughton 1993; Pastor et al. 1993; McNaughton et al. 1997; Ritchie et al. 1998). However, most experimental studies (Hector et al. 1999; Tilman et al. 2001) on the relation between biodiversity, community stability and ecosystem functioning have not taken herbivory into account, mainly due to experimental constraints (Duffy 2002). Herbivore size is a key axis along which impacts on community composition (Ritchie & Olff 1999a) and ecosystem functioning (Owen-Smith 1988; Hobbs 1996) can be understood. Therefore, herbivores of different sizes are expected to have different effects on spatial and temporal vegetation constancy. Larger species can store more reserves to overcome periods of scarcity (Mysterud et al. 2001), have longer lifespans (Peters 1983) and larger homeranges (Mysterud et al. 2001; Haskell et al. 2002), but at the expense of lower per capita metabolic and reproductive rates (Peters 1983). Body size therefore determines the responses of herbivores to spatial and temporal variability in their food resources (Ritchie & Olff 1999b) that is in turn induced by variability in plant resources (climatic and soil variability). Spatial patterns created by large species on the vegetation will be more coarse grained while the temporal vegetation changes they induce will be slower than of smaller species due to more

constant grazing pressure (Olff et al. 1999). Therefore, at small spatial scales, the spatial and temporal stability of community composition will increase with herbivore size.

In this study we test the hypothesis that spatial and temporal stability of plant community composition and biomass will increase with the body size of the herbivores present. We performed a field experiment in which we separated the effects of different herbivores (cattle, European rabbit and common vole) and measured the species richness and compositional constancy of the vegetation in space and time during seven years. In addition to existing soil heterogeneity, we applied a nutrient addition treatment to investigate how spatial heterogeneity in soil conditions affects the vegetation composition under large and small herbivore grazing.

Methods

Study site

The study was performed in the Junner Koeland, a 100 ha nature reserve including 50 ha floodplain grassland along the river Overijsselse Vecht in the Northeast of The Netherlands. The site is managed by the National Forest Service as a nature reserve. The area used to be communal grazing land for the farmers from a nearby village, and has probably been grazed by livestock since medieval times. Currently, cattle graze in the area from April till October with a density of 1 heifer per 2.5 ha. Naturally occurring vertebrate herbivores are European rabbits (*Oryctolagus caniculus*) and common voles (*Microtis arvalis*). Dominant graminoids in the floodplain meadows are *Festuca rubra*, *Agrostis capillaris* and to a lesser extent *Holcus lanatus* and *Luzula campestris*. Most abundant herbs are *Rumex acetosa* and *Stellaria graminea*. Nomenclature follows Van der Meijden (1997). Peak above ground standing crop of the grasslands amount to 432 g m^{-2} (E.S.Bakker unpubl. data) with herbs and graminoids contributing 3.5% and 96.5%, respectively (Bakker & Olff 2003). Previous work showed that plant growth in the site is generally nitrogen limited (Boekhoff 2000).

Exclosure design and nutrient treatment

An exclosure experiment was established using 5 blocks in May 1994. Each block consisted of three grazing treatments excluding herbivores stepwise from large to small. Outside the fences cattle, rabbits and common voles had free access to the vegetation. The first fence type consisted of two barbed wires at 0.5 and 1.0 m excluded only cattle, allowing access for rabbits and voles. The second fence type consisted of 1.0 m high chicken mesh excluding cattle and rabbits, leaving only voles to graze the vegetation. These different grazing treatments will be referred to as C+R+V for the cattle and rabbits and voles treatment, R+V for rabbits and voles and V for voles only. Each fence measured 12 by 12 m per block. Within each grazing fence 4 plots of 2 x 2 m were established following a randomized design. The plots received a nutrient treatment: two plots were fertilized and two plots were left as a

control. Nutrients were added twice a year during half April and early May from 1994 till 2001, at an amount of 15 g N, 5 g P and 10 g K per m^2 per year. Nutrients were dissolved in 10 l water and evenly distributed over the plots.

Vegetation measurements

The vegetation composition in all plots was recorded annually since 1995 in late July or early August (peak biomass) by visually estimating cover following the decimal Londo scale. Aerial cover was estimated visually per species by the same person in each year. Total cover could exceed 100% if species vertically overlapped. Vegetation height was measured using a stick with a scale and a polystyrene disc (10 cm diameter) that was dropped on the vegetation. The height at which the disc rested on the vegetation was taken as vegetation height. Vegetation height was measured in summer (July or August) from 1994 until 2001, except for the years 1996 and 1999. Vegetation height was recorded in a grid consisting of 4 x 4 cells (16 cells) of 50 x 50 cm wide regularly distributed over the plot, and averaged per plot before further analysis. In August 1998 vegetation height was measured in an extended grid of 10 x 10 cells of 20 x 20 cm wide, to capture vegetation heterogeneity on a finer scale than with the standard measurements.

Soil nutrient measurements

In November 1998 soil samples were taken to determine total nutrient contents of the soil in the different grazing treatments. In each exclosure two soil samples were taken per grazing treatment located in the opposite corners of the fences, but staying about 1.5 m away from the fences. Each sampling point consisted of a 50 x 50 cm plot from which the litter layer was removed and 5 soil samples were taken with an auger of 6 cm diameter and 10 cm depth. Samples were weighed and stored at 5°C till further processing the next day. From each soil core the upper 1 cm litter layer was removed as well as large roots before the 5 soil cores were homogenized. Total nitrogen, phosphorus and potassium were determined in air-dried soil (dried at 30°C for 48 hours) after digestion with sulphuric acid, selenium and salicylic acid. Nitrogen and phosphorus concentrations were measured colometrically using a continuous flow analyser (SKALAR). Potassium was measured by means of atomic absorption spectrometry (AAS). Soil moisture content was determined gravimetrically year round (every 6 weeks) from soil samples that were taken at the same sites as the other soil samples.

Ecosystem properties

We measured above ground standing crop and litter accumulation as characteristic ecosystem properties. In July 1995, 1999 and 2001 biomass samples were taken to determine above ground standing crop and litter accumulation. In 1995 and 1999 biomass was harvested in each vegetation plot by clipping a strip of 10 x 100 cm to soil level. Samples were sorted to live and dead. In 1995 only three of the five blocks were sampled. In 2001 1 random sample of the same size and dimension was taken

in each grazing fence outside the plots. All biomass samples were dried for 24 hours at 70°C and weighed.

Herbivore density

The number of cattle remained constant over the years at a density of 0.4 cow ha^{-1}. We assessed rabbit density through pellet counts (only C+R+V and R+V treatments). Pellets were counted approximately every three weeks from 1995 until 2001 and removed from the plot when counted. Rabbit densities were calculated for each year assuming a dropping rate of 400 pellets per rabbit per day (Lockley 1962).

Vole densities were assessed by trapping voles in the fall from 1998 until 2000, using Longworth live-traps baited with peanut butter, oats, carrots and apple. Traps were pre-baited for two days before a five-day trapping session started, and checked every 8 hours, resulting in 11 trap checks per session. Captured voles were weighed and individually marked by clipping a piece of the fur at different places on the back. Voles were then released. In 1998 an extra trapping session was performed in February and July to measure variability of vole densities over time. In February trapping lasted only 3 days. 90 Traps were used per session, evenly distributed over the grazing fences, which resulted in 6 traps per fence. Traps were spaced evenly in the treatments at 3-4 m distance. Vole trapping was approved by the committee for animal experiments (DEC) of Wageningen University (DEC no. 99112a).

To compare grazing impact between grazing treatments we translated herbivore densities into daily energy expenditure (DEE) at two times basal metabolic rate as DEE = 2 * 70 * (live body weight)$^{0.75}$ kcal day^{-1} (Demment & Van Soest 1985), where 1 kcal = 4.184 kJ. For cattle an average weight of 300 kg was assumed (heifers), for rabbits 1.5 kg (Wallage-Drees 1988) and mean vole weight was 0.017 kg (E.S. Bakker unpubl. data).

Data analysis

We selected the dominant species in the vegetation for the analysis of the temporal and spatial dynamics of individual plant species. A species was labelled as dominant when it had an average cover of > 5% in one of the grazing treatments averaged over all years and plots. This resulted in seven dominant species. The graminoids *Agrostis capillaris*, *Festuca rubra*, *Holcus lanatus* and the herb *Rumex acetosa* all had >10% cover in one of the grazing treatments, graminoids *Luzula campestris, Carex hirta* and the herb *Stellaria graminea* between 5-10% cover. In the graphical representation we used only five of these dominants for reasons of graphical clarity. In addition to the four dominants with >10% cover we included *Stellaria graminea* as extra herb, because of its strong year-to-year fluctuation.

Dissimilarity in vegetation composition between plots was calculated from Euclidean distances, using the cover of each plant species per plot according to the formula:

$$\sqrt{\sum_{i=1}^{s} \left(xi_1 - xi_2 \right)^2}$$

where s is the total number of plant species in plot 1 and 2 and xi_1 is the percentage cover of species i in plot 1, xi_2 is the percentage cover of species i in plot 2. To study the effect of nutrient addition on vegetation composition, we compared dissimilarity between the control and fertilized plots within each grazing treatment over the years. For each grazing treatment and each year we first calculated the dissimilarity between the two control and two fertilized plots within a fence. This yielded four dissimilarity values (a comparison between the first control plot and both fertilized plots and the second control plot versus both fertilized plots) that were averaged to yield one mean dissimilarity value per block per grazing treatment per year. Comparisons between the grazing treatments per year were thus based on five replicates. To obtain a measure of spatial variability, we calculated dissimilarity over time between the five blocks within a grazing treatment, using only the control plots. We first calculated dissimilarity between the first control plot of a block and all control plots of other blocks in that grazing treatment, then dissimilarity between the second control plot of that block and all other plots. This yielded 16 dissimilarity values, which were averaged before further calculations, yielding one dissimilarity value per grazing treatment per block per year.

Temporal similarity per grazing treatment is expressed as the coefficient of variance (standard deviation/average cover) of cover of each dominant plant species averaged per plot over years. The values for the dominant plant species were then averaged per plot and the values for the plots within a grazing treatment were then averaged per nutrient treatment within a block before further calculations. Therefore all calculations were performed on five replicates.

All measurements of soil nutrients and vegetation biomass were first averaged within a block per grazing treatment before further calculations. Therefore all calculations on soil nutrients are based on five replicates, calculations on vegetation biomass are based on three replicates since in 1995 only three blocks were sampled.

Results

Dynamics of dominant plant species in time and space

The temporal dynamics of the selected co-dominant plant species differed with grazing treatment (fig. 4.1). In the C+R+V treatment two grass species co-dominated the vegetation, *Festuca rubra* and *Agrostis capillaris*. The cover of both species gradually increased over the years, the others were present at a stable low cover. In the R+V treatment the dominance rank of the species was roughly the same as in the C+R+V treatment, but the cover of the species fluctuated more between years. In the V treatment none of the species was permanently dominant.
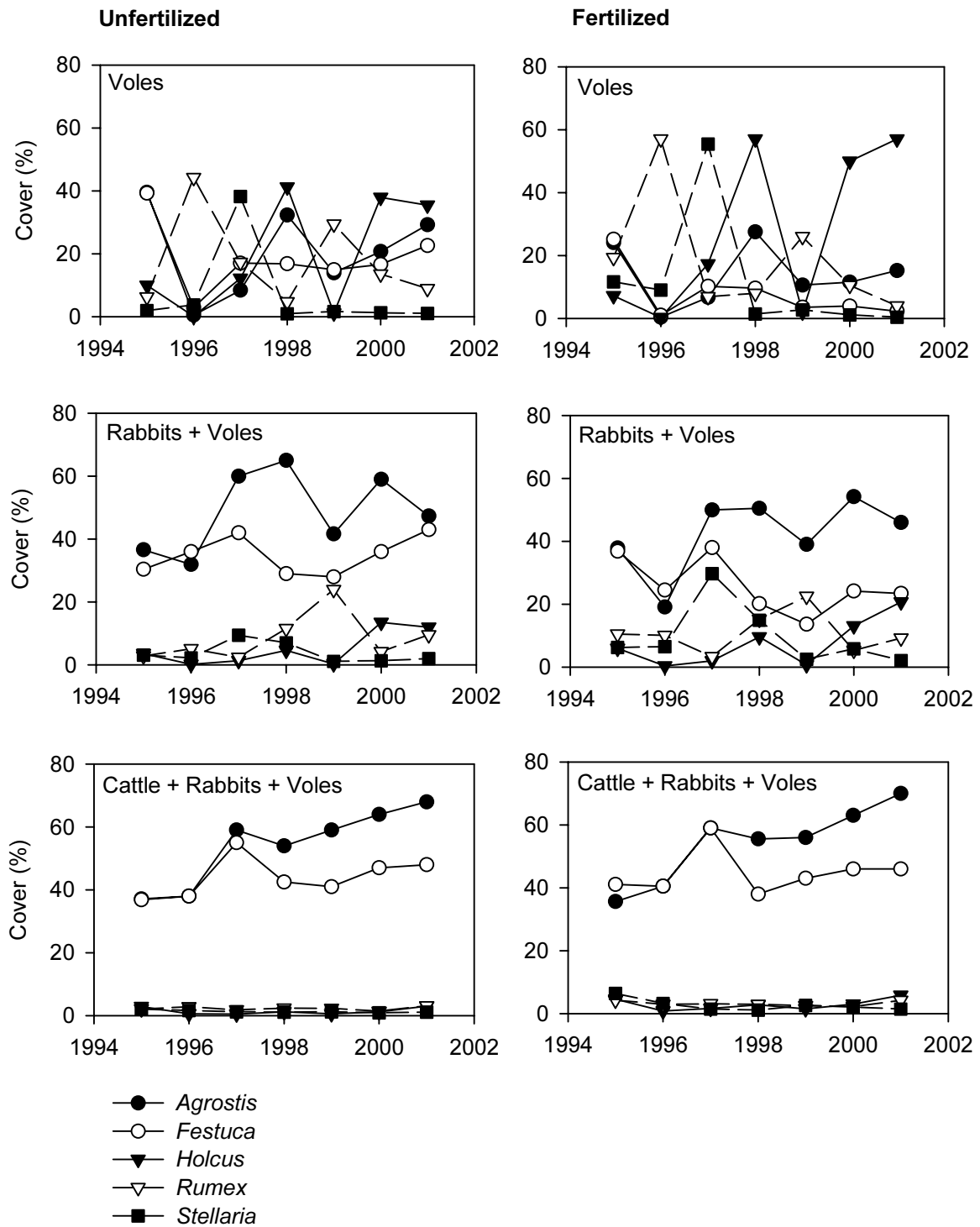
Figure 4.1 Temporal dynamics of 5 co-dominant plant species in the grazing treatments with and without nutrient addition. Each dot represents cover averaged over the 5 blocks. Species are the grasses *Agrostis capillaris*, *Festuca rubra* and *Holcus lanatus* and the herbs *Rumex acetosa* and *Stellaria graminea*.

There were large fluctuations between years in abundances of all species and three (*Rumex*, *Stellaria* and *Holcus* resp.) of the five species had outbreak years. No clear direction in vegetation development could be recognized after exclusion of cattle and rabbits. The patterns of temporal dynamics in the V treatment were similar in the fertilized plots, but the fluctuations were more pronounced. Peaks of the outbreaks were around 60% cover, whereas this was 40% in the control plots. In the C+R+V treatment addition of fertilizer did not have any effect neither on temporal dynamics of species nor on maximum cover (fig. 4.1).

In 2001 *Agrostis capillaris* was dominant in 6.5 of the 10 plots in the C+R+V treatment, *Festuca rubra* in the other 3.5 plots (tab. 4.1). Exclusion of cattle resulted in an increase of the number of plant species dominants, 5 compared to 2 in the C+R+V treatment after seven years. Especially *Holcus lanatus* became dominant in most fertilized V plots, together with several herb species.

Table 4.1 Characterization of plant community composition in the grazing treatments in 2001 with respect to dominant species. A species is dominant when it had the highest cover in a plot. Dominance is expressed as the number of plots where a species is dominant (n=10). In case of co-dominance in a plot the species received the value 1 / (number of co-dominants).

	Unfertilized			Fertilized		
	C+R+V	R+V	V	C+R+V	R+V	V
Agrostis capillaris	6.5	5	3	8	5	1
Festuca rubra	3.5	4	2	2	0.5	
Holcus lanatus		1	3		2.5	5
Carex hirta			1.5		1	1
Galeopsis tetrahit			0.5			
Sambucus nigra						1
Stachys palustris						1
Urtica dioica					1	1
No. of (co-)dominant species	2	3	5	2	5	6

Holcus cover and soil nutrients

We related the cover of the dominant plant species in the control plots (averaged over all years) to the soil variables measured in 1998. Soil total N correlated strongly positively with soil total P (Pearson correlation r=0.961, p<0.001, n=15), soil total K (r=0.550, p=0.034) and soil moisture (r= 0.981, p<0.001). Of the dominant species only *Holcus* responded significantly (p<0.05) to the soil parameters (except for soil K). We chose to represent the relation between *Holcus* and soil N here, because this relationship had the highest r-values and was also most significant. *Holcus* cover is significant negatively related to total soil nitrogen in all three grazing treatments (fig. 4.2). However, the response of *Holcus* to soil N is strongly suppressed in the C+R+V treatment whereas *Holcus* does strongly respond in the V treatment.
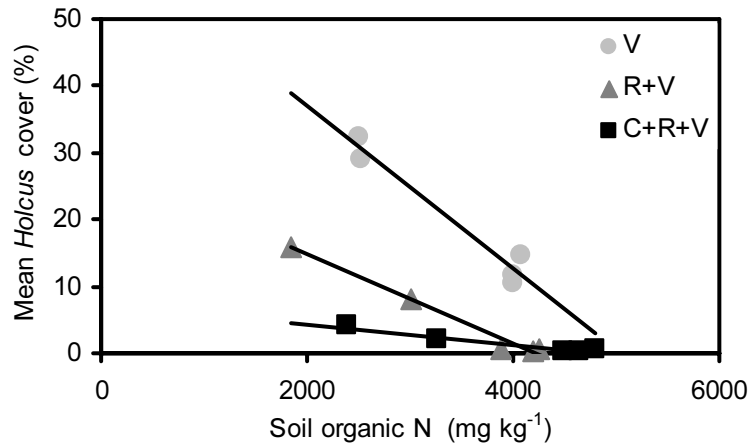
Figure 4.2 Relation between total soil nitrogen (mg kg^{-1}) in 1998 and cover (%) of *Holcus lanatus* averaged over all years in the grazing treatments. Regression analysis (n=5): C+R+V treatment: r^2 = 0.96, p=0.004, R+V treatment: r^2 = 0.99, p=0.001, V treatment: r^2 = 0.96, p=0.004. V: voles; R+V: rabbits + voles; C+R+V: cattle + rabbits + voles.

Dissimilarity in plant community composition

The dominant species in the V treatment showed larger variation over time than in the R+V and in the C+R+V treatment (fig. 4.3a). There was no difference in temporal variability between the fertilized and unfertilized treatment in any of the grazing treatments (Anova with grazing and nutrient treatment as fixed factors, block as random factor, grazing: $F_{2,8}$ = 32.024, p<0.001, nutrient: $F_{1,4}$ = 4.158, p=0.111, block: $F_{4,4.8}$ = 1.407, p=0.357, grazing*nutrient: $F_{2,8}$ = 0.900, p=0.444).

The effect of nutrient addition on changes in the vegetation composition depended on grazing treatment (fig. 4.3b). In the C+R+V treatment the initial difference between the plots remained constant over time. In the R+V and the V treatment the vegetation of the fertilized and control plots became more and more different during the experiment (fig. 4.3b).

The spatial variation between the plots in a grazing treatment also differed with grazing treatment. The vegetation of the control plots in the C+R+V treatment was rather similar in all five blocks (fig. 4.3c); the initial difference remained constant over time. In the V and the R+V treatment, the vegetation composition became more and more different between blocks over time.
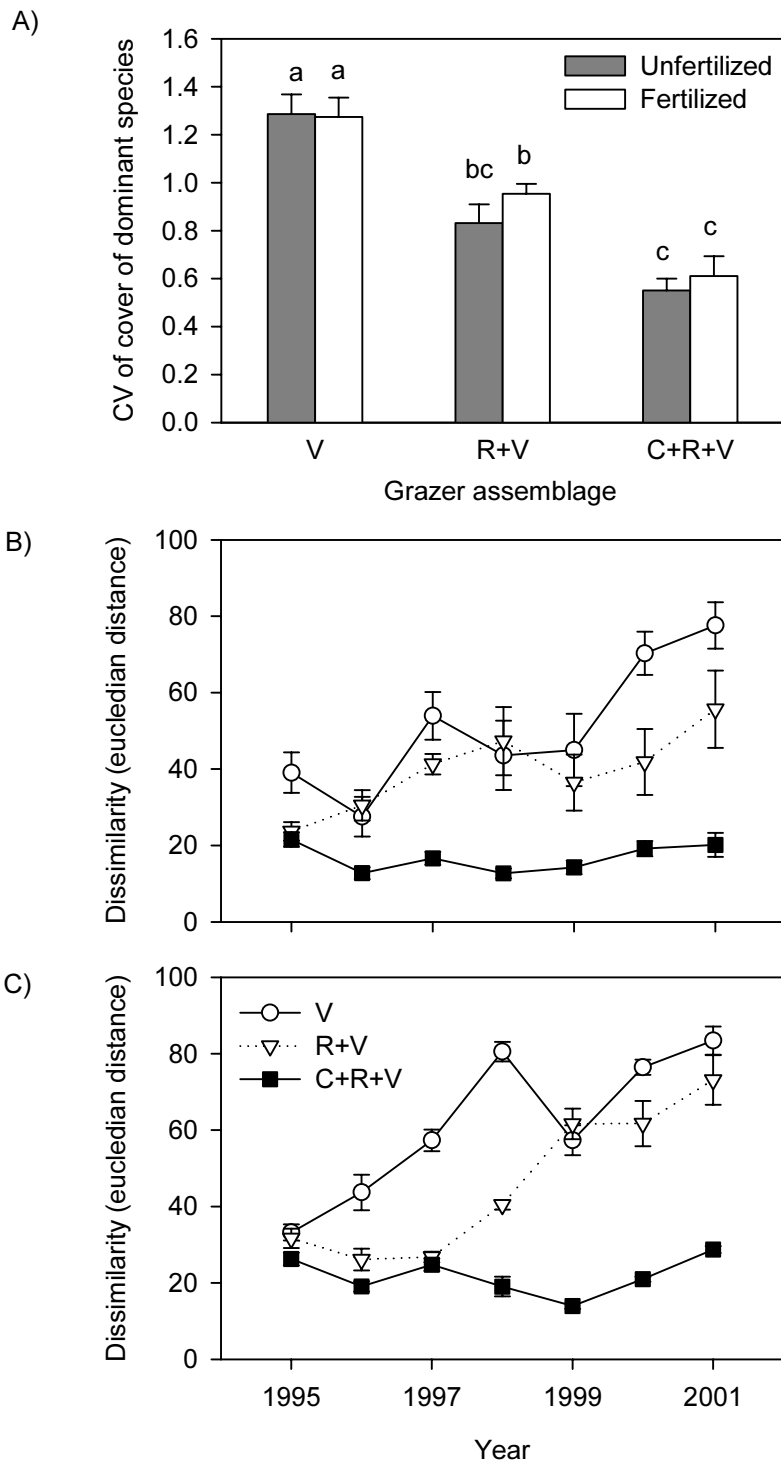
Figure 4.3 A. Temporal constancy of the vegetation in the grazing treatments under different fertilization regimes. The coefficient of variation is calculated per dominant species as the standard deviation divided by the average plant cover over years per grazing treatment. Values of the separate dominant species are averaged per plot. Data are means ± se. Different letters indicate statistically different CV's. V: voles; R+V: rabbits + voles; C+R+V: cattle + rabbits + voles.
B. Dissimilarity between fertilized and unfertilized plots over time in the grazing treatments.
C. Spatial heterogeneity expressed as dissimilarity between blocks within a grazing treatment. Only unfertilized plots were used for the analysis.
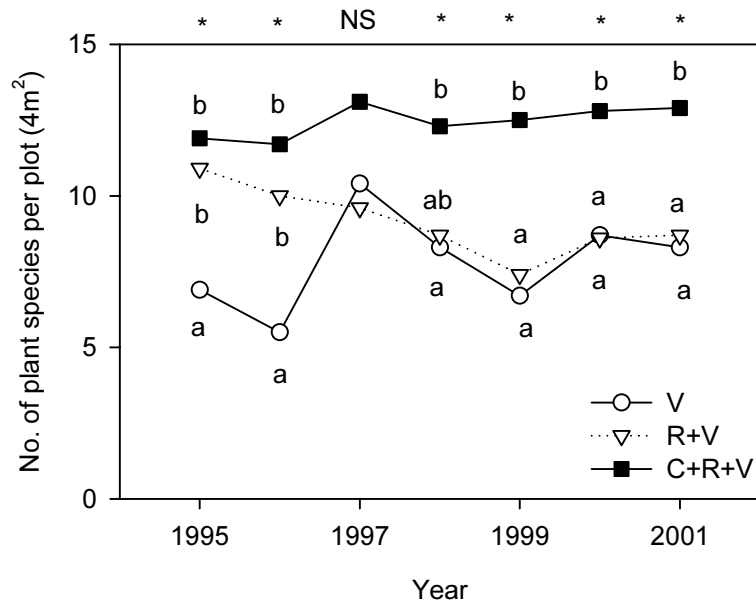
A) Unfertilized

B) Fertilized

Year
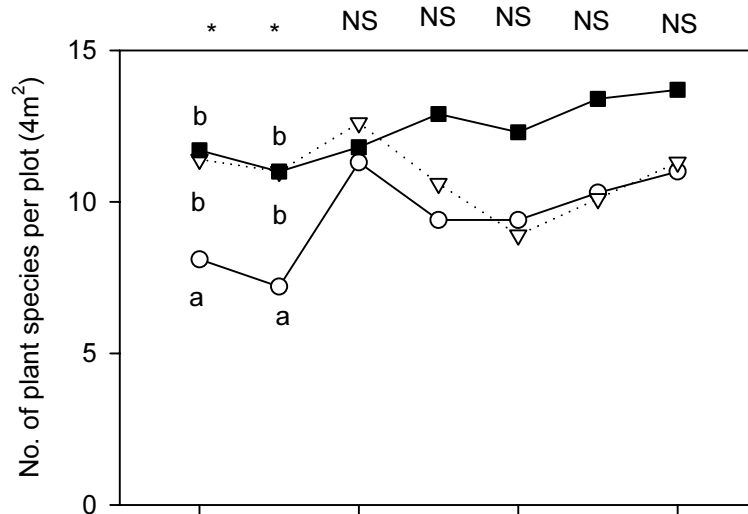
Figure 4.4 Plant species richness in (A) the unfertilized and (B) the fertilized plots under different grazing treatments. Different letters indicate significantly different numbers of plant species between the grazing treatments within a year (One-way Anova; p<0.05). V: voles; R+V: rabbits + voles; C+R+V: cattle + rabbits + voles.

Plant species richness

Grazing treatment affected plant species richness of the plots, but the impact varied with nutrient treatment (fig. 4.4, Anova after ln-transformation, fixed factors nutrients and grazing, random factors year and block, grazing: $F_{2,14.3}$ = 13.162, p=0.001, nutrients: $F_{1,5.7}$ = 25.424, p=0.003, nutrients*grazing, $F_{2,8.126}$ = 5.971, p=0.025). In the unfertilized plots, species number gradually increased in the C+R+V plots from 11.5 towards 13.5 species per plot. Species numbers in the R+V grazing plots were

identical to the C+R+V grazing plots in the first four years and then decreased towards the same numbers as in the V grazing treatment. Species numbers in the V treatment were lower than in the C+R+V treatment during most years. However, the effect of grazing depended on the year of study and was only significant in the first years (Fig. 4.4a, Anova, grazing: $F_{2,11.9} = 5.674$, p=0.019, year: $F_{6,6}$=2.685, p=0.121, grazing*year: $F_{12,48}$=2.431, p=0.015). In the fertilized plots the effect of grazing treatment on species richness was more pronounced (fig. 4.4b). Species richness was highest in the C+R+V treatment and from the third year on equally low in the R+V and V treatments (fig. 4.4b, Anova grazing: $F_{2,12.9}$=17.945, p<0.001, year: $F_{6,9.4}$=1.232, p=0.371, grazing*year: $F_{12,48} = 4.296$, p<0.001). In the C+R+V plots species number in the fertilized plots was not different from the control plots (Anova, fixed factor nutrients, random factors year and block, $F_{1,4.746} = 0.006$, p=0.942). In the R+V and in the V treatment species number was lower in the fertilized plots (R+V: $F_{1,6.160} = 10.848$, p=0.016, V: $F_{1,2.752} = 24.869$, p=0.019) compared to the unfertilized plots (fig. 4.4). We compared the temporal variability in the vegetation composition, calculated from the seven dominant species, with the achieved species richness in the last year of the study (2001) (fig. 4.5). Species richness significantly declined with temporal variability (Pearson correlation: r = -0.52, p=0.003, n=30).
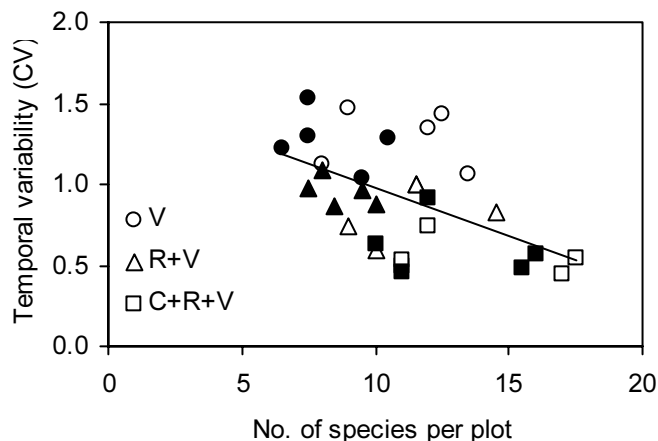

Figure 4.5 Relation between temporal variability (CV) and species richness. Both fertilized (open symbols) and unfertilized (closed symbols) plots were used for the analysis. Pearson correlation, r = –0.52, p=0.003, n=30. V: voles; R+V: rabbits + voles; C+R+V: cattle + rabbits + voles.

Vegetation height and structure

Vegetation in the grazing treatments became taller over time (fig. 4.6a). The effect of fertilizer differed between grazing treatments. In the V treatment the vegetation in the fertilized plot was higher, but only statistically different in one year. For the R+V treatment there was no difference between fertilized and unfertilized plots. The vegetation in the C+R+V treatment was on average lower in the fertilized plots compared to controls, only significantly in two years.

We randomly selected some unfertilized plots to illustrate differences in vegetation structure between the grazing treatments (fig. 4.6b). The vegetation in the C+R+V treatment was homogeneously short. Vegetation in the R+V treatment became taller, and also patchier, which was even more pronounced in the V treatment.

Above ground biomass and total cover

Temporal variation in living above ground biomass as well as total cover was higher in the V grazing treatment than both the R+V and C+R+V treatment (tab. 4.2). Variation in litter biomass was lowest in the R+V treatment and equal in the C+R+V and V treatment, but not significant.
Spatial variation in living biomass was lowest under large herbivore grazing and equally large in the R+V and V treatments. Spatial variation in total cover was equally low in the C+R+V and R+V treatment and higher in the V treatment. Variation in litter biomass was highest in the C+R+V treatment.

Table 4.2 Impact of different-sized herbivores on stability of ecosystem properties expressed as coefficient of variation (CV, calculated as standard deviation/mean). Data represent means ± se. Temporal variation in litter and living biomass is calculated as CV over three years and then CV is averaged over three blocks. Total cover variation is calculated as CV over seven years and then CV is averaged over all five blocks. For spatial variation in litter and living biomass average biomass of the three sample years is used. CV is calculated over three blocks and has therefore no standard error. For total cover averages of seven years were used and CV is calculated over five blocks. Different letters indicate significantly different grazing treatments.

	C+R+V	R+V	V	Grazing	P	Block	P
CV in time							
Litter biomass (n=3)	$0.53^a \pm 0.09$	$0.21^a \pm 0.07$	$0.51^a \pm 0.06$	$F_{2,4}=5.339$	0.074	$F_{2,4}=0.756$	0.527
Living biomass (n=3)	$0.39^a \pm 0.14$	$0.41^a \pm 0.28$	$0.58^a \pm 0.13$	$F_{2,4}=0.576$	0.603	$F_{2,4}=0.026$	0.026
Total cover (n=5)	$0.12^a \pm 0.01$	$0.14^a \pm 0.01$	$0.28^b \pm 0.04$	$F_{2,8}=14.344$	0.002	$F_{4,8}=1.694$	0.244
CV in space							
Litter biomass	0.65	0.16	0.27				
Living biomass	0.32	0.88	0.88				
Total cover	0.05	0.06	0.27				

Herbivore density

Cattle grazed in the area from half April till late October, at the same stocking rate each year (tab. 4.3). Data on cattle grazing pressure per block were not available. Rabbit density fluctuated slightly between years. Rabbits were more abundant where cattle grazed, especially during summer and autumn. But rabbits preferred the R+V treatment during spring. Voles hardly visited the C+R+V treatment, except for a few individuals in 1998. Most voles died over winter, resulting in a strong fluctuation in density over the year (tab. 4.3). When herbivore densities were expressed on basis of energy expenditure, energy use was roughly similar between the treatments (tab. 4.3). That means that the vegetation in the grazing treatments may have experienced a comparable grazing pressure, but from different combinations of herbivore species.

A) Vegetation height
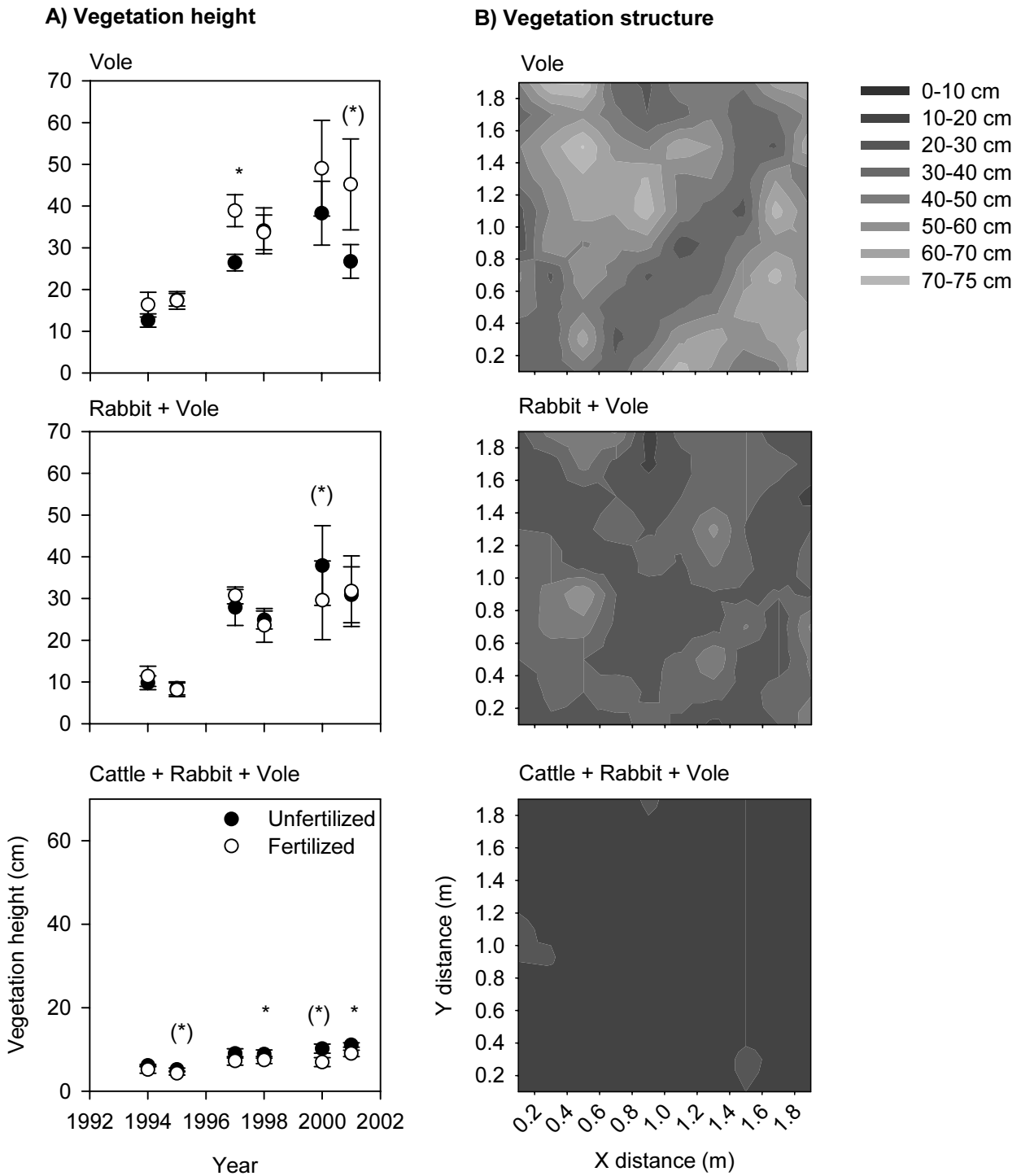
Vole

B) Vegetation structure

Vole

Figure 4.6 A. Development of vegetation height (cm) in the fertilized and unfertilized grazing treatments. An asterisk indicates statistically different vegetation height, * $p<0.05$, (*) $p<0.10$.
B. Vegetation structure in an unfertilized plot in August 1998. Shading represents classes of vegetation height (cm) at intervals of 10 cm.

Table 4.3 Estimated herbivore densities (individuals ha^{-1}) and daily energy expenditure (DEE) for 1998-2000 and densities per season for the year 1998. Data are means ± se.

	Cattle	Rabbits		Voles			DEE (MJ ha^{-1} day^{-1})		
In treatment	C+R+V	C+R+V	R+V	C+R+V	R+V	V	C+R+V	R+V	V
1998	0.4	12.4 ± 1.1	9.7 ± 1.1	278 ± 124	583 ± 261	1000 ± 447	34	24	28
1999	0.4	19.0 ± 1.6	10.3 ± 2.9	0	889 ± 398	1042 ± 467	32	33	29
2000	0.4	12.2 ± 1.2	7.8 ± 1.7	0	903 ± 404	764 ± 342	27	31	21
Per season (data from 1998)									
Winter	0	11 ± 1	7 ± 2	14 ± 14	0	28 ± 28			
Spring	0.4*	9 ± 2	16 ± 1	No data	No data	No data			
Summer	0.4	14 ± 2	3 ± 2	0	319 ± 64	833 ± 64			
Autumn	0.4*	19 ± 4	8 ± 2	278 ± 124	583 ± 261	1000 ± 447			

Discussion

The spatial and temporal compositional stability of the vegetation was differently affected by large or small herbivore grazing. Grazing by large herbivores resulted in very stable vegetation in time, while grazing by voles only caused very dynamic vegetation with frequent outbreaks of different plant species. Temporal variation of the vegetation expressed as CV was also highest in the V treatment. This could potentially be an effect of vegetation development after fencing of the plots, i.e. it is not surprising that the vegetation changed more inside the fence than outside. However, as expressed by the dominant species in the vegetation, these changes did not develop in a clear direction during the study period, but were rather characterized by repeated outbreaks of the same set of species. Therefore the conclusion that the vegetation shows more temporal fluctuations under vole grazing seems justified. We found that voles hardly used the vegetation outside the exclosures, although they had free access to it. It is therefore possible to compare the effects of voles as small herbivores with the combined effects of rabbits and cattle as large(r) herbivores. Since the R+V treatment shows intermediate responses for most measured variables, we will focus our discussion on the comparison between the V and the C+R+V treatment.

Nutrient addition had no effects on plant species dynamics in the presence of large herbivores while it had a major impact when these species were excluded. Adding nutrients when cattle and rabbits were excluded caused more chaotic vegetation dynamics, a different vegetation composition and lower species richness. This confirms other studies where grassland eutrophication in the absence of large herbivores is shown to destabilize competitive interactions (Inouye & Tilman 1995) or increase plant species turnover (Tilman & Wedin 1991a).

These results of the experimental nutrient addition are in agreement with the expression of the natural underlying soil variability in the vegetation. In ungrazed vegetation in other studies, the plant species composition reflects differences in soil nutrients or moisture in space and temporal variability in external factors, such as rainfall or flooding (Tilman 1987; Silvertown et al. 1994; Olff et al. 1997). This was also the case in the V treatment where the differences in vegetation composition

between the blocks that developed during the experiment reflected natural differences in soil conditions. In the C+R+V treatment differences in soil conditions were equally large, but not expressed in the vegetation composition. Apparently, grazing by small herbivores allowed expression of soil variability in the vegetation composition, while large herbivores suppressed underlying heterogeneity. In analogy we expect that temporal variability in growing conditions (such as interannual climatic variability) may also be more clearly expressed in the vegetation under grazing by small herbivores compared to large ones.

Our results suggest that large herbivores strongly regulate the local vegetation composition and structure, loosening its dependence on external factors as climate and soils. This results in high compositional stability and high plant species richness. On the other hand small herbivores mostly respond to vegetation composition and structure and allow the expression of climate and soils in the vegetation. This leads to low compositional stability and low plant species richness (fig. 4.7). We will discuss how the differential ability of large and small herbivores to exert topdown control may be explained by sensitivity to predation (fig. 4.7), the spatial position of biomass removal, timing of grazing, food selectivity and spatio-temporal scale of impact.

In our study system, the common voles were prey for many naturally occurring predators (mammals: ermine, polecat, fox, birds of prey: buzzard, kestrel) and therefore experienced a high predation risk (Pearson 1985), rabbits had less potential predators (polecat, fox, buzzard and goshawk) while cattle were only regulated in density by man. Despite our study was conducted in a highly managed landscape, we think that a decline with body size in the importance of predation in regulating herbivore populations is a general pattern. Densities of smaller herbivores are generally strongly regulated by predation (Krebs et al. 1999; Hanski et al. 2001). This implies that the interplay of small herbivores with the vegetation composition can only be understood if predation is taken into account (fig. 4.7) (Pusenius & Ostfeld 2000; Iason et al. 2002). However, larger herbivores can escape predation by increasingly being able to individually defend themselves i.e. megaherbivores (Owen-Smith 1988) or as a group or by escaping resident predators through seasonal migration (Drent & Prins 1987; Mduma et al. 1999). As a result these larger herbivores may become limited by food availability or quality, which causes them to exert a larger control over the vegetation composition and structure of their habitat (fig. 4.7).

The difference between large and small herbivores in ability to control vegetation structure and composition is not only caused by predation, but also by differences in the spatial position of biomass removal. Large herbivores graze the vegetation from above, which reduces competition for light (Bakker & De Vries 1992; Semmartin & Oesterheld 2001). Small herbivores such as voles preferably forage in high vegetation (Jacob & Brown 2000; Pusenius & Ostfeld 2000), where they graze the vegetation from below. As this does not prevent plant populations to compete for light, this type of grazing does not result in control over the vegetation composition.

The different-sized herbivores showed strong differences in the within-year timing of grazing caused by the correlation between body size, lifespan and migration distance (Peters 1983; Haskell et al. 2002). Voles were present in low densities in spring, gradually increased in number over summer and reached peak densities in autumn, whereas cattle were present in the area at the start of the growing season until late fall, reflecting natural migration patterns (WallisDeVries & Schippers 1994). This results in a mismatch in timing between growing season and vole peak density. By the time vole numbers are high enough to consume the vegetation production, the vegetation has already responded to the increased nutrient availability in spring. Most of this spring primary production will be literary out of reach since voles preferably harvest young shoots and leaves located at the soil level or just above. Due to the virtual absence of voles at the start of the growing season small random initial differences in plant size may be amplified (priority effects), that are otherwise soon removed by cattle through compensatory grazing. Through priority effects different species may become dominant varying with soil nutrient status under vole grazing. We conclude that large herbivores synchronize consumption with bulk plant production leading to increased control over the vegetation structure and composition.

The change with body size in food selectivity is an additional factor that explains why larger herbivores exert more control over the vegetation composition (fig. 4.7). However, this depends on the type of resource limitation for plant growth (Ritchie & Olff 1999a). Only in wetter, more nutrient-rich habitats, the dominant plant species are still sufficiently palatable and the large grazers will focus on these species. This reduces the competitive impact on subordinate plants through light competition and creates regeneration opportunities (Olff & Ritchie 1998; Bakker & Olff 2003) which enhances plant diversity. Smaller herbivores need a relatively higher quality diet (Demment & van Soest 1985) to account for their higher mass-specific metabolic rate, therefore they select nutrient rich plant parts, such as young leaves, or subordinate high quality herbs. This causes less impact of smaller herbivores on the vegetation composition and diversity (fig. 4.7).

A final factor explaining why larger herbivores in these habitats exert more control over the vegetation is their larger spatial and temporal scale of operation. The size of herbivore home ranges differs with body size. Large herbivores can respond to vegetation changes at a larger scale by locally increasing or decreasing grazing pressure within their home ranges, whereas small herbivores first have to discover the patch when it is outside their home range or built up numbers before they can increase grazing pressure. Larger species can store more reserves to overcome periods of scarcity (Mysterud et al. 2001) and have longer life spans (Peters 1983) but at the expense of lower per capita metabolic and reproductive rates (Peters 1983). Therefore year-to-year variation in population numbers is reduced in large versus small species resulting in a more stable grazing pressure from large herbivores. Population numbers of small herbivores as voles show strong dynamics within and between years (Dijkstra & Zijlstra 1997; Hanski et al. 2001), resulting in dynamic grazing pressure and less control over the vegetation.
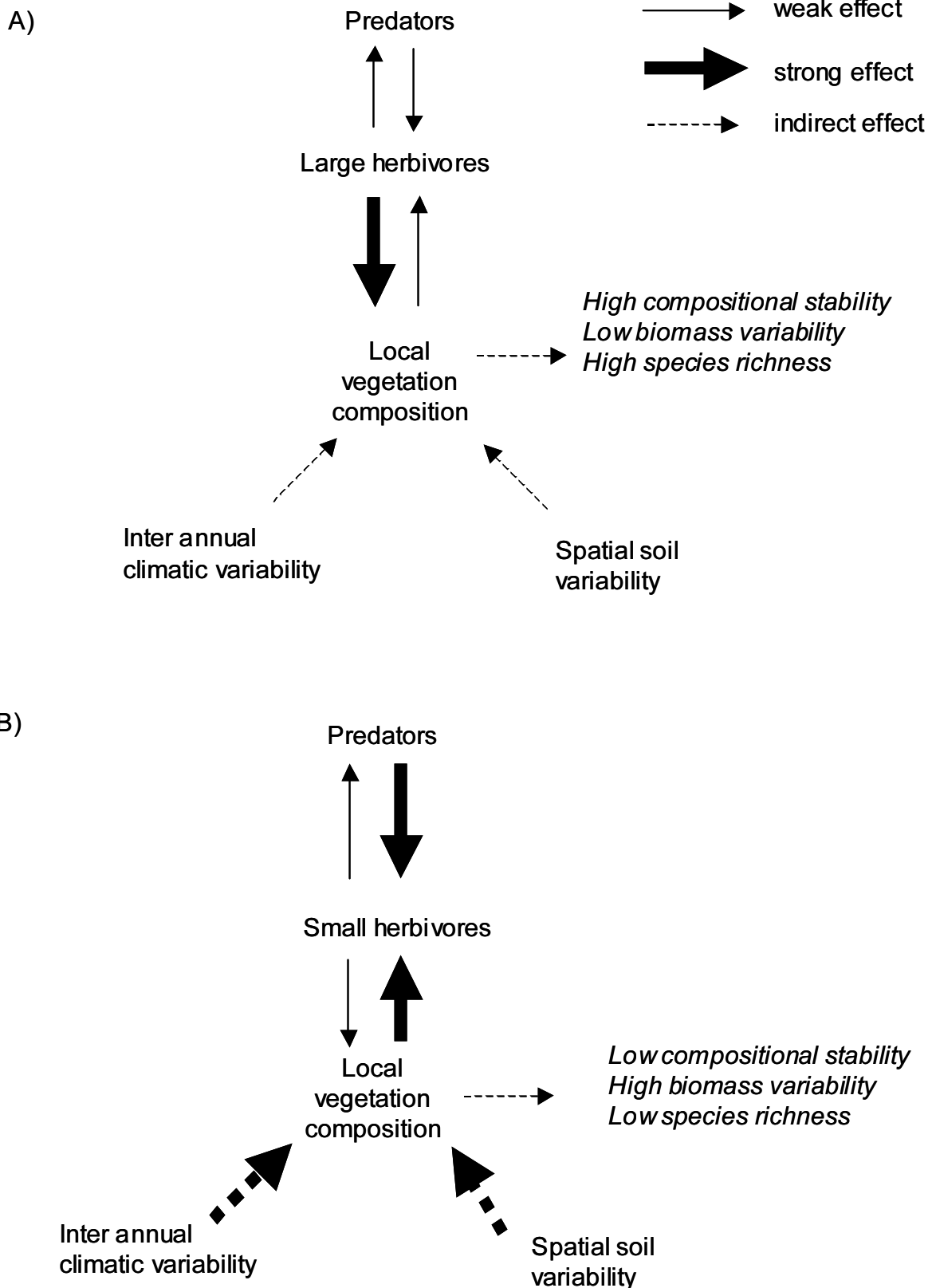
A)

Predators

Large herbivores

weak effect

strong effect

------▶ indirect effect

Local
vegetation
composition

- - - - ▶ *High compositional stability*
Low biomass variability
High species richness

Inter annual
climatic variability

Spatial soil
variability

B)

Predators

Small herbivores

Local
vegetation
composition

- - - - ▶ *Low compositional stability*
High biomass variability
Low species richness

Inter annual
climatic variability

Spatial soil
variability

Figure 4.7 Scheme of interaction between herbivores and vegetation composition for large and small herbivores.
A. Large herbivores have a large impact on the vegetation whereas other factors such as soil nutrient status and weather have less impact.
B. Small herbivores have less constant impact on the vegetation composition, here soil nutrients and climatic factors are relatively more important in determining vegetation composition.

The conclusion that large and small herbivores control the vegetation differently depends on the scale of observation. If the scale of observation was adjusted to herbivore size and for example the vegetation structure of several hectares was compressed to a few square meters, the grazing patterns created by cattle and voles may be very similar. At the landscape scale cattle may even create a structurally more diverse landscape because they prefer certain vegetation types and patches, resulting in vegetation with a mosaic of high and low patches. Vole grazing on a landscape scale may result in rather structurally homogenous vegetation, because vole grazing lawns are so small that they are hard to detect at a landscape scale.

A larger local compositional constancy coincided with higher plant species richness in this study. With the current design it is not possible to determine the causality of the relationship between stability and species richness. It is also possible that both a larger compositional constancy and higher species richness are a consequence of large herbivore grazing and not necessarily show a causal relationship (Sankaran & McNaughton 1999). Large and small herbivores had also different effects on the dynamics of ecosystem properties. Large herbivores had a stabilizing effect on plant living biomass and cover both in space and time. These results show that vertebrate herbivores can not be ignored in studies on the relations between plant diversity and ecosystem functioning in grasslands, and that different-sized herbivores have very different effects on spatial and temporal compositional stability of grassland communities.

Acknowledgements

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Chapter 5

Impact of herbivores on nitrogen cycling: contrasting effects of small and large species

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Abstract

Herbivores are reported to slow down as well as enhance nutrient cycling in grasslands. These conflicting results may be explained by differences in habitat or herbivore type. In this study we focus on herbivore body size as a factor that causes differences in herbivore effects on nitrogen cycling. We used an exclosure set-up in a floodplain grassland grazed by cattle, rabbits and common voles, where we subsequently excluded cattle and rabbits. Exclusion of cattle lead to an increase in vole numbers and a 1.5 increase in net annual nitrogen mineralization at similar herbivore densities (corrected to metabolic weight). In contrast, potential nitrogen mineralization rates measured under standardized laboratory conditions were the same for all three grazing treatments as well as all soil parameters, plant biomass and plant nutrient content measured in the field.

We suggest that microclimatic differences (i.e. soil temperature) may have caused part of the differences in mineralization rates. Timing and height of the mineralization peak in spring was the same in all treatments, but mineralization in the vole grazed treatment showed a peak in autumn, when mineralization had already declined under cattle grazing. The higher mineralization rates under vole grazing might be due to isolation from the larger litter layer in the vole grazed treatment resulting in more stable soil temperatures. Also nutrients could be returned through the increased litter layer. The mineralization peak in autumn coincides with a peak in vole density and high levels of nitrogen input through vole faeces at a fine-scale distribution, whereas under cattle grazing only a few patches receive all nitrogen and most experience net nutrient removal. We conclude that different-sized herbivores have different effects on nitrogen cycling within the same habitat. Exclusion of large herbivores resulted in increased nitrogen annual mineralization under small herbivore grazing.

Introduction

Herbivores can have a strong impact on plant productivity in grasslands (Huntly 1991; Milchunas & Lauenroth 1993; Hobbs 1996; McNaughton et al. 1997; Frank et al. 2002). In many terrestrial systems plant growth is nitrogen limited (Vitousek 1982; Aerts & Chapin III 2000). However, most nitrogen in terrestrial systems is locked in the soil organic matter, which has a slow turnover rate (Knops et al. 2002). Herbivores may affect plant productivity by modifying the rate of nitrogen cycling and therefore nitrogen availability for plant growth. The impact of herbivores on nitrogen cycling can lead to two alternative outcomes: herbivores can enhance nutrient cycling (Holland & Detling 1990; McNaughton et al. 1997; Tracy & Frank 1998; Frank & Groffman 1998; Sirotnak & Huntly 2000) or herbivores can decrease the rate of nutrient cycling (Pastor et al. 1993; Ritchie et al. 1998; Van Wijnen et al. 1999). Herbivores can have direct and indirect effects on nutrient fluxes (Bardgett et al. 1998; Ritchie et al. 1998; Sirotnak & Huntly 2000). Direct effects include return of nutrients through faeces (Bazely & Jefferies 1985; Ruess & McNaughton 1987; Pastor et al. 1996; Chesson 1997; Frank et al. 2000), indirect effects occur when herbivores influence the vegetation composition through grazing, resulting in changes in quality and quantity of nutrient input through litter (Pastor et al. 1993; Ritchie et al. 1998; Olofsson & Oksanen 2002). Despite the increasing number of studies of herbivore impact on vegetation composition and plant production, the question whether herbivores accelerate or slow down nutrient cycling is still debated. This calls for more research on the underlying mechanisms. We hypothesize that the balance between direct and indirect effects may depend on habitat type and herbivore size and density. In this study we investigate the role of herbivore body size in their effects on nitrogen cycling.

Figure 5.1 summarizes different pathways of N turnover in grassland ecosystems. Plants that remain ungrazed return nutrients via litter formation after natural death. The litter is decomposed, part of the nutrients become readily available, but most of it is added to the soil organic matter compartment, with slow turnover rates (Parton et al. 1987; Knops et al. 2002). Therefore we call this the slow cycle (fig. 5.1). By consuming plants herbivores create a shortcut in this slow nutrient cycle. Plants are fragmented and digested (decomposed) in the herbivore gut, resulting in deposition of readily accessible nutrients in faeces: the fast cycle (Ruess & McNaughton 1987; Pastor et al. 1993; Chesson 1997). The amount and quality of nitrogen input via both nutrient cycles directly affects nitrogen mineralization rates. However, also the physical conditions under which the soil microbes transform nutrients can have a strong impact on net mineralization rates. These indirect effects include soil temperature, soil moisture and aeration of the soil (Sierra 1997; Rustad et al. 2001; Wan et al. 2002). Herbivores can affect mineralization rates through their impact on these soil parameters: they can increase soil temperature through removal of isolating vegetation (Van der Wal et al. 2001; Wan et al. 2002) or decrease soil aeration through trampling (Jensen et al. 1996; Van Wijnen et al. 1999; Olofsson & Oksanen 2002). Also an increase in the amount of litter from ungrazed plants can

affect soil conditions: a litter layer can result in increased soil temperature and soil moisture. Herbivores can also exert indirect effects on N cycling through modification of the vegetation composition. A change in the species composition can induce changes in litter quantity or quality (Wedin & Tilman 1990; Pastor et al. 1993; Ritchie et al. 1998; Van der Krift & Berendse 2001).

The two pathways and indirect effects all include opportunities to enhance or slow down N-cycling (tab. 5.1). The importance of the different mechanisms mentioned in table 5.1 determines whether nitrogen cycling is enhanced or inhibited. The conflicting results of herbivore impact on nutrient cycling may be caused by a different relative importance of these mechanisms with varying habitat productivity or herbivore type and density. Habitat productivity is shown to affect the impact of herbivores on vegetation composition (Proulx & Mazumder 1998) and productivity (Milchunas & Lauenroth 1993). Herbivore density can influence effects of herbivores on nitrogen mineralization (Shariff et al. 1994; Kiehl et al. 2001) and productivity (Hik & Jefferies 1990). However, the importance of herbivore body size in their effects on vegetation and soils is only recently acknowledged (Olff & Ritchie 1998; Ritchie & Olff 1999). We hypothesize that the impact of herbivores on the proposed mechanisms (tab. 5.1) depends on herbivore body size. Body size is a critical parameter in the scaling of animal physiology, life history and behaviour (Peters 1983; Brown 1995), including metabolic rate and digestive capacity (Demment & Van Soest 1985), home range size (Haskell et al. 2002) and herbivore foraging parameters (Peters 1983; Belovsky 1997). When considering the proposed mechanisms affecting nutrient cycling (tab. 5.1), we hypothesize that herbivores of different sizes have different effects on vegetation and soil parameters. All herbivores return nitrogen through faeces and urine, enhancing N-cycling (Day & Detling 1991; Hobbs 1996; Frank et al. 2000). However, the scale of re-distribution of N is different: large herbivores deposit faeces in large amounts in few patches, whereas smaller herbivores produce small

Table 5.1 Overview of mechanisms that enhance or slow down nitrogen cycling through different pathways.

	Mechanisms enhancing N cycling	Mechanisms slowing down N cycling
Fast pathway	Microbial digestion in the herbivore gut: high temperature and moisture, fragmentation of plant material, profitable C/N ratio's[2]	Leaching or volatilization of nitrogen from faeces[3]
Slow pathway	Increased primary production, increased litter quality	Selective consumption of high quality species, decreased litter quality[1]
Indirect effects	Increased soil temperature[4] Increased/decreased soil moisture[6] Increased soil aeration[5]	Decreased soil temperature Increased/decreased soil moisture Decreased soil aeration

[1] Ritchie et al. (1998), Sirotnak & Huntly (2000), [2] Ruess & McNaughton (1987), Pastor et al. (1993) Chesson (1997), [3] Ruess & McNaughton (1988), [4] Sierra (1997), Wan et al. (2002), [5] Jensen et al. (1996)

[6] For soil moisture an optimum exists: more moisture in wet systems inhibits mineralization (Kiehl et al. 2001), whereas it enhances mineralization in water-limited systems (Wan et al. 2002).
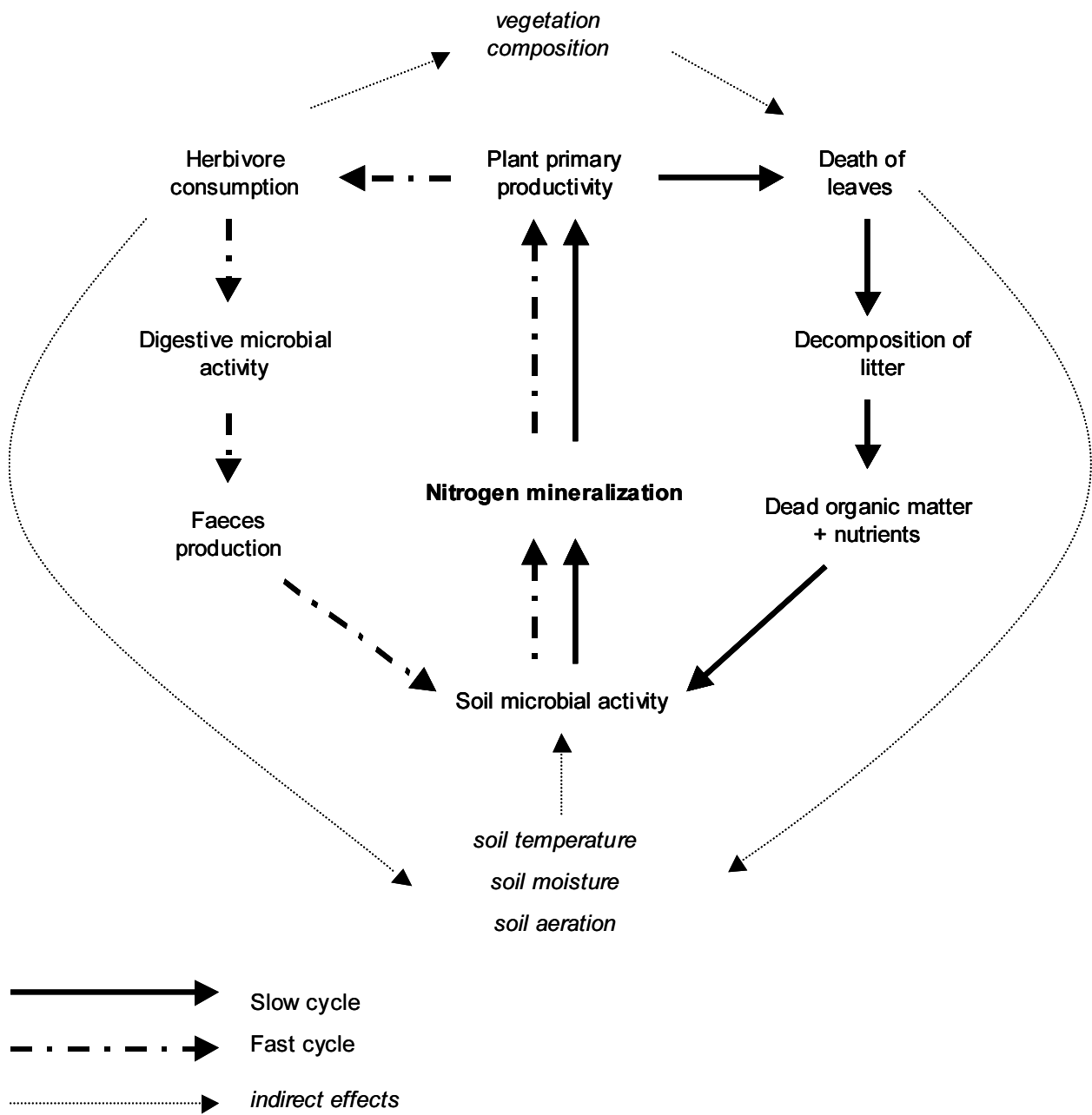
Figure 5.1 Scheme of nutrient cycling in grasslands under herbivore grazing.

pellets that are widely distributed. Nitrogen from small herbivore faeces may be easier available for plants. Small herbivores are better able to select plants of high quality and include more high quality plants in their diet than large herbivores, therefore potentially slowing down nitrogen cycling. Also the effects of different sized herbivores on physical soil parameters may differ. Trampling by large herbivores can cause soil compaction and reduced aeration, whereas many small herbivore species burrow (i.e. many rodents) thereby loosening the soil and increasing aeration.

Most natural grasslands are grazed by more than one species of herbivore. Herbivore species within a grazer assemblage can compete or facilitate for each other (Prins & Olff 1998; Van der Wal et al. 2000; Arsenault & Owen-Smith 2002). Facilitation can occur when herbivores show enough difference in body sizes to select different diets (Prins & Olff 1998): small herbivores may profit from grazing by large herbivores through improved quality or accessibility of their food source (Huisman & Olff 1998; Arsenault & Owen-Smith 2002). When herbivores select similar diets competition can occur, resulting in compensatory effects within an assemblage: the decline of one species of herbivore may be compensated by an increase in other species (Prins & Douglas-Hamilton 1990). The effects of combinations of different-sized herbivores on the vegetation can be additive or compensatory (Ritchie & Olff 1999). Therefore, the interactions between herbivores can have important consequences for the net-effect of the assemblage on the vegetation composition and nutrient cycling. The effects of a single herbivore species on the vegetation should thus be evaluated in a community context. In analogy, when working with an experimental design it is important to take into account behavioural and population responses of the remaining species to the exclusion of others.

In this study we focus on herbivore size as a parameter causing different outcomes of herbivore impact on nutrient cycling in a nutrient rich floodplain grassland. We used an exclosure set-up in a grassland grazed by cattle, rabbits and common voles, where we subsequently excluded cattle and rabbits. After five to seven years we measured nitrogen-mineralization, vegetation and soil characteristics, as well as realized herbivore densities.

Methods

Study site

The study was performed in the Junner Koeland area (52° 32'N, 6° 36'E), a 100 ha nature reserve including 50 ha floodplain grassland along the river Overijsselse Vecht in the Northeast of The Netherlands. The site is managed by the National Forest Service as a nature reserve. The area used to be common grazing land for the farmers from the village Junne, and has probably been grazed by livestock since the Middle Ages. Currently, cattle graze in the area from April till October in a density of 1 heifer per 2.5 ha. Naturally occurring grazers are European rabbits (*Oryctolagus caniculus*) and common voles (*Microtis arvalis*). Previous work showed that plant growth on Junner Koeland is generally nitrogen limited (Boekhoff 2000).

Experimental design

An exclosure experiment was established in 5 blocks in May 1994. Each block consisted of three grazing treatments from which herbivores were excluded following a 'Russian doll approach'. Outside the exclosure cattle, rabbits and common voles had free access to the vegetation. A fence consisting of two barbed wires at 0.5 and 1.0 m excluded only cattle allowing access for rabbits and voles. With chicken mesh rabbits were subsequently excluded leaving only voles to graze the vegetation. The different grazing treatments will be referred to as C+R+V for the cattle and rabbits and voles treatment, R+V for rabbits and voles and V for voles only. Each fence measured 12 by 12 m. Within each fence two plots of 2 x 2 m were established to monitor vegetation changes and rabbit presence.

Herbivore density

The number of cattle was similar between the years resulting in an average density of 0.4 cows ha^{-1}. Rabbit density was assessed through counting their pellets. Rabbit pellets were counted in the vegetation plots outside the exclosure and within the cattle fence approximately every three weeks from May 1994 until December 2001 and removed when counted. Rabbit density was calculated for each year using the pellet counts and assuming a dropping rate of 400 pellets per rabbit per day (Lockley 1962).

Vole density was measured by trapping voles at their peak density in October with live-traps from 1998 to 2000. Longworth live-traps were used, baited with peanut butter, oats, carrots and apple and filled with some hay. Traps were pre-baited for two days before a five-day trapping session started. Traps were checked for vole presence every 8 hours, resulting in 11 checks per trap. Captured voles were weighed and individually marked by clipping a piece of the fur at different positions on the back and then released. 90 Traps were used per session, evenly distributed over the grazing fences, which results in 6 traps per fence. Traps were spaced evenly with a distance of 3-4 m in between traps. Vole density turned out to be so high that after 4 days still new individuals were caught. This made it not possible to calculate potential vole density from the mark-recapture data. Therefore the number of individuals caught was interpreted as a minimum vole density. Vole trapping was approved by the committee for animal experiments (DEC) of Wageningen University (DEC no. 99112a).

To compare the impact of the different artificially created herbivore assemblages, we expressed cattle, rabbit and vole densities at the same scale. We expressed herbivore densities as daily energy expenditure (DEE) at two times basal metabolic rate: DEE = 2 * 70 * (live body weight)$^{0.75}$ kCal day^{-1} (Demment & Van Soest 1985) (1 kCal = 4.184 kJ). For cattle an average weight of 300 kg was assumed (heifers), for rabbits 1.5 kg (Wallage-Drees 1988) and vole weight was measured during catches as 0.017 kg (E.S. Bakker unpubl. data). Following this procedure, herbivore bodyweight was transformed to daily energy expenditure and this was multiplied by the measured average animal densities.

Nitrogen mineralization measurements

Net N-mineralization rates were measured in situ in all five blocks and grazing treatments. Measurements started in October 1998 and continued for a full year. The first three measurement periods during winter were eight weeks long; during the rest of the year periods of six weeks were used (resulting in 8 incubation periods). We sampled two subplots per grazing treatment, adjacent to the sampling points of the soil sampling (see below). Samples were taken using PVC tubes of 2.5 cm diameter and a depth of 10 cm. Per subplot a pair of tubes was used: one was taken directly to the laboratory and extracted with 50 ml 1 M KCl within 24 hours (the reference sample), the other tube was left in situ for eight or six weeks. The litter layer was removed before sampling a plot. Before incubation the top and bottom of the tubes were covered with plastic lids to prevent leaking of nitrogen from the soil tube. In the top of the tube, just below the lid and above the soil, two holes were present to permit aeration of the samples. After the incubation period the tubes were taken to the lab and weighed. The content of a tube was mixed and large roots were removed. A sample of 20 g field fresh soil was extracted with 50 ml 1M KCl, to determine plant available nitrogen content (NH_4^+ and NO_3^-), using a Continuous Flow Analyser (SKALAR). Net nitrogen mineralization was calculated as the difference between the NH_4^+ + NO_3^- content of the incubated sample and its paired reference sample. The pH was measured in the unfiltered KCl extract of the first series of reference samples (October 1998). Soil moisture was measured in a sub-sample of soil dried at 70°C for 48 hours as the weight difference before and after drying. Bulk density was calculated from the weight of the fresh reference sample, soil moisture content and tube volume. Soil moisture and bulk density were determined from the reference samples in all periods.

In addition to the in situ measurements of net nitrogen mineralization, potential mineralization rates were measured under controlled laboratory conditions during the first incubation period (starting October 1998). In each sampling plot a third sample was taken using the same PVC tubes as for the other samples. The tubes were incubated in a dark climate cell at 20°C with the air holes covered with tape to prevent desiccation of the sample. After eight weeks the samples followed the same extraction procedure as the other samples. Potential mineralization was calculated as the difference between the incubated sample and the paired reference sample from the field.

Soil nutrient measurements

In November 1998 soil samples were taken to determine total nutrient contents and organic matter of the soil in the different grazing treatments. In each exclosure two soil samples were taken per grazing treatment located in the opposite corners of the fences, but staying about 1.5 m away from the fences. Each sampling point consisted of a 50 x 50 cm plot from which the litter layer was removed and 5 soil samples were taken with an auger of 6 cm diameter and 10 cm depth. Samples were weighed and stored at 5°C till further processing the next day. From each soil core the upper 1 cm organic layer was removed as well as large roots before the 5 soil cores were homogenized. Total nitrogen was determined in air-dried soil (dried at 30°C for 48

hours) after digestion with sulphuric acid, selenium and salicylic acid. Nitrogen concentration was measured colometrically using a continuous flow analyser (SKALAR). Organic matter content of the soil was determined as weight loss of a sample dried during 24 hours at 105°C and then ignited for 3 hours at 550°C.

Vegetation measurements

Vegetation composition in the plots was recorded at peak standing crop (July or August) from 1995 until 2001 by visually estimating species aerial cover following the decimal Londo scale. In July 1995, 1999 and 2001 biomass samples were taken to determine standing crop and litter accumulation. In 1995 and 1999 biomass was harvested in each vegetation plot by clipping a strip of 10 x 100 cm to soil level. Samples were sorted to graminoids and herbs and to life and dead. In 1995 only three of the five blocks were sampled. In 2001 1 random sample of the same size and dimension was taken in each grazing fence outside the plots. In this sample a distinction was made between current-year standing dead and accumulated dead material (litter) from previous years. After clipping, a root sample was taken in the bare strip, using an auger of 8 cm diameter and 10 cm deep. The roots were washed in the lab and sieved using a 0.5 mm sieve. All biomass samples were dried for 24 hours at 70°C and weighed. The 2001 samples were also sorted to functional group and the nitrogen content of the plants was determined per functional group. These samples were subsequently ground and analysed for concentration of nitrogen in the lab after digestion with sulphuric acid.

Nitrogen content of faeces

Five samples of dung of all three herbivore species were collected in September 2000. Five cattle dung pats were sampled that were excreted a few hours earlier. Rabbit droppings were collected from five plots located near rabbit burrows. Droppings were collected daily to assure that they were fresh (less than 24 hours old) during five consecutive days and then pooled per plot. Vole pellets were collected from traps during vole trapping at the same time. Pellets were only collected from traps when the vole had produced a lot of pellets; pellets were pooled over the trapping days within a block (see methods vole trapping), resulting in five pooled samples. The samples were dried at 70 °C and analysed for concentration of nitrogen in the lab after digestion with sulphuric acid.

Data analysis

When two samples were taken per block per grazing treatment, these were averaged before further calculations, resulting in a sample size of 5 for all measurements. Data were ln-transformed prior to analysis to meet Anova assumptions. In most cases a two-way Anova was carried out with grazing treatment as fixed factor and block as random factor. When grazing effect was significant and block had not a significant effect, block was removed from the analysis and a post-hoc Tukey test was performed to determine differences between grazing treatments.

Results

Herbivore densities

During vole trapping 95% of all the catches (n = 1494) were common voles (*Microtus arvalis*), others were common shrew (*Sorex araneus*, 4.1%), wood mouse (*Apodemus sylvaticus*, 0.7%) and water shrew (*Neomys fodiens*, 0.2%). The herbivores did not distribute themselves equally over the treatments where they had access to (tab. 5.2). The response to the exclusion of cattle was different for rabbits and voles. Rabbits preferred the cattle grazed area, whereas voles did hardly visit it. When herbivore densities in the grazing treatments are compared based on daily energy expenditure at twice basal metabolic rate, the herbivore assemblages appeared to be very similar (fig. 5.2).

Soil characteristics

Net in situ annual mineralization was significantly reduced in the C+R+V treatment (fig. 5.3a, tab. 5.3). Especially NO_3^- -availability was lower. The difference in mineralization is present during most of the year and is not the result of a difference in timing of the start of the increase in mineralization rate in early spring (fig. 5.3a). During March a flux of nitrogen becomes available in all three grazing treatments simultaneously, which coincides with an increase in air temperature (fig. 5.3b). Mineralization is strongly reduced in June in the C+R+V treatment compared to the other treatments. Mineralization in the R+V and V treatment showed a strong peak in October, when the mineralization in the C+R+V treatment declined.

No significant differences in organic matter or potential N-mineralization were found between the grazing treatments (tab. 5.3). The total soil nitrogen pool was slightly higher in the C+R+V grazed treatment than in the other treatments, but the difference was not significant. The pH was significantly lower in the cattle grazed treatment. Bulk density was increased under cattle grazing, but not significantly. Soil moisture was not significantly different between the grazing treatments and not correlated with mineralization rates (fig. 5.3c), neither within the mineralization tubes nor outside.

Table 5.2 Distribution of herbivores over the grazing treatments in 1998, 1999 and 2000. Data represent mean number of individuals ha^{-1} ± se. V: vole grazed, R+V: rabbits + voles, C+R+V: cattle + rabbits + voles. [1] cattle graze from half April until half October, [2] average calculated from year round pellet counts (see methods), [3] measured at peak density late October.

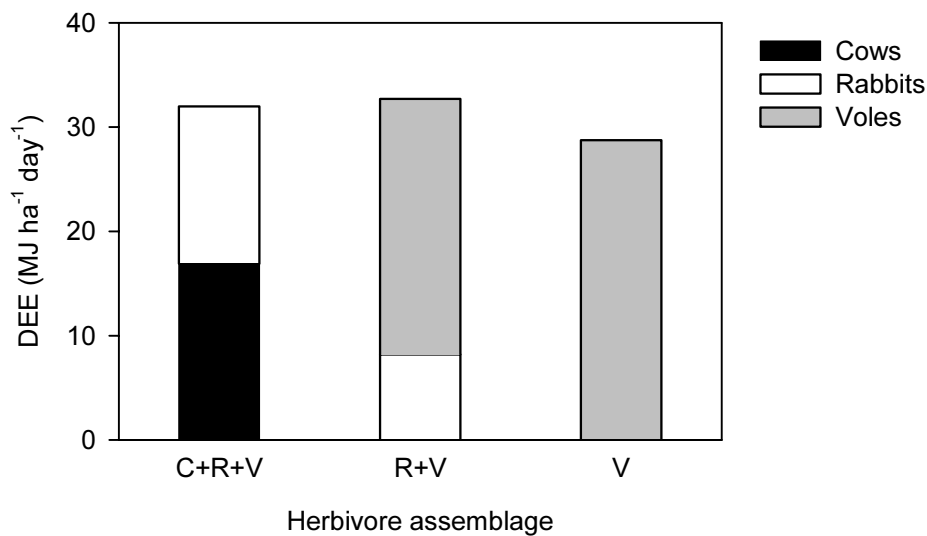| Grazing treatment	Year	Cows[1]	Rabbits[2]	Voles[3]
C+R+V	1998	0.4	12.4 ± 1.1	278 ± 124
	1999	0.4	19.0 ± 1.6	0
	2000	0.4	12.2 ± 1.2	0
R+V	1998	0	9.7 ± 1.1	583 ± 261
	1999	0	10.3 ± 2.9	889 ± 398
	2000	0	7.8 ± 1.7	903 ± 404
V	1998	0	0	1000 ± 447
	1999	0	0	1042 ± 467
	2000	0	0	764 ± 342

Figure 5.2 Daily energy expenditure (MJ ha^{-1} day^{-1}) of the herbivores at maintenance level (two times basal metabolic rate) in the artificial grazing treatments in 1999. Herbivore energy expenditure is calculated using measured herbivore densities (tab. 5.2) and substituting in the formula from Demment & van Soest (1985), see text for details. Grazing treatments: V = vole grazed, R+V = rabbits + voles, C+R+V = cattle + rabbits + voles.

Above and below ground biomass

Above ground living biomass was rather similar for all sampling years (fig. 5.4a). There is considerable local variation in living biomass, but no significant differences between the grazing treatments (Anova grazing: $F_{2,8.1}$ =1.241, p=0.339, year: $F_{2,3.0}$ =6.782, p=0.076, interaction grazing*year $F_{4,12}$ =1.663, p=0.223, block: $F_{4,7.4}$ =0.022, p=0.999). The major difference between the grazing treatments is the accumulation of litter (fig. 5.4b). Litter production varied considerably between years (Anova: interaction grazing*year $F_{4,12}$ =9.707, p=0.001). In 1995 and 1999 large amounts of litter were accumulated especially in the vole grazed treatment (One-way Anova on data grouped by grazing and year: $F_{8,30}$ =10.167, p<0.001, different letters in fig. 5.4b indicate differences between groups at the 0.05 level). By 2001 the large amount of litter from 1999 had completely disappeared.

The amount of roots decreased significantly after exclusion of cattle and rabbits (tab. 5.4). More root biomass was found in the C+R+V treatment in absolute terms compared to the V treatment, but also relatively when compared to above ground living biomass. Nitrogen content of biomass was not significantly different between grazing treatments for all functional groups (tab. 5.5). Legumes were not separately tested because the amount of legumes was too small for a nutrient analysis.
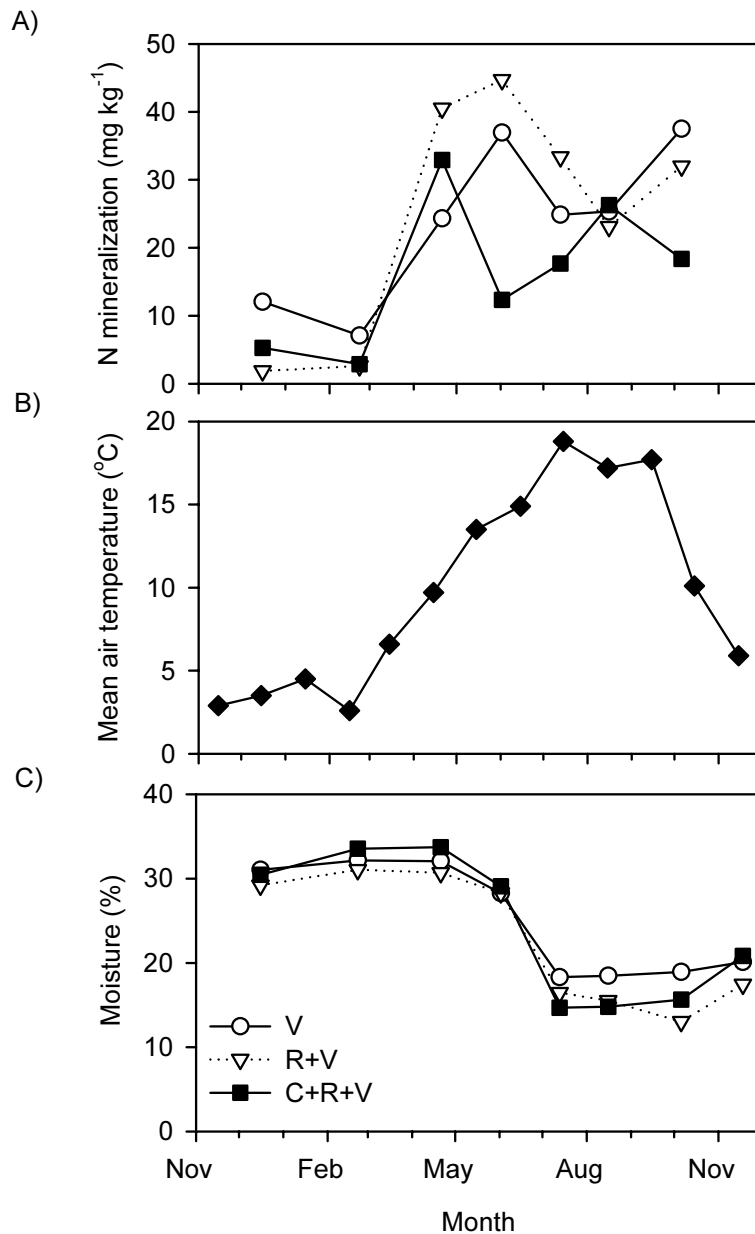
A)

B)

C)

Figure 5.3 A. In situ net nitrogen mineralization (ammonium + nitrate) from October 1998 till November 1999. Nitrogen mineralization is measured at a depth of 0-10 cm.
B. Daily mean air temperature (°C) in Twenthe from November 1998 till November 1999 (measured at 1.5 m above ground level, KNMI). The weatherstation Twenthe (52°16'N, 6°54'E) is located approximately 50 km southeast of the study area.
C. Moisture content (%) of the soil in the different grazing treatments.

Table 5.3 Soil characteristics 1998-1999 of the upper soil layer (0-10 cm deep), n= 5 for all parameters. Data are means ± se. Data were tested through a two-way Anova with fixed factor grazing and random factor block. Significant grazing effects (p<0.05) are highlighted, different letters indicate significant differences.

	C+R+V	R+V	V	Grazing		Block	
				$F_{2,8}$	P	$F_{4,8}$	P
N mineralization in mg kg^{-1}							
N-mineralization (NH$_4^+$ + NO$_3^-$ in mg kg^{-1} year^{-1})	120.6^a ± 13.5	185.3^{ab} ± 21.7	189.4^b ± 22.4	4.815	**0.042**	1.172	0.391
NH$_4^+$ -accumulation (mg kg^{-1} year^{-1})	18.5^a ± 14.0	10.5^a ± 6.2	7.1^a ± 14.3	0.141	0.871	0.673	0.629
NO$_3^-$ -accumulation (mg kg^{-1} year^{-1})	102.6^a ± 13.5	174.8^b ± 16.0	182.4^b ± 16.7	11.414	**0.005**	1.656	0.252
Potential N-mineralization (NH$_4^+$ + NO$_3^-$ in mg kg^{-1})	115.2^a ± 11.8	99.5^a ± 19.6	105.0^a ± 35.8	0.348	0.716	0.800	0.558
N pool soil (g kg^{-1})	3.9^a ± 0.5	3.4^a ± 0.5	3.4^a ± 0.4	3.514	0.080	30.316	<0.001
Soil N turnover (g g^{-1} N year^{-1})	0.033^a ± 0.005	0.058^b ± 0.010	0.055^b ± 0.003	5.991	**0.026**	0.921	0.497
Relative nitrification rate (%)	85.7^a ± 10.6	95.5^a ± 2.8	98.9^a ± 7.1	0.882	0.451	0.473	0.755
Other soil properties							
Bulk density (g cm^{-3})*	1.08^a ± 0.027	1.04^a ± 0.020	0.99^a ± 0.018	3.484	0.092	12.836	0.003
Soil moisture outside mineralization tubes (g g^{-1} soil)*	0.024^a ± 0.024	0.023^a ± 0.022	0.025^a ± 0.013	0.967	0.411	7.713	0.006
Soil moisture in mineralization tubes (g g^{-1} soil)*	0.028^a ± 0.023	0.028^a ± 0.024	0.028^a ± 0.017	0.240	0.792	23.484	<0.001
Soil organic matter (g g^{-1} soil)	0.096^a ± 0.009	0.100^a ± 0.089	0.091^a ± 0.014	0.128	0.882	0.184	0.940
pH-KCl	3.9^a ± 0.06	4.0^{ab} ± 0.11	4.3^b ± 0.11	5.768	**0.028**	2.021	0.184

* Bulk density and moisture values were tested over all periods, three-way Anova with grazing fixed, block and period random factor: for bulk density factor period $F_{7,8.6}$=0.819, p=0.596, for soil moisture factor period: in tubes $F_{7,17.0}$=18.226, p<0.001, outside tubes: $F_{7,19.0}$=64.495, p<0.001. Degrees of freedom for bulk density: grazing df=2,6.7, block df=4,6.3; moisture in tubes: grazing df=2,8.0, block df=4,12.3, outside tubes: grazing df=2,10.6, block df=4,8.8.
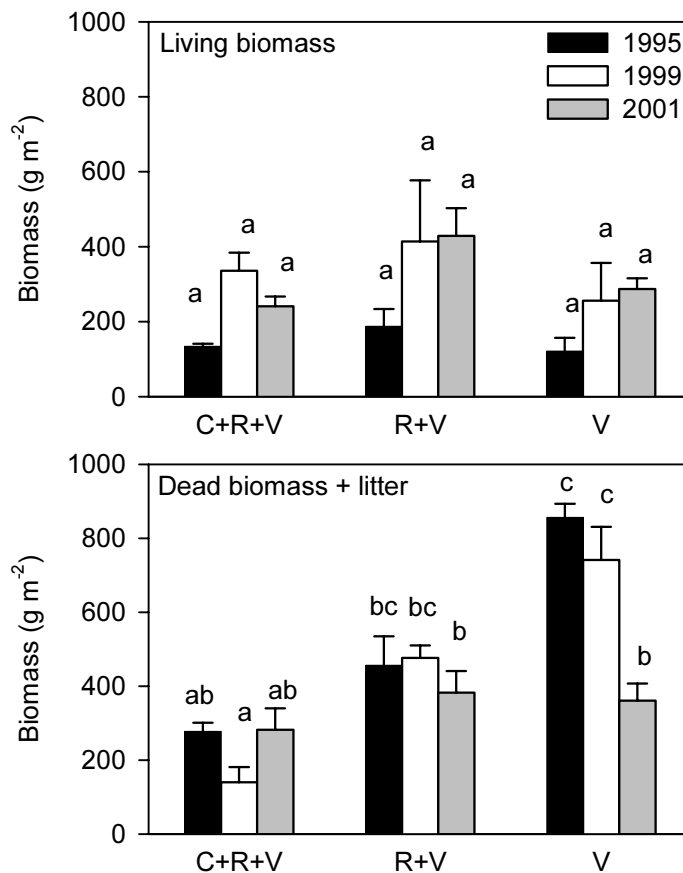
Figure 5.4 Biomass of vegetation harvested in August 1995, 1999 and 2001. Data are means ± se (g dry weight m^{-2}). Living biomass includes all green vegetation, dead + litter is standing dead + the litter layer that has accumulated since the start of the experiment. Different letters indicate statistically different amounts of biomass (p<0.05).

Table 5.4 Biomass of vegetation harvested in August 2001. Data are means ± se (g dry weight m^{-2}). Different letters indicate statistically different amounts of biomass (two-way Anova with grazing as fixed factor, block as random factor).

	C+R+V	R+V	V	Grazing		Block	
				$F_{2,8}$	P	$F_{4,8}$	P
Standing dead	192^a ± 19	146^a ± 31	175^a ± 40	1.234	0.341	2.278	0.150
Standing life	241^a ± 26	429^a ± 74	287^a ± 29	2.880	0.114	0.633	0.653
Graminoids life	228^a ± 29	388^a ± 88	263^a ± 23	0.550	0.597	1.175	0.391
Legumes life	7^a ± 4	0^a	0^a	3.107	0.100	1.000	0.461
Herbs life	13^a ± 4	41^a ± 14	24^a ± 7	3.281	0.091	1.012	0.456
Litter	90^a ± 41	236^a ± 45	186^a ± 34	3.086	0.102	0.894	0.510
Roots	294^a ± 82	143^{ab} ± 64	78^b ± 32	10.555	**0.006**	4.584	0.032
Total standing crop	726^a ± 43	718^a ± 115	540^a ± 42	2.138	0.180	1.327	0.339

Table 5.5 Nitrogen concentration of plant biomass harvested in August 2001. Data are means ± se (mg N g^{-1} dry weight). Different letters indicate statistically different amounts of nitrogen (two-way Anova with grazing as fixed factor, block as random factor).

	C+R+V	R+V	V	Grazing $F_{2,8}$	P	Block $F_{4,8}$	P
Grass life	20.7^a ± 3.1	19.2^a ± 1.3	19.2^a ± 3.1	0.538	0.604	2.871	0.095
Herb life	30.5^a ± 2.4	24.1^a ± 4.3	22.7^a ± 2.9	1.920	0.227	1.879	0.233
Life standing crop	21.3^a ± 1.3	19.5^a ± 1.3	19.5^a ± 3.0	0.660	0.543	2.691	0.109
Standing dead	15.0^a ± 0.5	15.1^a ± 0.5	14.8^a ± 2.0	0.133	0.878	0.894	0.510
Litter	17.0^a ± 1.1	18.3^a ± 1.0	16.9^a ± 2.2	0.854	0.466	2.434	0.144
Roots	10.7^a ± 0.5	14.1^a ± 1.2	13.5^a ± 1.0	4.027	0.062	1.083	0.426

Changes in vegetation composition

The vegetation composition changed during the 7 years of the experiment (fig. 5.5). After exclusion of cows the cover of herbs increased, whereas legumes completely disappeared. A few woody species established in the vole grazed treatment. Large year-to-year fluctuations occurred in the V treatment in the cover of graminoids and herbs. After 7 years no statistical differences in biomass were found between the grazing treatment for the different functional groups (tab. 5.4).

Nitrogen concentration in faeces

Nitrogen concentration was highest in rabbit droppings, cattle and vole faeces had a similar nitrogen concentration (tab. 5.6).

Table 5.6 Nitrogen concentration (mg kg^{-1}), weight of droppings and nitrogen return via faeces (g ha^{-1} day^{-1}) in 1999. Data are means ± se. Nitrogen concentration in droppings of the different herbivores is tested with an One-way Anova, $F_{2,12}$ = 13.904, p=0.001.

	Nitrogen content of faeces (g kg^{-1})	Dry weight of a single dropping[1] (g)	Faeces production[2] (g day^{-1})	Fecal N[3] (g ha^{-1} day^{-1}) C+R+V	R+V	V
Cow	21.95^a ± 0.73	220	2200	19	0	0
Rabbit	32.42^b ± 1.73	0.0943	37.72	23	13	0
Vole	25.00^a ± 1.65	0.00318	5	0	111	130
Total				42	124	130

[1] Weight of cattle dung estimated, fresh weight ranges from 1.5 to 2.7 kg (Haynes & Williams 1993), percentage dry weight is 13% (E. S. Bakker unpubl. results). Weight of rabbit and vole droppings is determined by weighing 20 droppings.

[2] Faeces production for cows is estimated at 10 dung pats per day (e.g. Haynes & Williams 1993). For rabbits dropping rate is estimated at 400 droppings per day (e.g. Lockley 1962). For voles we estimated pellet production at 5 g per vole per day. This value is based on literature values. Pastor et al. (1996) used a value of 7g pellets per animal per day for *Clethrionomys gapperi* faeces production. Young Owl & Batzli (1998) report digestible intake for *Microtus pennsylvanicus* and *Microtus ochrogaster* fed on a grass diet of approximately 0.3 g g body mass^{-1} (average of both species). Since digestibility is roughly 50% (Young Owl & Batzli 1998), excretion of dry matter is also 0.3 g gbm^{-1} day^{-1}, yielding 5.1 g pellets per vole of 17 g per day.

[3] Fecal N is calculated by multiplying animal density (tab. 5.2) with faeces production and nutrient content of faeces
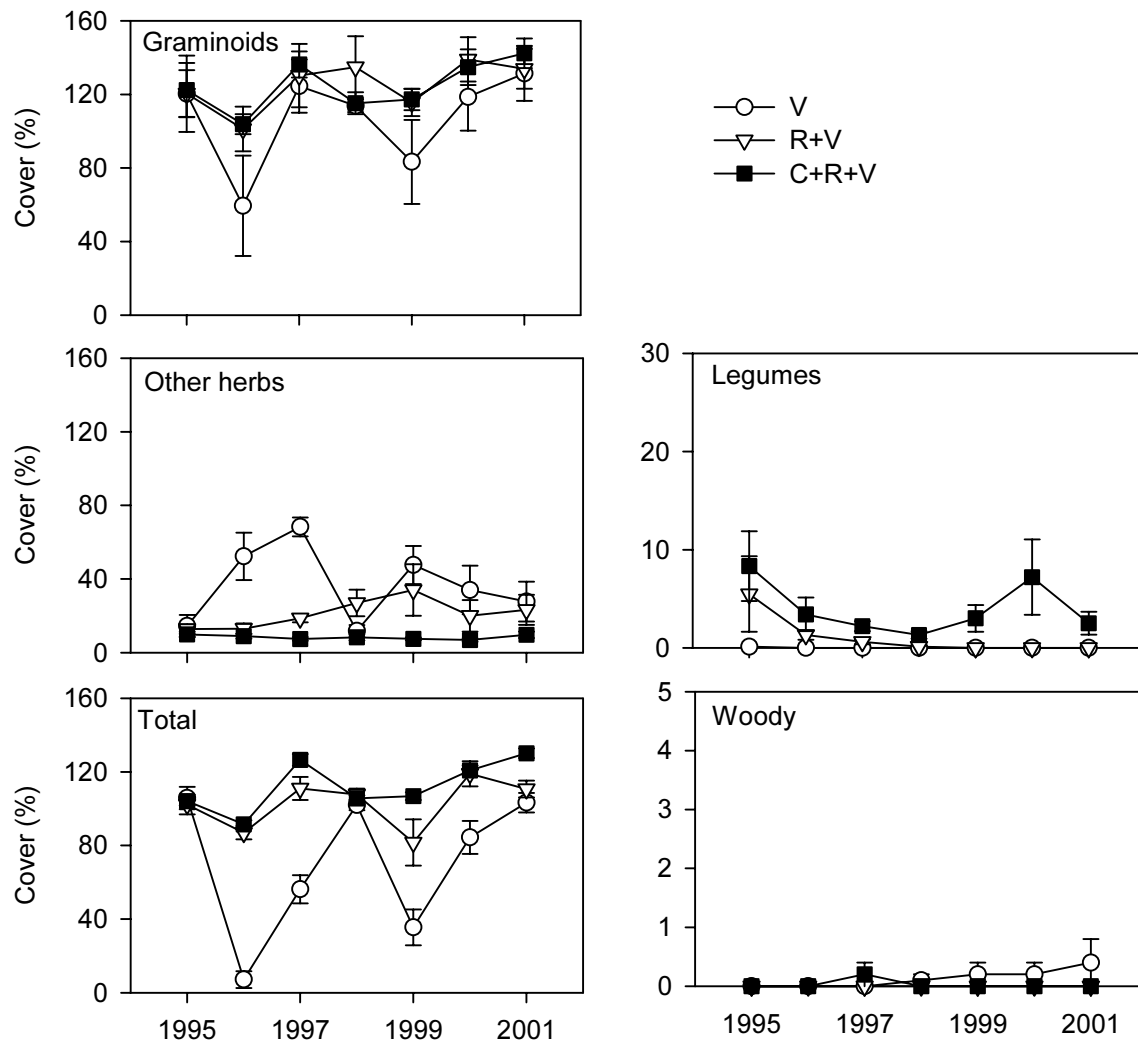
Figure 5.5 Abundance of functional groups in the grazing treatments as percentage cover from 1995-2001. Data are means ± se

Discussion

Strong compensatory effects were observed in the artificial grazing treatments: exclusion of cattle lead to an increase in vole numbers in such a density that the total amount of energy spent remained constant. Energy expenditure given in figure 5.2 gives only a rough indication of actual consumption on a daily basis, since digestibility of the vegetation for the different herbivores depends on body size as well (Demment & Van Soest 1985). The density of all herbivore species is likely to be underestimated; the number of rabbits and voles are minimum estimates (see methods) whereas cattle density is given for the whole study area and will be slightly higher for the plots surrounding the exclosures (E.S. Bakker, pers. obs.). More trapping sessions during the year are required to express the measured vole densities on a yearly basis, since peak density only lasts for a few months. The

measured vole densities of approximately 1000 individuals per hectare at the study site are very high (note that these are absolute minimum estimates). Other studies report effects of vole grazing on grassland vegetation at densities of 395 individuals ha^{-1} (Batzli & Pitelka 1970), 50 individuals ha^{-1} (Sirotnak & Huntly 2000) or 500 individuals ha^{-1} (Leutert 1983). Voles are true grazers, since they consume mainly green plant material supplemented with seeds in summer (Batzli & Pitelka 1971; Leutert 1983)(E.S. Bakker, unpubl. results). Therefore we conclude that at peak density, voles are likely to exert a considerable grazing pressure at the vegetation, that is in the same order of magnitude as cattle grazing pressure. Except for a few individuals in 1998, voles did not enter the C+R+V treatment, although they had free access to it (tab. 5.2). Rabbits on the other hand, were facilitated by cattle. Most measured parameters show intermediate values for the R+V treatment, or values similar to the V treatment. Therefore we focus the discussion on a comparison between cattle + rabbit grazing (C+R+V treatment) versus vole grazing (V treatment).

The created herbivore assemblages had a pronounced different effect on nitrogen mineralization rates. Net annual in situ mineralization was 1.5 times as much in the V and R+V grazed treatment compared to the C+R+V grazed treatment (tab. 5.3). This difference is most pronounced in the accumulation of nitrate in the treatments without cattle. Turnover of nitrogen was significantly lower in the treatment with cattle grazing (tab. 5.3). The onset of increased mineralization in spring follows the increase in air (and presumably soil) temperature accurately in all grazing treatments (fig. 5.3ab). The major difference in mineralization rate is found in early summer and autumn. Both the treatments with only voles or rabbits showed a prolonged mineralization season with a peak in October, whereas mineralization had decreased by then in cattle grazed treatment (fig. 5.3a). By contrast, potential mineralization rates from samples taken in October (1998) and measured under lab conditions were equal for the three grazing treatments. Soil moisture was the same in all grazing treatments measured over the whole year and showed no relation with mineralization rates (fig. 5.3), whereas bulk density was slightly higher under large herbivore grazing, although not significantly ($p=0.09$). The total soil nitrogen pool seemed slightly higher in the C+R+V treatment, although the difference was not significant ($p=0.08$). Summarizing these results we conclude that the measured soil properties could not be responsible for the differences in annual net nitrogen mineralization in the field.

The major factor that was standardized during the potential mineralization measurements was temperature, a physical soil property that we did not measure in the field. We hypothesize that this parameter may have been responsible for (part of) the differences in mineralization rates between the grazing treatments in the field. Soil temperature is an important parameter influencing mineralization rates (Sierra 1997; Wan et al. 2002). Soil temperature in the R+V and V grazed treatment could potentially be higher due to accumulated litter that can have an isolation effect both against heating and cooling down. The V and R+V treatment indeed contained more litter than the C+R+V treatment in the year of mineralization measurements (1999, fig. 5.4b), before this effect had disappeared in the last sampling year. Grazing by herbivores generally prevents litter accumulation (Bakker et al. 1983; Bazely & Jefferies 1986; Van Wijnen et al. 1999). However, small rodent populations show

strong fluctuations in population density over time (Batzli & Pitelka 1971; Dijkstra & Zijlstra 1997; Hanski et al. 2001) resulting in a fluctuating grazing pressure as well. Although vole grazing does reduce litter formation relative to ungrazed vegetation (Sirotnak & Huntly 2000), the irregular grazing pressure by voles will result in more litter formation than grazing by larger herbivores. Also the way voles graze can contribute to litter accumulation. Voles harvest flower stems from which they consume the seeds and discard the stems. The left-overs pile up and form little hay piles. Voles also collect vegetation for nest material or food storage (Leutert 1983).
The increase in litter production in the V and R+V treatments could stimulate mineralization both through improving local climate as well as increasing nutrient input. When leafs die, a short flux of nitrogen is released that is readily available for plants and microbes (Schaffers et al. 1998). Most of the plant litter decomposes slowly and is incorporated in the soil organic matter, where it is slowly decomposed (Parton et al. 1987; Knops et al. 2002).

Small and large grazers differ in food selectivity (Demment & Van Soest 1985) and could therefore have a different impact on vegetation composition (Ritchie & Olff 1999). The vegetation composition differed between the V treatment and the C+R+V treatment (fig. 5.5), but there is strong year-to-year variation in the V treatment. Changes in vegetation composition can induce changes in plant litter quality (Ritchie et al. 1998; Berendse 1998; Olofsson & Oksanen 2002). Olofsson & Oksanen (2002) found that grazing by reindeer promoted growth of species with easily decomposed litter, resulting in a stimulus of nitrogen cycling. Increased nitrogen cycling through overcompensation of the grazed species (Hik & Jefferies 1990) or a shift towards species with higher turnover rates (Olofsson & Oksanen 2002) is found (Frank & McNaughton 1992; Frank & McNaughton 1993) or suggested (De Mazancourt et al. 1998) to be an important mechanism of increased nitrogen cycling under grazing. However, grazing could also lead to selection against a palatable plant species, instead of stimulating its growth (Pastor et al. 1993; Ritchie et al. 1998; Sirotnak & Huntly 2000). Especially plants with a high nitrogen content, such as legumes, are preferred food for herbivores. Ritchie et al. (1998) and Sirotnak & Huntly (2000) showed that after exclusion of herbivores legume abundance increased, resulting in increased litter quality and nitrogen fixation and therefore stimulation of nitrogen cycling. However, in our study legumes reached only 4% cover on average and during the mineralization measurements they were exclusively present in the C+R+V treatment that had the lowest mineralization rates. The different grazing treatments did not result in differences in plant nitrogen content nor in quality of litter. Therefore herbivore-induced changes in vegetation composition could not be the cause of the measured differences in annual net mineralization in this study.

Recycling of nitrogen through dung and urine instead of recalcitrant plant litter is often given as an explanation of increased mineralization rates under grazing (Ruess & McNaughton 1987) since herbivore dung is more easily decomposable than most plant litter through the favorable C/N ratio of dung (Pastor et al. 1993; Hobbs 1996; Frank & Groffman 1998). In table 5.6 we calculated the amount of nitrogen deposited in g dung ha^{-1} day^{-1} for the year 1999 for the different herbivores (see table for

methods). Under the current assumptions, the nitrogen return in the R+V and V grazed treatment is about 3 times higher than in the C+R+V treatment during peak densities of voles (tab. 5.6). This flux of nitrogen through vole excreta could be an explanation for the observed peak in mineralization rate in autumn (fig. 5.3a), that coincides with a peak in vole abundance. It depends on the distribution and size of faeces how fast faecal nitrogen becomes available (Williams & Haynes 1995; Pastor et al. 1996). The herbivore species distribute their dung in different ways: cattle dung pats include large amounts of nitrogen concentrated on a small spot, whereas rabbits place their droppings dispersed during foraging or in latrines and voles deposit small heaps of a few clustered droppings mainly in runways or feeding stations scattered through the vegetation (Williams & Haynes 1995). Pastor et al. (1996) showed that nitrogen from vole droppings was much faster mineralized than from the same amount of moose droppings. The smaller the pellet, the more rapidly it disintegrates and the faster nitrogen becomes available for plants again. Also, rabbit pellets, but especially vole faeces, are expected to redistribute nitrogen at a fine scale, where most of it is easily available at the individual plant level. Nitrogen from cattle dung can leach (Haynes & Williams 1993), the vegetation below it can die and much of the nitrogen can accumulate for months or years in the soil under a dung pat before being used by the vegetation (Williams & Haynes 1995). Cattle grazing may result in net nutrient removal in most areas, since nutrients are returned on a small fraction of the total area (Afzal & Adams 1992). Rabbit pellets are shown to locally fertilize the vegetation via latrines (Willott et al. 2000), but little is known of the effects of pellets that are scattered through the vegetation during foraging. For smaller herbivores even less is known of the effect of the spatial redistribution of nitrogen through faeces on nutrient cycling. First calculations by Pastor et al. (1996) and in this study show that small herbivores can potentially have a large impact on nitrogen return via faeces.

Summarizing, the fact that herbivores create a shortcut in the nitrogen cycle, does not mean that enhancement of the nitrogen cycle is necessarily the net outcome of the complex interaction of processes involved in nitrogen cycling (fig. 5.1). We hypothesized that the relative importance of mechanisms influencing nitrogen cycling under grazing depends on habitat type and herbivore species. This study shows that different-sized herbivores have different effects on nitrogen cycling under nutrient rich conditions. Explanations for these differences can be improved physical soil conditions (i.e. soil temperature) due to litter accumulation under vole grazing. Also the difference in pattern in nitrogen return can contribute to the differences: cattle remove nitrogen in most patches, and return it in high concentrations in small spots. In the vole grazed treatment nitrogen is returned via litter and vole excreta that both occur in a more evenly distributed spatial pattern, but more concentrated in time.

Acknowledgements

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Chapter 6

Experimental manipulation of food quality and apparent predation risk in the European rabbit: bottom-up versus top-down control in a central-place foraging herbivore

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Abstract

We investigated the relative importance of predation risk and food quality on the timing and spatial location of grazing by a central-place foraging herbivore: the European rabbit. Dropping counts and vegetation measurements revealed that grazing intensity by rabbits declined with increasing distance from their burrows under thorny shrubs into the meadow, thereby creating a gradient in increasing vegetation height, biomass and decreasing plant nutrient content. When nitrogen content of the vegetation was experimentally increased with 150% through fertilizing and mowing, rabbits visited these plots four times more frequent than the untreated control plots. Rabbits preferred fertilized plots more strongly with increasing distance from burrows. Addition of predator scent in the form of small amounts of mink pellets (to increase their apparent predation risk by their natural mustelid predator in the area, the polecat) did not result in different total visitation rates by the rabbits. The combined addition of fertilizer and mink pellets yielded the same results as addition of fertilizer alone. However, the mink pellets did tremendously change the timing of the visitation of rabbits to the plots, as measured with infrared detectors. Rabbits were predominantly nocturnal but increased their activities during the day at the expense of night activities when mink pellets were added, resulting in equal activities during night and day. This shift in activity pattern may be an adaptation to increased perceived predation risk by mustelid predators, which are mainly nocturnal. Instead of accepting more risk (forage further away) in situations with higher expected returns, the rabbits coped with the increased risk by shifting their timing of foraging. We conclude that rabbits are sensitive to perceived predation risk, but that this does not influence the average spatial distribution of their grazing pressure. The observed natural rabbit grazing gradient can to be explained by intraspecific facilitation. Food quality was highest close to the burrows, therefore rabbits selecting for high quality food should forage most intensely close to the burrows and only move further away for higher quality items or when the vegetation close to their burrows is depleted. Through intensive grazing close to the burrows rabbits facilitated for themselves either through stimulating fresh protein rich re-growth or return of nutrients through faeces or both.

Introduction

Patch selection by feeding herbivores has been explained through optimal foraging rules, which predict that herbivores forage there where they experience the highest energy return per time unit (Charnov 1976; Stephens and Krebs 1986). In the absence of mortality costs of patch selection, this foraging strategy is expected to maximize fitness. The risk of predation imposes an additional constraint on patch selection (Lima and Dill 1990) and affects which patch selection strategy will lead to the highest fitness. Perceived risk of predation has been shown to affect a range of foraging decisions, such as the distance from cover that animals travel to forage (Anderson 1985; Holmes 1991; Banks et al. 1999), group size (Cassini 1991), foraging selectivity (Lima and Valone 1986) and timing of foraging (Kotler et al. 1991; Jacob et al. 2000). Species that are sensitive to predation often create their own save haven, such as burrow system (e.g., rabbits, voles) or a nest (e.g. beaver). This causes these species to forage from a central-place, which causes further restrictions to patch selection, as the animal has to return after each foraging bout to the same location. Central-place foragers are predicted to first deplete nearby patches before exploiting patches further away (Orians and Pearson 1978; Schoener 1979) From the perspective of optimal foraging theory, the animals should select more distant patches until the travel costs start exceeding the energy returns (Fryxell 1999). However, moving further away will also impose a higher (perceived) predation risk, which may put an additional limit to their foraging range. Central-place foraging herbivores are mostly small to medium sized herbivores that live in burrows or in between rocks. To feed, these herbivores have to move out of their burrows and venture into the surrounding grasslands. Gradients in foraging intensity, as expected from the previously outlined theory, has so far been observed in pikas (Huntly et al. 1986), collared pikas (Holmes 1991), cavies (Cassini 1991) and rock hyraxes (Kotler et al. 1999): they graze mostly close to burrows or cover and gradually less further away. This decline in foraging intensity with increasing distance from burrows has been found to coincide with changes in plant community composition and species richness for European rabbits (Zeevalking and Fresco 1977) and pikas (Huntly 1987). Although the maximization of energy return, and the balancing of predation risk have both been suggested to explain spatial decisions of herbivorous central-place foragers, the relative importance and interaction of these two factors is still unclear. Most studies are descriptive; only few studies try to address this issue experimentally (Holmes 1991; Kotler et al. 1999).

We studied European rabbits as a good example of a social central-place foraging herbivore. We tested the interplay between perceived predation risk and food quality through manipulating both factors separately and in concert in an experimental set-up. We had the following expectations on the interplay of predation risk and food quality: if predation risk is a dominant factor than increasing the perceived predation risk will cause rabbits to stay closer to their burrows (avoid high-risk areas). If food quality is the dominant factor explaining their spatial distribution, than experimental increase in food quality will increase rabbit visits to the plots, even if these are further

away. If food returns and predation risk are jointly evaluated by the animals, then rabbits will still visit high food quality patches that are far away even under high predation risk ('worth the risk') while poorer food quality patches that are far away will be abandoned under conditions of increased predation risk. We compare the results from the experiment with rabbit grazing behaviour on control plots to see whether the outcome of the experiment explains natural grazing behaviour of rabbits. We manipulated predation risk through applying predator scent, using mink faeces. We manipulated food quality through applying fertilizer and mowing vegetation.

Methods

Study site

The study was performed on Junner Koeland, a 100 ha grassland reserve in the North-East of The Netherlands. The site is owned by the National Forest Service (Staatsbosbeheer) and managed as a nature reserve. The site is grazed by cattle at a density of 1 heifer graze at 2.5 ha from April through October. Naturally occurring vertebrate herbivores are European rabbits (*Oryctolagus caniculus*) and common voles (*Microtus arvalis*). In the study area natural predators of rabbits are polecat, fox and several birds of prey, mainly buzzard (*Buteo buteo*) and goshawk (*Accipiter gentilis*). The reserve includes 50 ha of floodplain grassland, where the experiment was performed. We selected three locations where clear signs of inhabited burrows were present. In each site rabbit burrows were located on the edge of shrubs (*Prunus spinosa)* and grassland. Rabbits mostly had to move out of their burrows into the meadow for foraging since the soil under the shrubs and woodlands was mostly bare. We made sure that the nearest inhabited burrows in the meadow or on the edge of another cluster of shrubs were at least >100 m away so interference with the foraging gradient would be minimal. Each site had their own local rabbit groups, two sites were 300m apart but separated by continuous woodland, the third site was located at 800m distance on the other side of an old oxbow of the river Overijsselse Vecht. Dominant plant species in the grasslands were the grasses *Festuca rubra*, *Agrostis capillaris*, *Holcus lanatus* and the herb *Rumex acetosa*.

Experimental design

On these natural grazing gradients we manipulated predation risk and food quality. Food quality was manipulated through mowing and fertilizing the vegetation, perceived predation risk was manipulated by applying predator scent on the vegetation. On each site we established four transects of 40m long starting from the rabbit burrows and running into a meadow. On each transect we placed 5 plots of 3 x 3 m wide at regular intervals, the exact distance was measured for each plot and plots were assigned numbers 1 (closest to burrows) through 5 (furthest) (fig. 6.1). On these plots we applied four different treatments: (i) improved food quality (NPK-fertilizer and mowing), (ii) applied predator scent, (iii) the combination of improved food quality and predator scent and (iv) an untreated control. We will refer to the treatments as (i) food, (ii) predation, (iii) food + predation and (iv) control. Each

treatment was applied on each distance along a transect. To minimise the effect of the predator scent on the control plots and the plots in which we only improved food quality, the four different treatments were divided into two main groups: control and food in one group and predation and food + predation in the other group. The distance between the two groups was kept at about 30m. The groups were randomly assigned to a set of transects. The experimental treatments were randomly assigned to the plots within these two groups at each distance (fig. 6.1). The three selected sites within the study area each represented one replicate of this design (blocks).
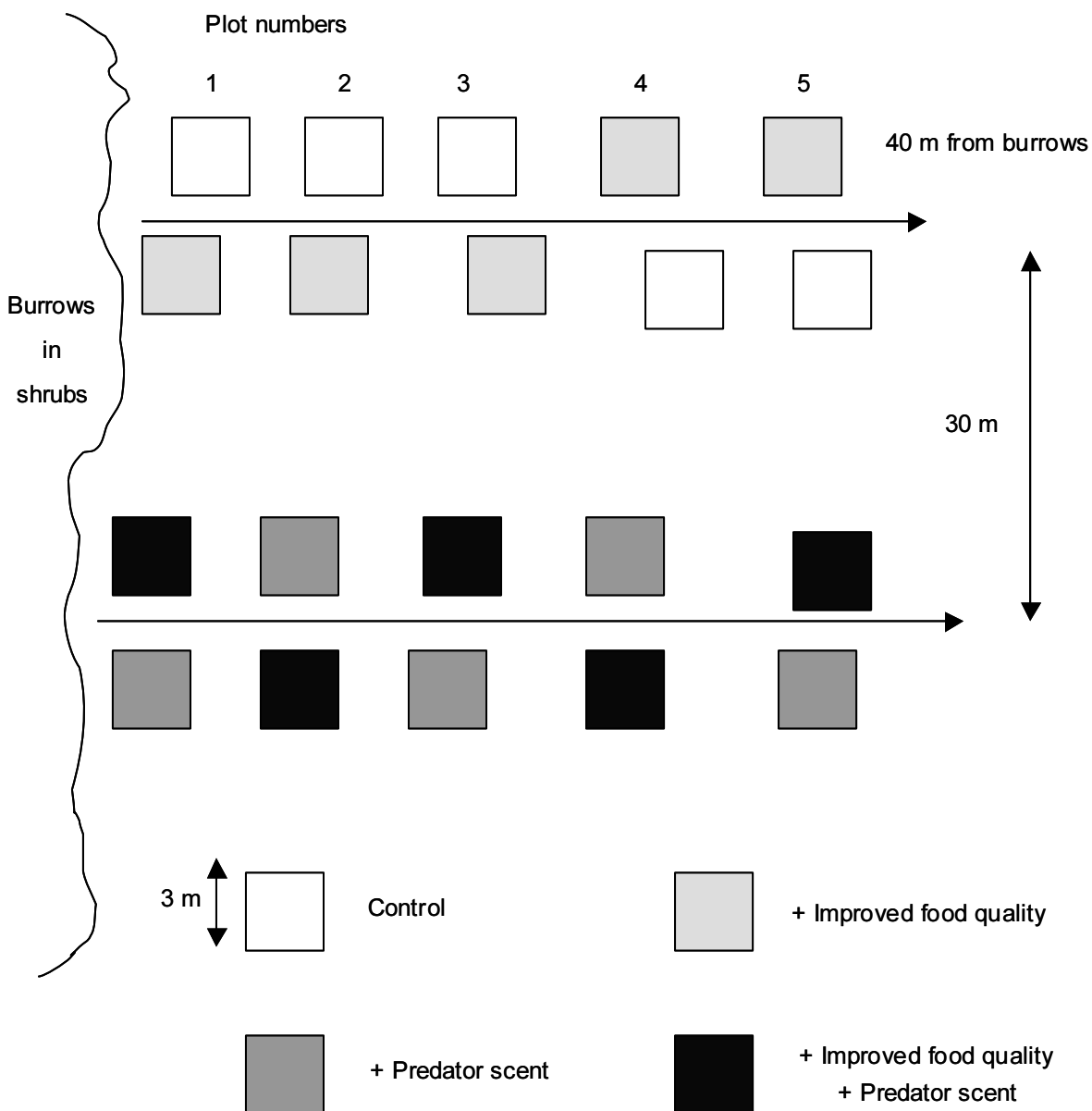
Figure 6.1 Design of the experiment. This design was replicated at three different sites.

The plots were established on 28 June 2001. By then all plots were cleared from rabbit pellets that were counted when removed. Also vegetation height was measured at 16 points in a regular grid in all plots. Both measurements were used to check whether there were initial differences between the transects before the experimental treatments were applied. The experiment ran for 12 weeks and ended on 23 September 2001.

Experimental treatments

Pellets of mink were used to increase perceived predation risk for the rabbits. We used mink pellets because it is a mustelid just as polecat and it is easy to collect a large amount of their pellets. We obtained fresh pellets from a commercial mink farm. The pellets were distributed over the plots by placing one spoon full in every corner (50 cm from the edges of the plot) and one in the middle of the plot. Fresh pellets were applied every two weeks. The small and local amount added avoided any nutrient addition effects of the added predator scat.

Manipulation of the food quality consisted of mowing the vegetation in combination with applying a fertilizer. Mowing results in fresh re-growth of the vegetation that has a high nitrogen content. Fertilizing with nitrogen, phosphorus and potassium enhances this effect. The plots were mown at the start of the experiment in late June to a height of about 5 cm. Fertilizer was applied as slow-release grains in late June after mowing, at half of July and half of August. This added up to 15 g N m^{-2}, 12.5 g P m^{-2} and 25 g K m^{-2} in total.

Measuring rabbit visitation of the plots

Rabbit visitation of the plots was measured in two ways: via an indirect and a direct method.

We used rabbit dropping counts as an indirect measure of rabbit presence (Taylor and Williams 1956). Rabbit pellets were counted and removed every two weeks in all plots. Latrines were counted separately, since these serve as a territory mark, whereas pellets scattered over the plots are more likely to represent foraging time rabbits may have spent on the plots. Here we present the dropping data excluding the latrine pellets from the data for analysis. However, we performed all tests both with and without the latrine droppings and it did not make a difference in level of significance in any test, with the exception of the relation with the infrared records (see below and in results). The relation between the amount of droppings and rabbit grazing pressure was measured through counting the amount of grazed leaves in late July. Leaves were counted along a 1m stick that was randomly placed in a plot. At every 5 cm for the nearest leaf to the stick was recorded whether it was grazed. This was repeated by replacing the stick five times until a hundred leaves were scored. Only the plots that were not mown and fertilized were included in the measurements. The amount of grazed leaves was compared with the amount of droppings counted in the plots just before the leaf measurements.

For the direct method we used infrared movement detectors (Trailmaster TM550, Goodson & Associates, Inc., Lenexa, KS, USA) to record the presence of rabbits in a plot. This equipment consists of two small devices, a transmitter and a receiver. The

receiver counts the times the infrared beam is interrupted and thus counts every interruption of the beam as an event. We placed the beamer and receiver on wooden stakes at two opposite corners of a 3 x 3 m plot. The infrared beam was at a height of 20 cm above soil level and formed a diagonal line over the plot. The rabbits that passed the diagonal were counted, which gives an impression of the frequency of rabbit visitation of the plots. The equipment also registers the time of interruption of the beam and therefore it also recorded information on the activity pattern of the rabbits. We established 20 plots (four treatments times five distances) at each of our three sites and used 20 infrared devices available. We monitored all plots in one site simultaneously and switched all equipment to another site at two-week intervals. The experiment lasted for 12 weeks; in this time each site was measured twice with 4 weeks in between measurements at each site. To make sure that the equipment counted rabbits and not the cows that grazed the area, the area with the infrared equipment was surrounded by an electric fence (a wire at 1m height) to keep the cows out without affecting the entrance of the rabbits. The fence was moved with the infrared equipment, therefore plots were not longer than two weeks in a row excluded from cattle grazing. We assumed that most of the counts were rabbits since there are not many other larger animals present, only a few foxes and an erratic polecat or roe deer. Observations during two evenings starting before dusk and continuing into darkness with night vision binoculars revealed that the plots were frequently visited by rabbits and once by a kestrel that was perching on the stakes that marked the corners of the plots.

The sensitivity of the equipment was set that the infrared beam had to be interrupted for 0.25 second before it registered an event. The receiver could register up to 1000 events. The higher readings usually were in the range of several hundred observations (<500) in two weeks. Occasionally the receiver had registered 1000 observations over a very short period i.e. a few hours. It could be due to an insect that sat on the receiver or sometimes a flowering stalk had grown in front of the devices, which are sensitive to a combination of infrared and movement. We treated such an observation as an outlier, since we could not check whether it had been a rabbit or not and it greatly affected the spread of the data. As a consequence 5 of the 120 observation bouts (20 devices times 6 time periods) were removed and during the last observation period a set of batteries gave out, causing another 7 plots to be excluded from the analysis (see data analysis). Most of these plots did have proper observations during one of the two measurement periods for each site and missing values were located at different sites, distances and treatments.

Vegetation measurements

At the end of July vegetation samples were taken in all plots by clipping a strip of 1 by 0.1 m to soil level. The biomass was sorted in the categories living grasses, herbs and dead material. The biomass was dried at 70°C, weighed and the living grasses were grinded for analysis of total nitrogen, phosphorus and potassium content. Samples were digested with sulphuric acid, selenium and salicylic acid. The nitrogen and phosphorus concentrations were measured colometrically using a continuous

flow analyser (SKALAR). Potassium was measured by means of atomic absorption spectrometry.

Data analysis

We used the cumulative amount of rabbit droppings over all counting periods for each plot as a response variable, as we were not interested in the temporal variability within the observation period. For the analysis of treatment effects, we summed the number of droppings for all five plots in a transect, since we expected a gradient in droppings from the burrows that would cause large variation in the numbers per transect. For the infrared detectors we could not use the cumulative number of observations due to missing values (see above). Therefore we first averaged the data per plot over the two measuring periods and then averaged the values within a transect before further calculation. No distance gradient could be detected in the recorder data (see results); therefore this method could be used.

To test for treatment effects, we applied a ln-transformation on the data to obtain homogeneity of variance for all data and performed a one-way Anova followed by a posthoc Tukey-test, with n=3 (the sites). The effect of distance to burrows on any parameter was tested using the measured distances for each plot in a logarithmic regression with non-transformed data. All plots at the same position from the burrows, for example the plots number 1, were on a slightly different actual distance. If we had to compare plots within one position, for example ratio of droppings found in improved food versus control plots at different distances, we used the plot number as a measure for actual distance.

We assigned the time records that we collected with the infrared equipment to a day and night period to make it possible to compare the day-night patterns between the different treatments. We treated the period from 06:00 until 21:00 as a day of 15 hours and the other 9 hours as night. True light and dark times shifted during the research period, with sunrise at 05:20 and 07:25 and sunset at 22:04 and 19:37 on 28 June and 22 September respectively. The choice to work with standard day-night times enabled us to calculate expected values when the rabbits would visit the plots randomly during day and night.

Results

Experimental manipulation

The amount of rabbit droppings removed from the plots before the experiment started revealed that the transects were equally visited by rabbits before the treatments were applied (total number of droppings per treatment: One-way Anova, $F_{3,8}=0.149$, $p=0.928$, n=3). There was a strong negative relation between the distance from the burrows and the amount of droppings found (logarithmic regression: $r^2=0.34$, $p<0.001$, n=60). This same gradient was present in the height of the vegetation that became significantly higher with distance (logarithmic regression $r^2=0.30$, $p<0.001$, n=60). Vegetation height increased from 7 cm (± 1 se) closest to the burrows to 14 cm (± 1 se) high on average at the farthest distance. The height of the vegetation

was also not different between the treatments at the start of the experiment ($F_{3,8}$= 0.394, p=0.761, n=3).

The application of fertilizer and mowing resulted in a 150% increase in nutrient content of the vegetation (fig. 6.2a, for nitrogen: $F_{3,8}$=179.404, p<0.001, n=3, phosphor and potassium did yield the same results). Nitrogen concentration in the fertilized plants was about 3.5%, which is just slightly more than the highest values of nitrogen concentration in the unfertilized vegetation close to the burrows (3.1%, fig. 6.2b). The plant nitrogen content dropped with distance from the burrows in the unfertilized gradients (fig. 6.2b, r^2=0.22, p=0.009, n=30) and showed the same trend in the fertilized plots, although this was just not significant (r^2=0.13, p=0.051, n=30). Biomass increased with distance from the burrows in the unfertilized plots from 106 (± 12 se) to 292 (± 49) g dry weight m^{-2} (r^2=0.26, p=0.004, n=30), whereas it showed no trend with distance in the mown and fertilized plots changing from 108.0 (± 32.7) to 162 (± 25) g dry weight m^{-2} (r^2=0.03, p=0.315, n=30).
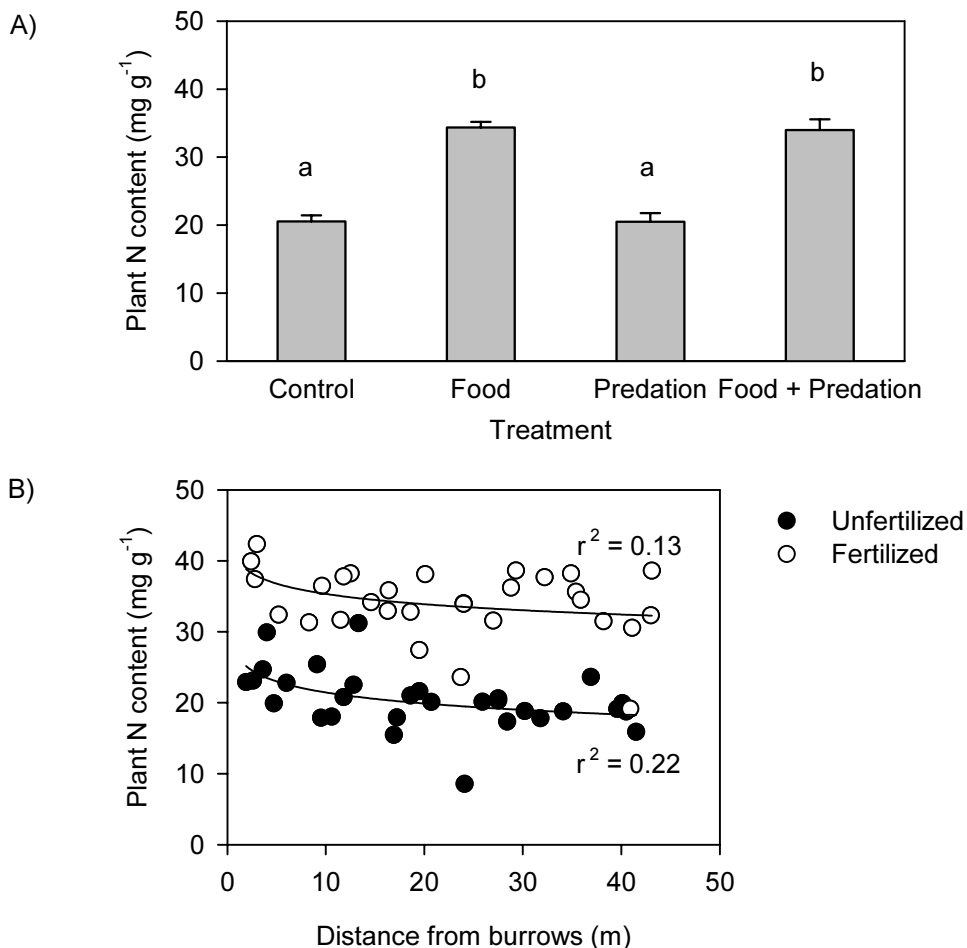
Figure 6.2 A. Effect of fertilization (food) and application of mink pellets (predation) on the nitrogen content of grass life biomass. Different letters indicate statistically different concentrations of nitrogen (n=3) (p<0.05).
B. The relation between distance from the rabbits burrows and plant nitrogen content in the unfertilized (control and predation) and fertilized (food and food + predation) plots. A logarithmic regression revealed a significant decrease in nitrogen content in the unfertilized plots (p=0.009) and the same trend for the fertilized plots, but just not significant (p=0.051).

Rabbit visitation

About four times as many rabbit droppings were found on the fertilized plots compared to the unfertilized (fig. 6.3a, One-way Anova $F_{3,8}$=9.172, p=0.006, n=3). The application of mink pellets had no effect on the number of droppings found in the plots. With increasing distance from the burrows, fewer droppings were found in the control plots (fig. 6.3b, logarithmic regression, r^2=0.558, F_{13}=16.26, p=0.001). In the other three treatments distance had no effect on the amount of droppings (p>0.1). The preference of rabbits for the improved food plots increased significantly with distance from the burrows (tab. 6.1). This relation was also found in the improved food + predation plots. Rabbits showed no increasing preference or dislike for the predation plots with increasing distance (tab. 6.1). There was no relation between the amount of grazed leaves and distance from burrows (r^2=0.09, p=0.116, n=30). The amount of droppings counted in the plots in the period just before the time of measurement of the grazed leaves was positively correlated with the amount of grazed leaves (r=0.39, p=0.035, n=30).
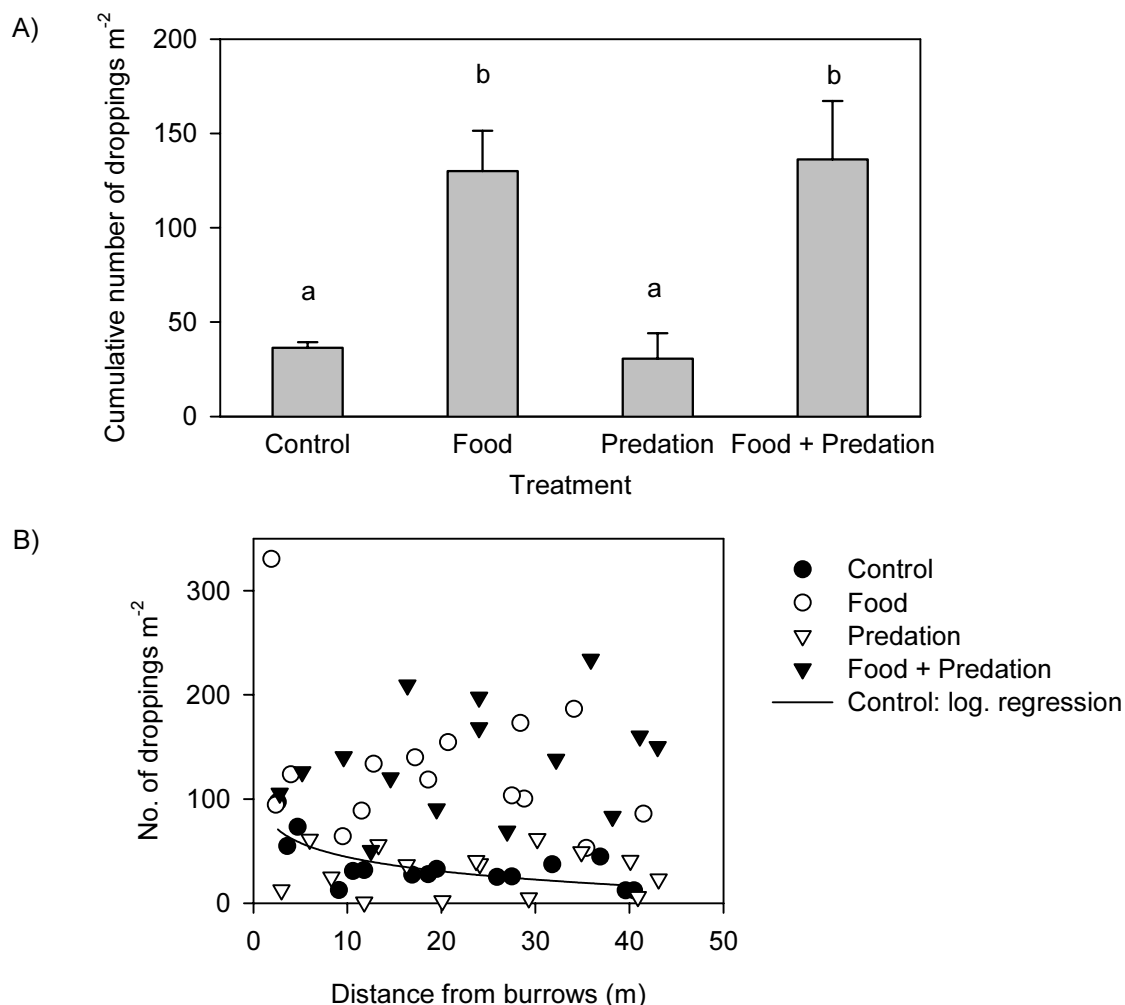
Figure 6.3 A. Effect of fertilization (food) and application of mink pellets (predation) on the cumulative amount of droppings collected from the plots. Different letters indicate statistically different amounts of droppings (n=3)
B. The relation between distance from rabbits burrows and recorded total amount of droppings found on the plots. The relation between distance and amount of droppings was tested for each treatment and was only significant (p<0.05) for the control treatment (logarithmic regression, r^2=0.56, p=0.001).

Table 6.1. The preference of rabbits for treatments relative to the control as a function of the distance from the burrows. Preference for each treatment is calculated at each distance as the percentage of droppings found in the treatment plot relative to the total amount of droppings found in the treatment + adjacent control plot. A value of more than 50% indicates a preference for the treatment over the control. A linear regression was performed on these preference percentages with plot number as a measure for distance as the independent variable. A significant relationship indicates an increasing preference for the treatment plot with increasing distance (all relations were positive).

Test variable relative to the Control treatment	R^2	$F_{1,13}$	P
Preference for Food plots	0.43	9.779	0.008
Preference for Predation plots	0.08	1.130	0.307
Preference for Food + Predation plots	0.276	4.950	0.044

A)

B)
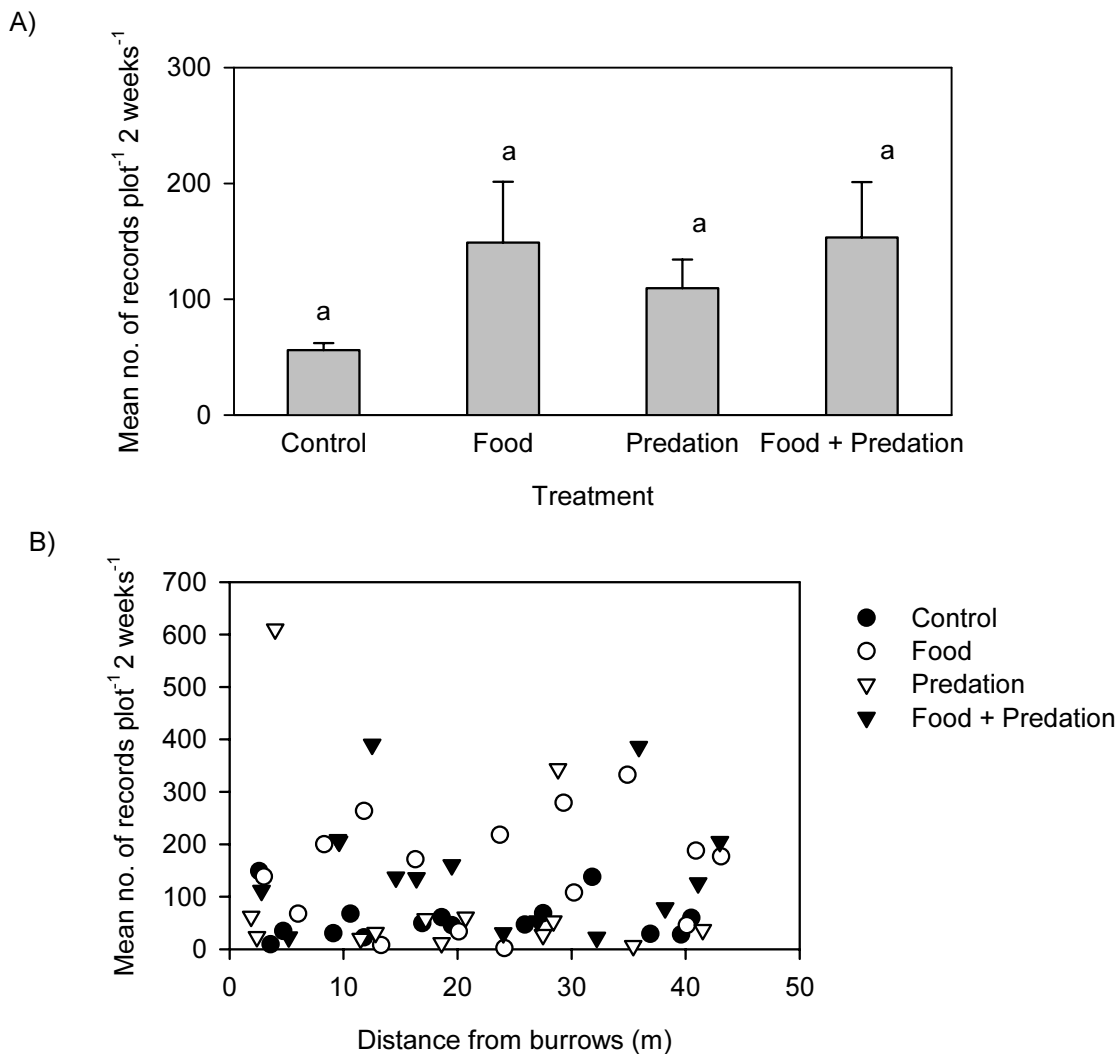
Figure 6.4 A. Number of records of rabbit activity in the different treatments. Data represent the mean (+ se) of records plot^{-1} two week period^{-1} (n=3). The differences between the treatments were not significant.
B. The relationship between the distance from the burrows and the number of records in the different treatments. The relationships were not significant.

The infrared detectors also revealed an increased visitation of the improved food plots and slightly more on the predation plots (fig. 6.4a), but the differences were not significant due to large variation in the data ($F_{3,8}=1.512$, $p=0.284$, $n=3$). Theoretically the effect of the mink pellets could be transient, being strongest shortly after application and then fading away either because of a reduced smell or habituation by the rabbits. However, a separate test of the treatment effect during the first 30 hours after application of the mink pellets did also show no significant differences between the treatments ($F_{3,8}=0.478$, $p=0.707$, $n=3$). There was no relation between the number of records and the distance from burrows for any of the treatments (fig. 6.4b, $p>0.3$ in all cases). The number of records in each observation period was positively related to the number of droppings counted in the plots in that same period (Pearson correlation $r=0.23$, $p=0.015$, $n=108$). In this case including the latrine droppings resulted in a tighter relation with the amount of recordings (Pearson correlation $r=0.26$, $p=0.006$, $n=108$).

Rabbits were mainly active during the night in the control plots with peaks at dawn and dusk (fig. 6.5). Fertilization resulted in a steep increase in the visits at night, whereas the number of daytime visits remained similar (tab. 6.2). On the plots where the mink pellets were added no pattern in timing of visits could be detected (fig. 6.5). Rabbits seemed to visit the plots equally often at each hour of the day or night. On the plots that were fertilized, but also had received mink pellets, the rabbits made an equal amount of visits to the plots during the day, but the amount of visits at night increased steeply. Mowing and fertilization resulted in a significant increase in nighttime foraging by the rabbits relative to the control (tab. 6.2). However,

Table 6.2 The distribution of the records over day and night expressed as percentage of the total number of records collected per treatment (n). Daytime was defined as the time from 06:00 until 21:00 (15 hours); the remaining 9 hours were defined as night. The actual number of records was used to test whether the records were randomly distributed over the day and night (then 38% of the records should be found at night) using a Chi-square test. Subsequently the amount of day and night records was tested against the distribution of records in the control treatment.

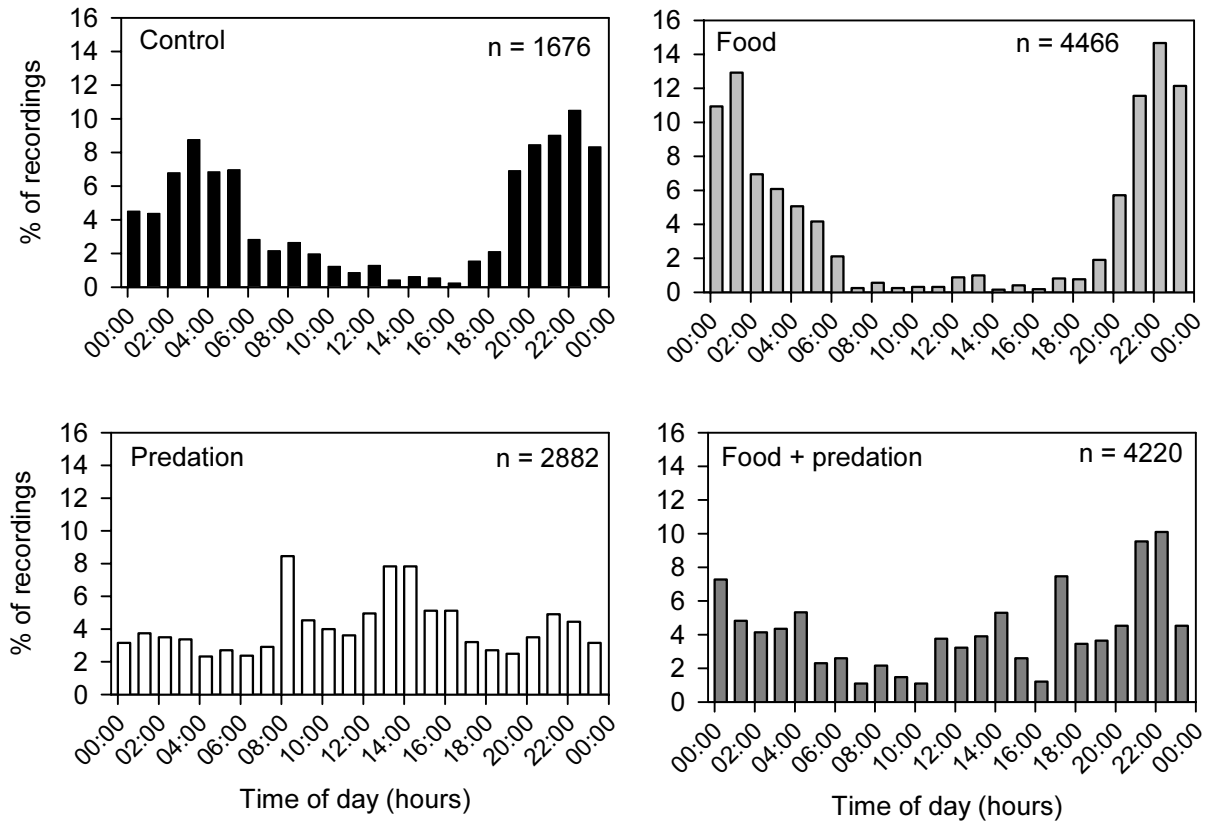|	No. of records (%)		Preference for night or day	P	Difference with Control treatment	P
Treatment	Night	Day	χ^2_1		χ^2_1	
Control (n=1676)	1106 (66%)	570 (34%)	565	<0.001	-	-
Food (n=4466)	3751 (84%)	715 (16%)	4197	<0.001	669	<0.001
Predation (n=2882)	893 (31%)	1989 (69%)	39	<0.001	1296	<0.001
Food + Predation (n=4220)	2194 (52%)	2026 (48%)	321	<0.001	282	<0.001
% Night and day per 24 hours	38%	62%				

Figure 6.5 Activity pattern of rabbits in the different treatments. Data are the number of records per hour expressed as the percentage of the total amount of records in one treatment (n). The data are corrected for missing values.
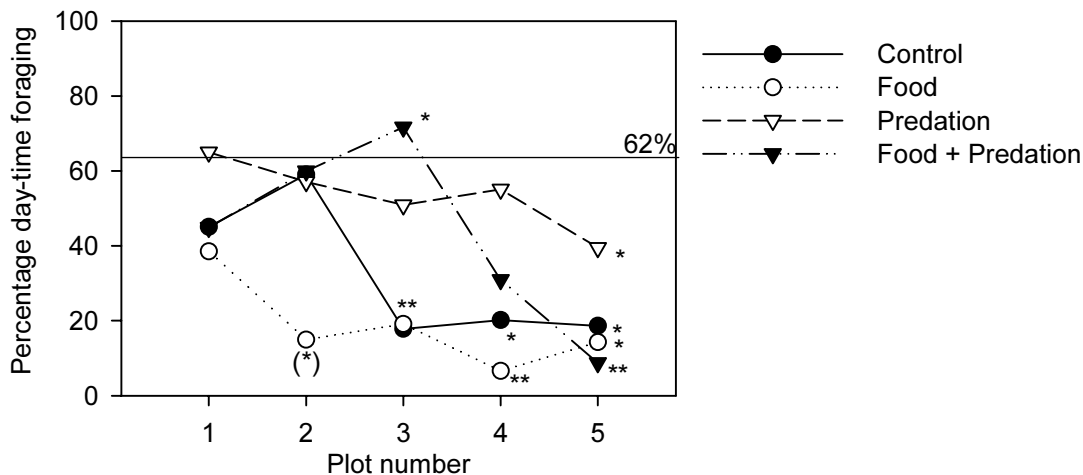

Figure 6.6 The percentage of day-time foraging in relation to the distance from the burrows. The plot number is used to express distance in this graph to allow the presentation of the averages, number 1 being closest to the burrows and 5 (at about 40m) furthest away. Each dot represents the mean percentage of observations during day-time relative to the total number of records obtained from the plots at that distance (n=3). The line at 62% indicates the relative amount of day-time of the total observation time. For each treatment, the average of each plot number was tested against the value 62 with a one-sample t-test to determine whether the percentage day-time foraging was significantly different from the amount of day-time available. (*) p<0.1, * p<0.05, ** p<0.01.

of mink pellets had the opposite effect, resulting in a reversal of the rabbit foraging pattern, which shifted from 66% nighttime records to 69% daytime records where mink pellets were applied. The combination of fertilizing and mink pellets still shifted the balance significantly to daytime activity compared to the control treatment (tab. 6.2). The amount of day or nighttime foraging was dependent on the distance from the burrows and experimental treatment (fig. 6.6, Anova, treatment: $F_{3,6.025}=5.399$, p=0.038, site: $F_{2,6.032}=1.381$, p=0.321, covariate plot number: $F_{1,44}= 18.063$, p<0.001). At the closest two plots from the burrows foraging was not significantly more during the day or night in all treatments, but at larger distances foraging occurred progressively more at night and at the farthest plot significantly more night visits were recorded in all treatments (fig. 6.6).

Discussion

A 150% increase in food quality resulted in four times as many rabbit droppings as in the untreated plots. An increase in predator scent on the other hand did not result in a change in the number of rabbit droppings. The combination of increased food quality with increased predator scent still resulted in a four times increase in rabbit droppings. Concluding from the rabbit droppings, rabbits in the experiment were therefore strongly affected by food quality and not by predation risk. The number of records from the infrared monitors for each treatment is in line with these conclusions, although the differences between the treatments were not significant. The data from the monitors and the dropping counts were significantly positively related, but the relationship was not very tight (r=0.23, p=0.015). This might be partly due to large variation in both types of data, but also because both measurement methods do not necessarily measure the same behaviour. The droppings counts without the latrines are probably most closely related to the time a rabbit spent on the plot, presumably mostly foraging. The counts with the infrared beams however, express how often a rabbit passed through the plot and through the beam, since rabbits could also pass through the plot without interrupting the beam. This could be during foraging, but also when engaged in territorial or social activities. In that respect it is illustrative that the dropping counts including the latrines did match more closely with the infrared counts, although still loosely (r=0.26, p=0.006). Observations during two evenings revealed that rabbits frequently chased each other and in their pursuit ran through a number of infrared beams.

The lack of response in visitation rate to the increased predator scent could be because rabbits did not associate the mink pellets with a predator. In general, experiments with rodents (Jedrzejewski et al. 1993; Herman and Valone 2000) and hedgehogs (Ward et al. 1997) have shown that they are well able to recognize predator scent and respond with behavioural adaptation. In our study rabbits did strongly respond to the mink pellets in the timing of their visits by increased visitation during the day. If mink pellets were associated with a mustelid predator by the rabbits, this behaviour could be explained as follows. The only regular visiting mustelid predator in this area is the polecat, which frequently consumes rabbits. Polecats are mainly active during darkness, extending to dusk and dawn (Lode

1995). Therefore increased daytime foraging would be a good strategy to avoid a polecat. In the untreated controls and the fertilized plots on the other hand rabbits mainly foraged during the night. Apparently daytime foraging is disadvantageous. That could be due to the presence of a pair of goshawks and several pairs of buzzard that both exclusively hunt during daytime (on one of the field days we walked into a goshawk that had just caught a rabbit). As long as there are no signs of recent polecat activity rabbits may therefore avoid predation risk from raptors by foraging during the night and adapt this pattern when polecats are known to be around.

Jacob & Brown (2000) obtained similar results for foraging common voles that adapted their foraging response to the type of predators that they encountered during the day (raptors) or night (owls and mustelids).

Rabbits might also be restricted in their possibilities to change absolute visitation rates. Rabbits are strictly territorial and have a strong social hierarchy (Lockley 1961). It may therefore not be possible to switch to other parts of the meadow when these are already occupied.

Central-place foragers are predicted to forage in a gradient, with more intensive foraging close to the burrow and lesser the further away. Such a gradient was found for the rabbits where the number of droppings counted significantly decreased with distance in the untreated control plots. The dropping count that was done before the experiment started and the vegetation height measurements confirmed that this gradient was already naturally present and was therefore not an experimental artefact.

Central-place foragers are predicted to forage close to their burrows because of several reasons. First we explore the hypothesis of reduced predation risk close to the burrows. Rabbits usually do run back to their burrows when escaping from a predator and therefore travelling distance and thus time exposed to predation does increase with distance. However, it can be questioned whether predation risk was indeed larger at larger distances. The vegetation height increased with distance but in general the vegetation was low and only 14 cm high on average at the farthest distances. This may have restricted the view of rabbits somehow, but may not be very suitable for a predator to approach their prey unseen. The burrows on the other hand, were located under shrubs, which were very suitable to hide in, also for a predator. Shrubs may also seriously block the view on oncoming predators. A well-known hunting strategy for goshawks is to use landscape structures and suddenly appear from behind them to take their prey by surprise. It is therefore hard to say how predation risk varies with distance. Kotler et al. (1999) came to the same conclusion in studying foraging behaviour of rock hyraxes in South Africa. They foraged least at the closest distance from their hiding places, which Kotler et al. (1999) attributed to the presence of sentinels that perched on the kopjes (15m high rocks) and had a good view at further distances, but could only partly scan their immediate surroundings. The only adaptation to predation risk at various distances that we observed was that rabbits adapted their timing of foraging by foraging more at night at larger distances. However, experimentally increased perceived predation risk did not cause a pattern in the total amount of visits at any distance. Therefore predation risk did not affect the spatial foraging gradient.

Another explanation is that central-place foragers experience the highest energy return when they forage close to the burrows and therefore minimize travel time. This might be more important in animals that carry food to a central-place as in feeding young at a nest or compiling a winter storage and therefore make a lot of foraging bouts in a short time, whereas this is not the case in rabbits. Another determinant of energy return is the food intake rate. Herbivores might achieve the highest intake rates at the highest standing crop (Gross et al. 1993). However, with the increase in standing crop also the amount of dead material increases which subsequently increases searching and handling time (Van der Wal et al. 1998). At similar plant nitrogen levels Iason et al. (2002) found in experimental trials that European rabbits obtained the highest intake rates at an intermediate standing crop of approximately 5-10 cm height (140 g m^{-2}). This contrasted with a field experiment where they found that rabbits consequently visited the plots with the shortest vegetation (2 cm) and therefore suboptimal food intake rates (Iason et al. 2002). In our experiment most droppings in the untreated plots were found closest to the burrows where the vegetation was shortest, which was around 7 cm in height and 106 g dry weight m^{-2}. According to Iason et al. (1999) this is well in the range of optimal cropping rates for rabbits. Another reason to select for a low to intermediate standing crop is that with increasing standing crop often the plant nutrient concentration drops. With the mown and fertilized treatment we therefore provided the rabbits both with vegetation height (5 cm) that allowed optimal intake rates combined with the highest nutrient concentrations. In our experiment rabbits strongly selected for this treatment. The preference of rabbits for the mown and fertilized plots increased with distance. This is in accordance with central-place foraging theory, where animals are predicted to become more selective with travelling distance and either take more or more valuable items. In the untreated gradient, the highest nitrogen values and the most attractive vegetation height were found closest to the burrows. Therefore if rabbits selected strongly for nitrogen content or optimal intake rates they could best stay close to the burrows. If they would move further from the burrows where average plant quality drops they should forage more selectively on high quality plants such as herbs or legumes or occasional shorter patches. This would result in a pattern of foraging that matches the observed undisturbed foraging gradient. Therefore a selection for the highest nitrogen intake rates alone could explain the observed spatial foraging pattern of the rabbits.

Central-place foraging in herbivores may actually operate differently than in other central-place foragers. Through grazing herbivores can modify their environment. Repeated grazing can stimulate fresh re-growth and increase the nitrogen content of the vegetation. Therefore, through grazing, herbivores are able to facilitate for themselves or for other herbivorous species. Facilitation through keeping the vegetation in a nutrient rich state through repeated grazing has been shown to occur in barnacle geese (Ydenberg et al. 1981), lesser snow geese (Hik et al. 1990) hares (Van der Wal et al. 2000) and is the basis of the existence of grazing lawns (McNaughton 1984). Herbivores can thus qualitatively improve the vegetation while depleting it, which contradicts the assumptions of central-place foraging theory. Combined with grazing, herbivores that frequently visit certain patches of vegetation

may also fertilize these patches by defecating (Ruess et al. 1989). Herbivores are shown to enhance nitrogen mineralization in grazed compared to ungrazed vegetation (Frank et al. 1993; McNaughton et al. 1997), which may also stimulate re-growth of the vegetation. Vegetation on prairie dogs towns for example, contains plants with a higher nutrient content than the vegetation off-town (Coppock et al. 1983). On the other hand herbivores can also decrease vegetation quality through selective grazing on high quality species that subsequently disappear from the vegetation (Huntly 1987; Ritchie et al. 1998; Ritchie et al. 1999a). The ultimate effect of herbivores on the vegetation nutrient content may depend on factors both in plants as in herbivores and are not yet fully understood. The properties of plants and herbivores determine whether the best food will be close to the burrows or far away and therefore whether central-place foraging herbivores have to choose between food and safety.

Acknowledgements

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Chapter 7

Ecological anachronisms in the recruitment of temperate light-demanding tree species?

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Abstract

All of the current-day adaptations that we see in plant-animal interactions are the resultant of past selection pressures. Therefore plant traits that we view today may be adaptations to animal partners or ecological conditions that are no longer present and may even be no longer functional. Such ecological anachronisms are however difficult to test, especially if specific plant or animal partners went extinct. We suggest that light-demanding trees (Oaks) and thorny shrubs (Blackthorn) in temperate vegetations provide a testable ecological anachronism. These plant traits may reflect adaptations to now-extinct large grazers as aurochs and tarpan. We explored the ecological functions of these traits in areas grazed by their domesticated descendants, cattle and horses. Specifically we tested the hypothesis that grazing induces shifting mosaics of grassland, shrub thickets and woodlands through the key process of associational resistance: the protection of palatable young trees by thorny shrubs. A cross-site comparison of four floodplain woodlands in north-western Europe showed that Oak can regenerate in the presence of large herbivores through spatial association with Blackthorn, a clonal thorny shrub. We performed an exclosure experiment with transplanted Oak seedlings to test the importance of protection from grazing and landscape position in a cattle grazed area. Oaks grew best in grassland exclosures and on the edge of thorny shrub thickets that received most light. Oak survival was severely reduced in Oak woodland. The high success of young Oaks among young thorny shrubs thus reflects an optimal balance between sufficient protection from herbivores and sufficient light availability. However, in two of the four sites, no young trees could be found. These sites both experienced a high rabbit density over the past ten years. Rabbits graze both on young ramets of Blackthorn as well as on young Oaks. In a survey with aerial photographs of 50 years of shrub and tree expansion on one of the sites we found that expansion of Blackthorn shrubs coincided with low rabbit abundance and not with livestock density. Therefore we conclude that the process of associational resistance did not work against rabbits, because they consume the Oak seedlings as well as the thorny shrubs that have no thorns when they are young. Under grazing by large herbivores, a shifting mosaic of grassland, shrubs and trees may develop that has high conservation values due to the positive correlation between heterogeneity in vegetation structure and biodiversity of e.g. insects and birds.

Introduction

Most experimental research on understanding current ecological adaptations of organisms to different environments is based on the implicit assumption that rates of environmental change, adaptation and changes in community structure are matched. However, adaptation through natural selection is a slow process, while environmental change and species extinctions can be very rapid. Current traits that are no longer optimal for current environmental conditions because they reflect adaptations to past environmental conditions are called ecological anachronisms. For example, C_4-plants may have evolved under low CO_2 levels during the Miocene (Ehleringer et al. 1991; Cowling 1999) (but see (Pagani et al. 1999)) whereas C_4 photosynthesis has no identifiable advantage under current CO_2 levels (Ehleringer et al. 1991). Very large neotropical fruits, such as avocados, are viewed as adaptations to seed dispersal by now-extinct gomphoteres and other extinct large pleistocene herbivorous mammals (Janzen and Martin 1982). Thorny, divaricate New Zealand shrubs with small leaves are thought to have evolved in response to browsing by Moas, large herbivorous birds that were driven to extinction by medieval Polynesian man (Atkinson and Greenwood 1989; Cooper et al. 1993). Several species of temperate North-American and European thorny shrubs may have evolved under browsing by now-extinct large pleistocene herbivores, since the presence of the taxa like *Prunus*, *Crataegus*, *Rhamnus*, *Rhus* and *Juniperus* goes back through the Pleistocene even as far as the early Tertiary (Tallis 1991).

These ecological anachronisms are hypotheses that are often hard to proof (Barlow 2002; Howell et al. 2002). Indirect evidence may be collected from current ecological conditions to understand adaptations to past environmental factors, but not for interactions for which the original partner or its ecological equivalents went extinct. In these cases only paleo-ecological evidence can be used. Gomphoteres and moas went extinct, leaving no modern ecological proxies. However, proxies are available for the large herbivores that co-evolved with thorny shrubs in temperate regions. Large domestic herbivores as cattle and horses, domesticated from their extinct ancestors aurochs and tarpan, make it possible to test historical ecological partners of species in current experimental research. We hypothesize that these past ecological interactions between woody species and large grazers are not only reflected in the traits of thorny shrubs, but also in the recruitment strategy of more palatable woody species that are spatially associated with these shrubs under grazed conditions.

Several species of Oak (*Quercus* sp.) have been reported to show major difficulties in regenerating in current temperate forest reserves (Rackham 1980; Vera 2000; Johnson et al. 2002; Smit 2002), that generally lack large grazers. Oaks are light-demanding trees that can germinate in the shade, but need open conditions for long-term survival and growth of the seedlings (Crow 1992; Kelly 2002). Temperate woodlands and meadows are also home to a variety of thorny shrubs (i.e. *Prunus*

spinosa, *Crataegus* sp., *Rosa* sp. etc.) that are also light-demanding, and currently mostly occur along forest edges (Stortelder et al. 1999). Several species of thorny shrubs exhibit a divaricate growth-pattern, for example *Prunus spinosa* and *Crataegus monogyna*, which has been viewed as an adaptation to cope with large herbivores (Greenwood and Atkinson 1977). This branching pattern causes a dense canopy to develop after browsing, which is more difficult to penetrate for a large herbivore, especially when the branches terminate in a sharp thorn, such as in *Prunus spinosa*. The widespread occurrence of tree species that depend on local openness of the landscape and the presence of thorny, divaricate shrubs leads us to the hypothesis that both may be viewed as an adaptation to now extinct partners: large grazers of the temperate zone. The taxon *Quercus* (Oaks) was present in Europe from before the Pleistocene (Tallis 1991) and thus oak species could have evolved in the presence of the Pleistocene and earlier fauna. After the extinction of most of the Pleistocene large herbivores (including many grazers i.e. horses, bison, mastodon, mammoth, woolly rhinoceros, a ground sloth, camels and the cave bear), only aurochs, tarpan and American bison were left as large grazers in present-day temperate regions together with several deer and antelope species and European bison as browsers/mixed feeders (Graham and Lundelius 1993; Vereshchagin and Baryshnikov 1993; Kahlke 1994). With the expansion of human settlements aurochs and tarpan went globally extinct in 1627 (Szafer 1968; Van Vuure 2002) and the mid-nineteenth century (Vereshchagin et al. 1993), respectively, through overexploitation and competition with livestock, leaving no native large bulk grazers in Europe. However, cattle and horses were introduced, and replaced the two wild ancestral species from which they were originally domesticated. They lived close to each other up to the Middle Ages (Clason 1967; Szafer 1968; Lauwerier 1988; Lebreton 1990). Therefore, the temperate woodlands and meadows with Oaks and thorny shrubs remained grazed despite extinction of the original partners. This provides us with a system that allows testing of hypotheses on past ecological interactions, with consequences for current biodiversity patterns.

Herbivores generally have a negative impact on regeneration and growth of woody species (Crow 1992; Crawley and Long 1995; Rousset and Lepart 2000; Kuiters and Slim 2002; Smit 2002). Plants can directly defend themselves physically or chemically (Herms and Mattson 1992; Twigg and Socha 1996), through indirect defence by spatial association with defended species (associational resistance) ((Olff et al. 1999; Callaway et al. 2000; Milchunas and Noy-Meir 2002) or by tolerating herbivory (Rosenthal and Kotanen 1994). Direct defences can be permanent or inducible (Karban 2003). Inducible mechanical defence (such as thorns and divaricate branching in trees) and tolerance (e.g. grass regrowth strategies) are expected to evolve under conditions of high resource availability, because of the high carbon and nutrient requirements needed for these strategies (Bryant et al. 1983; Coley et al. 1985). Interestingly, large herbivores foraging in landscapes are expected to select drier sites with high soil nutrient availability, because these sites will have the highest forage quality (WallisDeVries 1994; Olff et al. 2002). This may have enforced the evolution of thorns and divaricate branches and the occurrence of indirect defence especially under these conditions (Coley 1987; Myers 1987).

The interplay of domesticated large grazers, Oaks and thorny shrubs in the drier parts of fertile riparian areas in the temperate zone may represent such a system with important direct and indirect defences of plants against herbivores. In most places large grazers were removed from woodlands to benefit timber production. However, in a few remaining isolated nature reserves in Western Europe, so-called wood-pastures, such as New Forest, Borkener Paradies and Junner Koeland, the natural interplay of herbivores and plant defences may be still at work. In these sites, Oaks are generally surrounded by thorny shrubs (Burrichter et al. 1980; Pott and Hüppe 1993). It has been hypothesized that the thorny shrubs protect the palatable Oaks and other tree species from grazers (Vera 1997, 2000), an example of associational resistance (Olff et al. 1999). Subsequently the trees will grow out and outshade the shrubs that facilitated their establishment. However, this prevents further recruitment of tree species causing the system to return to grassland upon death of mature trees. When this cyclic succession becomes spatially asynchronized it will result in a shifting mosaic of grassland, shrub thicket and woodland patches (Vera 1997; Olff et al. 1999; Vera 2000). Due to the positive correlation between heterogeneity in vegetation structure and biodiversity of e.g. insects, birds and shrubs (Cody 1975; Kollmann and Schneider 1999; Kruess and Tscharntke 2002), such shifting mosaics have a high conservation value. However, the key mechanisms of this cyclic succession have not yet been experimentally proven. Associational resistance only operates when herbivores lack the ability for small-scale discrimination between palatable and the unpalatable species, i.e. cannot avoid the unpalatable species when trying to eat the palatable one, and in the absence of fire. This makes the effectiveness of protection of palatable trees by thorny shrubs dependent on herbivore body size; associational resistance only works against large herbivores. Small herbivores as rabbits and voles will go underneath and in between thorny shrubs and consume palatable tree seedlings (Manson et al. 2001). Therefore, different-sized herbivores have potentially very different effects on tree recruitment, but field data supporting this are yet lacking.

In this study we will first explore the generality of the occurrence of associational resistance in different grazed woodlands in Germany, the Netherlands and Great Brittain, analyzing the role of various thorny species and of different-sized herbivores. Next we will describe the spatio-temporal dynamics of vegetation mosaics maintained by these mechanisms using historical aerial photographs. Finally we will test the proposed protective mechanism of palatable trees by thorny shrubs in a field experiment.

Methods

Study sites

Five riverine floodplains were chosen as study sites (tab. 7.1). In the Netherlands Junner Koeland, Prathoek and the Hui, along the river Overijsselse Vecht, the Borkener Paradies in Germany, bordering the river Ems and a riverine floodplain in

New Forest in the UK, bordering Beaulieu River were sampled. All five sites are nature reserves and good examples of the natural vegetation bordering most of the larger rivers in the temperate zone before canalisation and intensive cultivation. They all contain hardwood forest in a grass-shrub-tree mosaic. At all sites livestock has been grazing for centuries (Burrichter et al. 1980; Putman 1986; Bokdam 1987; Tubbs 1997). Due to canalisation of the Overijsselse Vecht and the river Ems, the floodplains are only flooded under extreme conditions, only a few times last century. In table 7.1 the density and species composition of livestock is given. In Borkener Paradies and the Dutch sites grazing by deer (only roe deer) is erratic and therefore not considered.

Table 7.1 Characteristics of the study areas.

Study site	Size (ha)	Size of hardwood habitat (ha)	Number of grazers ha^{-1}	Presence of rabbits
Junner Koeland (NL)	100	30	0.4[1]	++
Prathoek (NL)	22	22	0.4[1]	++
Hui (NL)	12.5	1	1.8[1]	+−
Borkener Paradies (G)	50	30	0.4[2]	−
New Forest (UK)	40000	320[4]	1.9[3]	−

[1] cattle, [2] cattle and horses, [3] cattle, ponies and red deer (Putman 1986) [4] only vegetation containing *Prunus spinosa* shrubs is considered (data from Putman 1986)

Transect surveys

In Junner Koeland, Prathoek, Borkener Paradies and New Forest we measured shrub- and tree presence along transects across the shrubs using a plotless sampling technique. Transects started in open grassland approximately 20 m from the shrub edge. Starting points were along a line parallel to the shrub edge, each 20 m apart. Transects ran perpendicular from the starting line into the shrubs. On the transect line sampling points were at every 20 m from the starting point. A sampling plot consisted of a circle with 10 m radius from the sampling point on the transect. Shrub- and tree presence was determined using the point-centered quarter method (Mueller-Dombois and Ellenberg 1974). Each circle at a sampling point is subdivided in four quarters following the compass direction and a second line perpendicular to it. The presence of the nearest tree and shrub within 10 m from the sampling point is recorded for each quarter. If a quarter contained more trees or shrubs, these were ignored; if no tree or shrub was found within 10 m a value of zero was recorded. Tree and shrub species were determined and of each individual the diameter (trees at breast-height and shrubs 20 cm above the ground) was measured and height was estimated. Only shrubs and trees taller than 50 cm were recorded. Transects were sampled in spring and summer of 1998 for all sites with 57 sampling points in Junner Koeland, 73 for Prathoek, 106 for Borkener Paradies and 75 for New Forest.

Association between trees and thorny shrubs was calculated by comparing the incidence of trees occupying a quadrat together with a thorny shrub with the incidence of quadrats with trees and no (thorny) shrubs and with thorny shrubs but no trees. A Chi-square test was used to test whether trees were significantly

associated with thorny shrubs. The Chi-square test was only performed when five or more trees and thorny shrubs were recorded (not necessarily in the same quarters).

Shrub expansion and tree establishment

Shrub expansion and tree establishment was studied in Junner Koeland using aerial photographs. On Junner Koeland, the shrubs consisted almost entirely of Blackthorn (*Prunus spinosa*); trees were mostly Penduculate Oaks (*Quercus robur*). On the photographs, with a scale of 1:2500, the Blackthorn shrubs and individual Oak trees were easily recognizable. Photographs from 1945 onwards were available. For this study photographs from 1945, 1961, 1972, 1983, 1989 and 1995 were digitized and spatially referenced. Calculations on the area of shrub cover were made using IDRISI (Anonymous 1999). Periods of tree establishment were calculated using a calibration curve (age (years) =0.7*diameter (centimeters) + 3.28, linear regression: r^2=0.92, p<0.001, n=13). The calibration curve was established by counts of tree rings and measured tree diameters after sampling Oaks in Junner Koeland with a small core (Jorna 1984).

Herbivore density in Junner Koeland over the last decades

Densities of cattle over the last fifty years have varied considerably due to enlargement of the reserve and changing ideas on the desired grazing intensity. Information on cattle density in the different decades was obtained through personal communication with the site managers and local farmers. Rabbit counts were available from 1979 onwards. Rabbits were counted twice a year during mid-summer along a transect that was traveled by foot. The transect was approximately 1 km long and provided a clear view on the floodplain meadows, excluding the forest patches. Rabbits were counted using binoculars on a calm summer evening right before dusk. The higher of the two counts was taken as relative number of rabbits for that year. This number gives a relative estimate of rabbit density, the transect was surveyed by JM Gleichman during all years. No measures of rabbit density before 1979 were available. Therefore we used density of rabbit burrows as counted from the aerial photographs as a relative measure of rabbit numbers. Rabbits burrowed mainly on the sandier patches that resulted in clear white spots on the black-and-white photographs. Photographs of 1951, 1961, 1972, 1981, 1982, 1983, 1989, 1992, 1994, 1995 and 2000 were used to count burrows on the area surrounding the hardwood forest patches, measuring 12.5 ha of grassland. Burrow density was significantly correlated with the rabbit counts in the eight years when both photographs and transect counts were available (Pearson correlation, n=8, r=0.79, p=0.02). We therefore used burrow densities averaged over the different time periods as a measure of rabbit presence over the fifty years.

Transplantation experiment

A transplantation experiment was performed with one-year-old Penduculate Oaks to study the interaction between abiotic conditions and grazing on survival and growth of Oak seedlings. The experiment was performed in Junner Koeland and the Hui, to

compare a site with high rabbit density with a site with low rabbit density. We purchased 432 Oak seedlings from a commercial firm in early spring 2002. The acorns were sown the previous year and the Oaks were delivered with a clump of soil attached to the roots; the tap root of the seedlings was cut after about 7 cm to make the seedling fit in a small pot. The seedling consisted of one woody stem with buds, no leafs or branches were present yet. The seedlings were stored at 5ºC until planting.

In each study site we planted 216 Oak seedlings at the end of April 2002. Seedlings were planted in four structure types: grassland, young Blackthorn, old Blackthorn and Oak woodland arranged in a transect. The grassland site was located about 2-4 m from the edge of the shrubs. Young shrubs were located at the edge of the thicket and consisted of low, very spiny branches that had sprouted from rhizomes a few years ago. Old shrub was the higher and older inner part of the thicket, located 3-10m from the young shrubs. Old shrub mostly lacked understorey, the first branches started at about 50 cm from soil level. The woodland site was located 3-5 m from the old shrubs and consisted mainly of mature Oak trees with very little understorey.

Per study site 3 transects were established. Each transect ran through the four structure types and contained 2 plots in each type: one exclosed plot and one plot that was accessible for herbivores. Each plot measured 1 m^2 and received 9 transplanted Oak seedlings. The exclosures consisted of chicken mesh wrapped around four poles with a height of 50 cm. On top of the exclosures a broad-mazed roof (mesh 5 x 10 cm) was constructed to prevent cattle grazing in the exclosures. At 15 October 2002 all seedlings were harvested, separated in stem, current-year growth of branches and leaves, dried at 70ºC and weighed. In the lab was also determined which trees were alive or dead.

We measured light availability (PAR) in the different structure types in June using a one-meter long probe containing 64 photodiodes equally spaced over its length (SunScan Canopy Analysis System, Delta-T Devices Ltd, U.K.). We took two measurements per plot, just above the leaves of the tranplanted Oaks (about 50 cm above ground level), that were averaged before further calculation.

In June we collected two soil samples per plot to measure extractable soil nitrogen. The samples were taken with a 2.5 cm diameter polyvinyl chloride tube (PVC) with a depth of 10 cm. Mineral N was extracted from fresh soil within 24 hours after sampling using 1 M KCl. The NH_4^+ and the NO_3^- content of the soil was measured colometrically using a continuous flow analyzer (Skalar).

Next to the grassland exclosures a plot of 2 x 2 m was established where droppings of rabbits were counted to determine relative rabbit density. Droppings were counted every four weeks and removed from the plots when counted. Droppings were counted from April till July. Rabbit density was calculated through assuming a dropping rate of 400 droppings per rabbit per day (Lockley 1962).
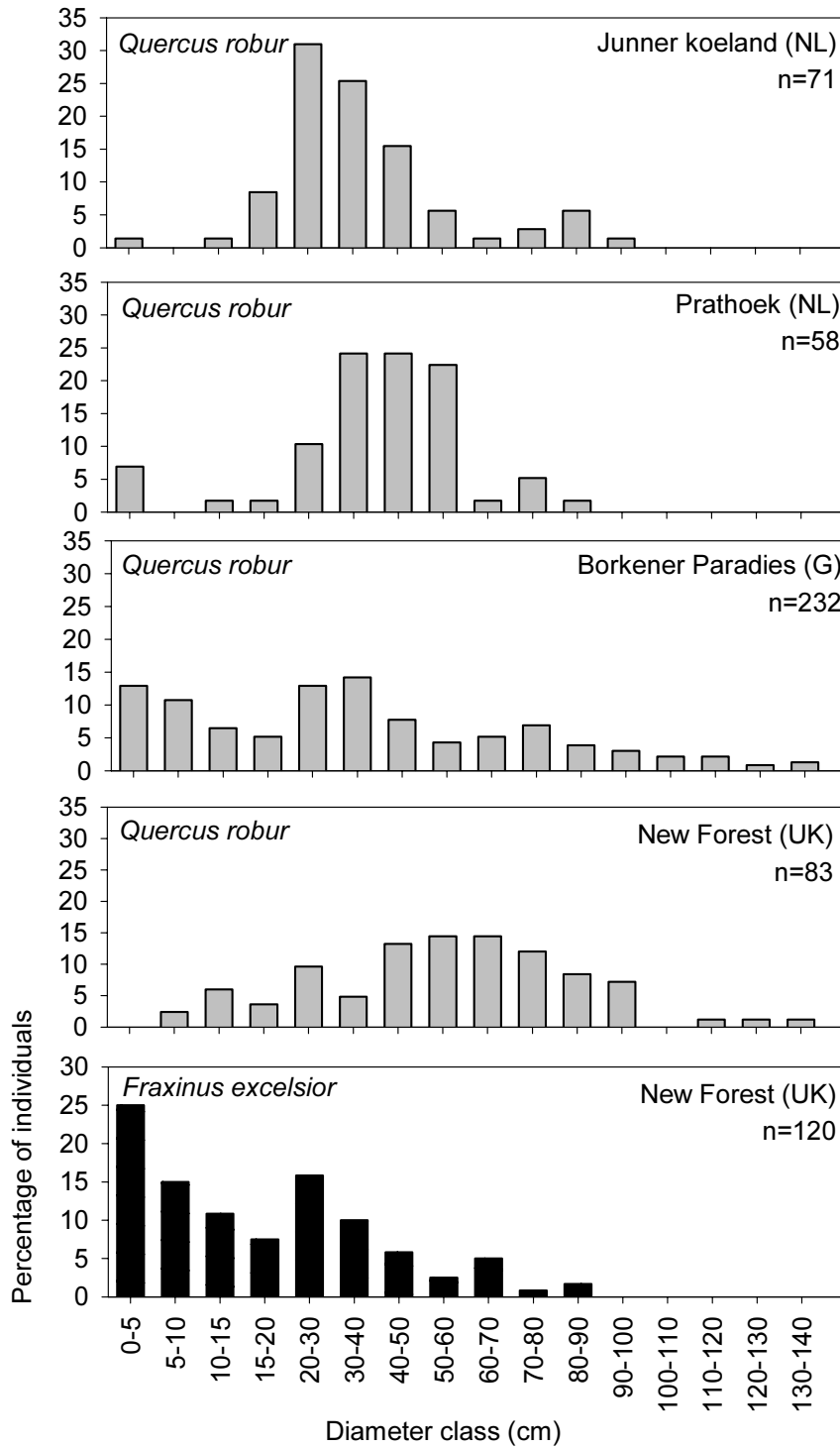
Figure 7.1 Frequency distribution of Oaks (*Quercus robur*) and Ash (*Fraxinus excelsior*) in the study sites as percentage of total number of individuals per tree species per site (n).

Results

Establishment of trees

We found 688 trees representing 10 species in all sites together. Junner Koeland and Prathoek harboured fewer trees per sampling point than Borkener Paradies and New Forest, and also less small (< 2m) trees (tab. 7.2). In Junner Koeland, Prathoek and Borkener Paradies *Quercus robur* was the dominant tree species (> 75% of all trees). In the New Forest *Fraxinus excelsior* (44%) was co-dominant with *Quercus* (31%); together they represented 75% of all trees. The age distribution of the dominant trees (measured as stem diameter) was different for the study sites (fig. 7.1). In Junner Koeland and Prathoek, the Oaks were approximately between 20 and 60 centimetres thick, whereas smaller and larger trees were missing in those areas. Dominant trees in Borkener Paradies and New Forest showed a more equal distribution of trees over size classes, with ample young trees.

In the transects 1054 shrubs were recorded distributed over 20 species. Of these 20 species 7 were labelled as thorny species: *Crataegus monogyna, Ilex aquifolia, Prunus spinosa, Rhamnus catharticus, Rosa sp., Rubus sp.* and *Ruscus aculeatus.* On average 48% of the shrub species were thorny in each site (tab. 7.2). *Prunus spinosa* was dominant (>=60%) in Junner Koeland and Prathoek, in Borkener Paradies it was co-dominant with *Crataegus monogyna* (together >60%) and in New Forest both species shared dominance with *Ilex aquifolia* (together 70%). The average number of shrubs per sampling point was equal for all study sites.

Table 7.2 Number of trees and shrubs recorded in the study sites, number of species and number of trees and shrubs per sampling point (maximum is 4 per sampling point, see methods).

Site	No. of tree species	Total no. of trees	No. of trees <2m (% of total)	No. of trees/ sampling point	No. of shrub species (% thorny)	No. of shrubs (% thorny)	No. of shrubs/ sampling point
Junner Koeland	4	79	1 (1%)	1.4	9 (44%)	183 (81%)	3.2
Prathoek	3	74	4 (5%)	1.0	9 (44%)	222 (65%)	3.0
Borkener Paradies	8	262	28 (11%)	2.5	12 (50%)	362 (85%)	3.4
New Forest	7	273	25 (9%)	3.6	13 (54%)	287 (96%)	3.8

Associational resistance

Young Oaks were found 49% more often together with Blackthorn than expected if the occurrences would be independent (tab. 7.3) in Borkener Paradies, the only site with enough small Oaks to test the relationship. At this site young Oaks were significantly associated with Blackthorn, whereas tall Oaks were significantly negatively associated with Blackthorn in most sites. The relationship between Oaks and *Crataegus* was the other way around: a negative association was found with small trees (but not significant) and positive relations for tall trees (tab. 7.3). Measurements from the other thorny shrubs had to be pooled since not enough data were available for these species. The results are in line with the data for *Crataegus*. Only in New Forest enough Ash trees were found to test its relationship with thorny

A)

	grass
	1945
	1961
	1972
	1983
	1989
	1995

B)

Figure 7.2 A. Cover of *Prunus spinosa* shrubs in Junner Koeland from 1945-1995
B. Position of Oaks in 1972. Oaks (black dots) were mostly established within the *Prunus* shrubs (grey) and hardly in open grassland (white).

Table 7.3 The association between Oak (*Quercus robur*), Ash (*Fraxinus excelsior*) and thorny shrubs for small (<2m) and large trees (>2m). The percentage gives the percentage of cases in which trees were found more or less (the negative values) often than expected. The expected value is based on a random distribution of trees over quadrats containing thorny and no (thorny) shrubs. The table reads as follows: in Borkener Paradies small Oaks (< 2m) were found 49% more often in the same quadrat as *Prunus spinosa* than expected, whereas tall Oaks (>2m) were found 1% less often than expected in a quadrat containing Blackthorn. Values were not determined (ND) when there where less than 5 trees or shrubs in a category. The association was tested using the number of quadrats were trees and shrubs (co-)occurred with a χ^2 test, (*) p<0.1, **p<0.01, ***p<0.001.

		Number of trees		*Prunus spinosa*		*Crataegus monogyna*		Other thorny shrubs	
	Tree-height	*< 2m*	*> 2m*	*< 2m*	*> 2m*	*< 2m*	*> 2m*	*< 2m*	*> 2m*
Tree species	Site								
Quercus robur	Junner Koeland	1	70	ND	-13%***	ND	ND	ND	3%
	Prathoek	4	54	ND	-15%**	ND	4%	ND	ND
	Borkener Paradies	23	211	49%***	-1%	-16%(*)	10%***	-4%	1%
	New Forest	4	84	ND	-10%***	ND	13%***	ND	13%***
Fraxinus excelsior	New Forest	20	101	2%	7%***	4%	-2%	4%	-2%

shrubs. The results were different from Oaks, young Ash trees were not positively or negatively associated with Blackthorn shrubs, tall trees were positively associated with Blackthorn shrubs (tab. 7.3).

Shrub expansion and tree establishment in the Junner Koeland

Expansion of Blackthorn shrubs in Junner Koeland varied considerably with time (fig. 7.2a). Figure 7.3a summarizes the increase of shrub area for the different time periods. In some periods the shrubs expanded rapidly (e.g. 1961-1972 and 1972-1983), whereas in other periods hardly any shrub expansion occurred (e.g. 1945-1961 and 1983-1995). Calculation of the establishment period of the Oaks from the transect data resulted in a very similar establishment pattern as the increase in Blackthorn shrubs over time (fig. 7.3b). The correlation between the number of established Oaks and hectares of shrub expansion is highly significant and positive (Pearson correlation, n=5, r=0.98, p=0.003).
The cattle density continuously declined over the years from 1945 onwards (fig. 7.3c) either because of a reduction in the number of cattle or because of an enlargement of the grazing area. Rabbit density, measured as number of burrows, increased over time (fig. 7.3d).

Survival and growth of the transplanted Oak seedlings

Survival of the transplanted Oaks was significantly reduced through grazing in grassland and Oak woodland at both study sites (fig. 7.4). In Junner Koeland 85% of the transplanted Oaks survived in the grassland exclosure and in the young Blackthorn both with and without grazers. Grazing reduced survival to 26% in grassland and 11% and 15% in old Blackthorn and Oak woodland where survival in
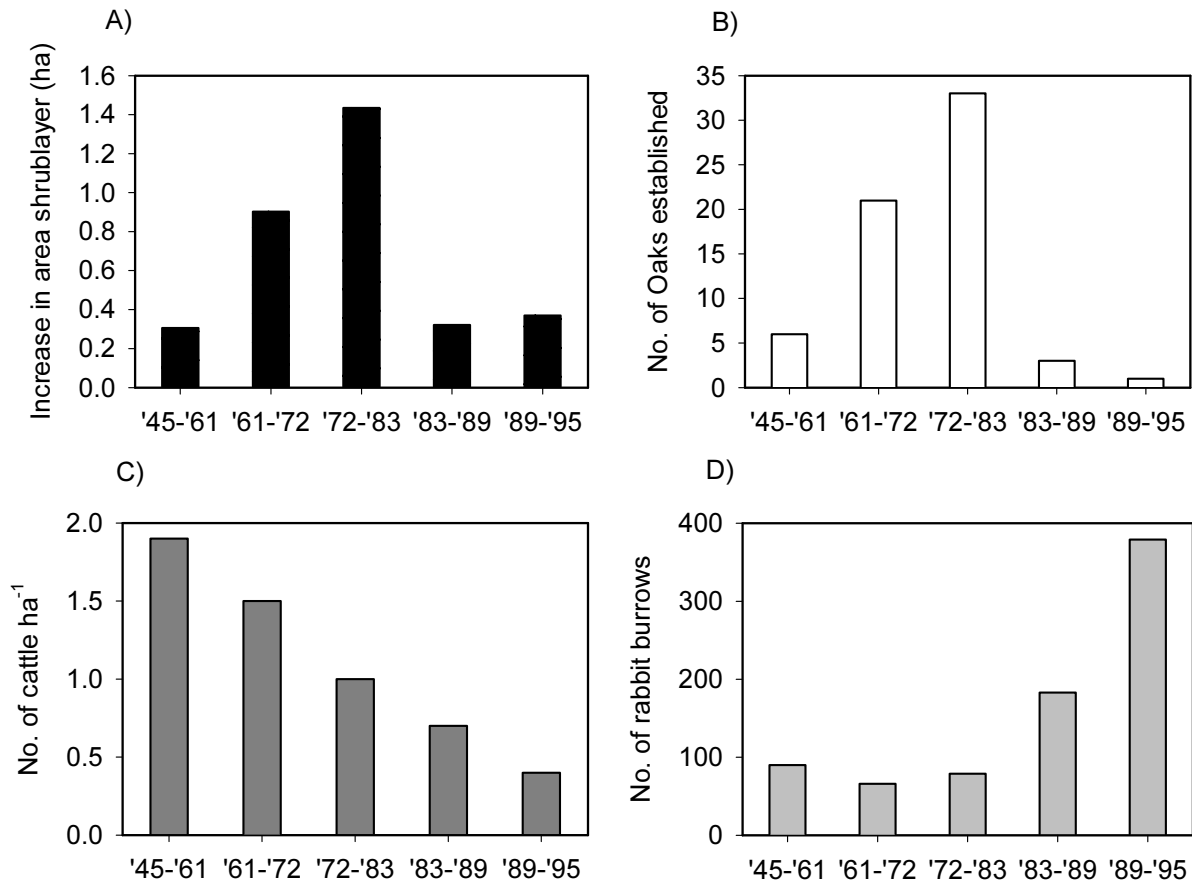
A)

B)

C)

D)

Figure 7.3 A. Increase of area *Prunus spinosa* shrubs per time period.
B. Establishment of Oaks per period
C. Cattle stocking density
D. Relative rabbit density as average number of burrows

exclosures was already reduced to 33% and 48% respectively. Seedling survival in the Hui was reduced under grazing in grassland and woodland, where 26% and no seedlings survived respectively (fig. 7.4). Survival in young Blackthorn was about 80% and not affected by grazing, which was also true for old Blackthorn where both grazed and ungrazed plots resulted in 48% survival.
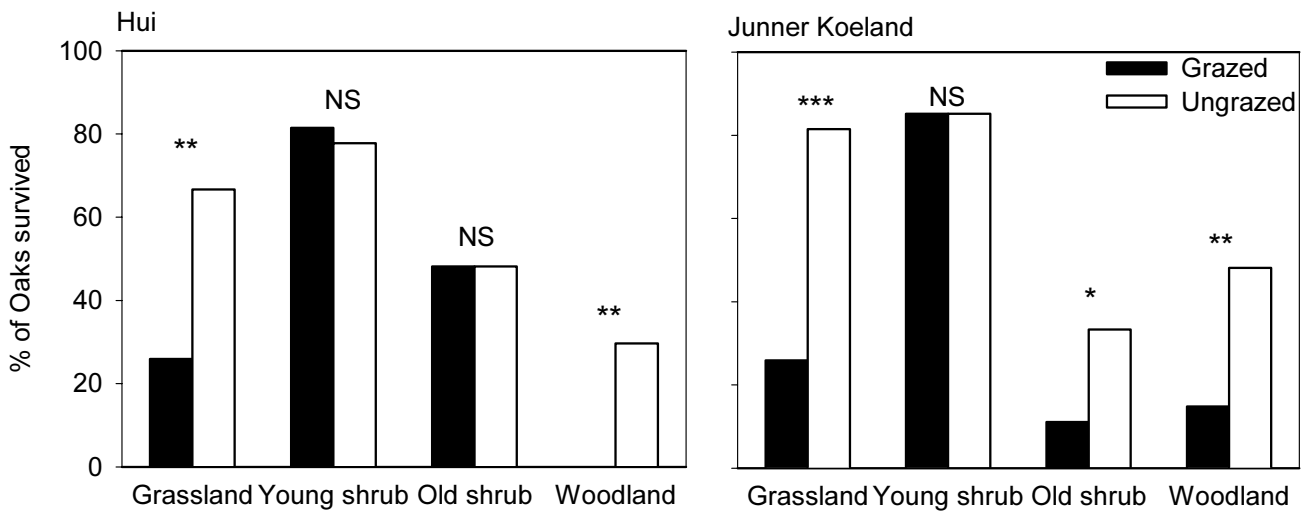
Figure 7.4 Effect of grazing on survival of transplanted Oak seedlings in different structure types (n=27). Chi-square test, * p<0.05, ** p<0.01, *** p<0.001, NS p > 0.05.
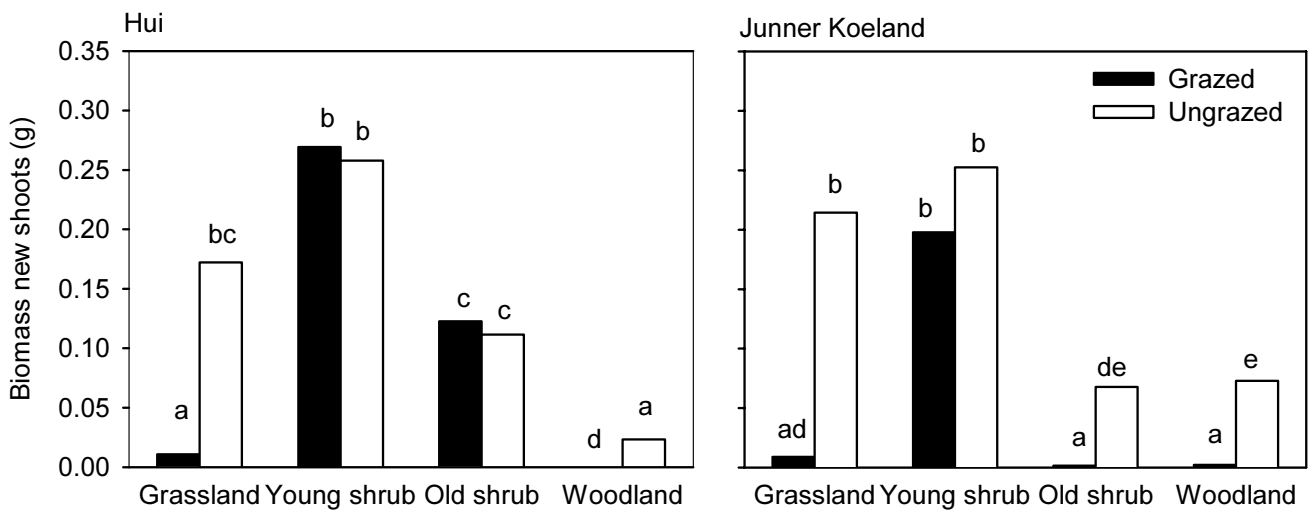
Figure 7.5 Effect of grazing on biomass (g/seedling) of current year shoots of transplanted Oak seedlings in different structure types. Different letters indicate statistically different biomasses at the level p<0.05, One-way Anova.

Abiotic conditions in the vegetation structure types

In the Junner Koeland extractable soil nitrogen increased from grassland to Blackthorn to Oak woodland (fig. 7.6a). This pattern was less clear in the Hui, the effect of structure type depended on the study site (interaction structure type*site $F_{3,6}$ = 7.728, p=0.017). When soil nitrogen levels were tested per structure type per study site, values were highest in woodland and lowest in grassland and young Blackthorn on Junner Koeland, the other plots had intermediate nitrogen levels (Oneway-Anova, $F_{7,16}$ = 4.435, p=0.006, fig. 7.6a).

Light levels were much lower in Blackthorn and Oak woodland compared to grassland at both study sites (fig. 7.6b). In the young Blackthorn approximately 20% of the light was still available and about 10% in old Blackthorn and Oak woodland. The effect of structure type was similar in both study sites (Anova after ln-transformation, site effect: $F_{1,2}$= 0.385, p=0.598, structure type: $F_{3,6}$=79.315, p<0.001, site*structure type: $F_{3,6}$ = 0.978, p=0.463).
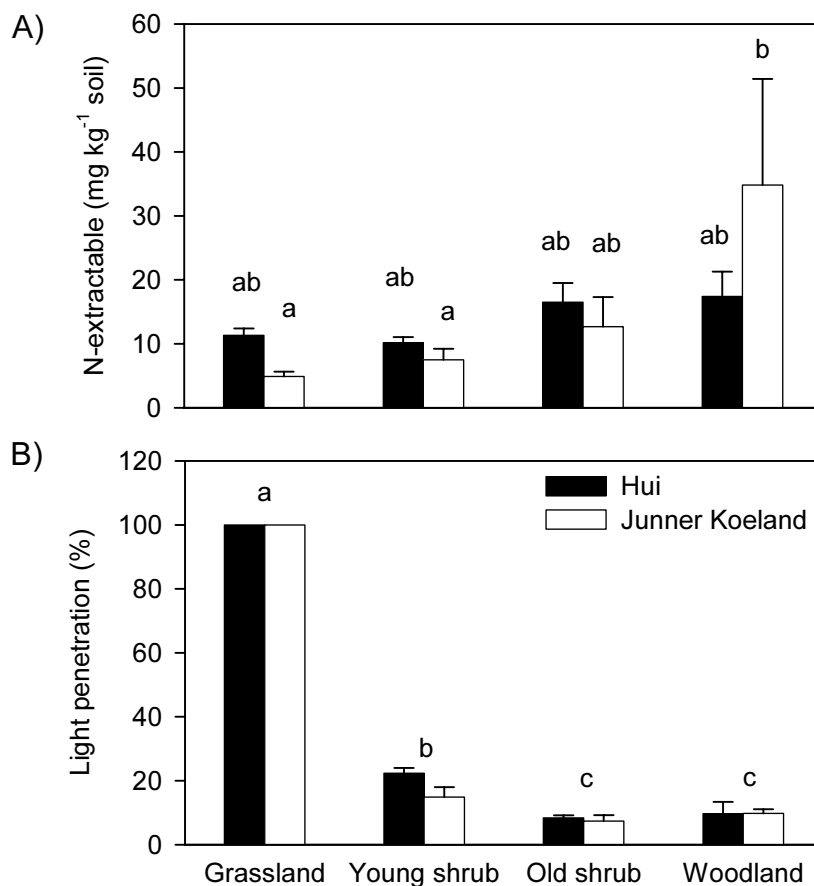
Figure 7.6 A. Extractable soil N content (mg kg^{-1}) in the different structure types. Soil nitrogen level depended on structure type and study site (Anova after ln-transformation: structure type*site $F_{3,6}$ = 7.728, p=0.017). Soil nitrogen levels were grouped per structure type per study site and tested with an Oneway-Anova, $F_{7,16}$ = 4.435, p=0.006. Different letters indicate significant differences (p<0.05).
B. Light penetration at crown level of the transplanted Oaks, approximately 25 cm above soil level. Light penetration is expressed as percentage of the light level in open grassland exposed to direct irradiation. The effect of structure type was similar in both study sites (Anova after ln-transformation, site effect: $F_{1,2}$= 0.385, p=0.598, structure type: $F_{3,6}$=79.315, p<0.001, site*structure type: $F_{3,6}$ = 0.978, p=0.463). Different letters indicate significant differences (p<0.05).

Discussion

Although the operation of associational resistance has been frequently suggested in grazed ecosystems (Vera 1997; Olff et al. 1999; Callaway et al. 2000; Rousset et al. 2000; Vera 2000; Milchunas et al. 2002), experimental evidence was yet scarce (Callaway 1992; Callaway and Davis 1998). We found that in the presence of both cattle and rabbits, survival and growth of the transplanted Oaks was severely suppressed both in grassland and Oak woodland. Only in the young, thorny Blackthorn shrubs the transplanted Oaks survived and grew equally well in and outside exclosures, showing the importance of facilitation through associational resistance. Thus herbivory induced an indirect positive interaction between two otherwise competing woody species, at least in the recruitment phase. In the absence of herbivores survival and growth was lowest in the Oak woodland, probably due to insufficient light (Kelly 2002). The high success of young Oaks among young thorny shrubs thus reflects an optimal balance between sufficient protection from herbivores and sufficient light availability.

On a landscape scale, this may explain why Oaks were spatially associated with Blackthorn shrubs (Junner Koeland). In this area, temporal variation in the establishment of Oaks during the last decades seemed to be explained by variation in expansion of area covered by Blackthorn shrubs. In Borkener Paradies the occurrence of young Oaks was also positively associated with the presence of Blackthorn shrubs. This further supports that associational resistance provides an important mechanism for the regeneration of palatable trees in grazed systems.

However, the effectiveness of associational resistance as protection against grazing depends on herbivore size and foraging preferences. The transplanted Oaks were not protected against rabbit grazing in the old Blackthorns that have few spines at the bottom of their stems, allowing access by rabbits that consume the Oak seedlings. We suggest that the main differences in current tree regeneration between the four riverine floodplains that we studied is due to differences in rabbit densities over the past ten years and not to differences in large herbivore densities.

Rabbits can have a strong impact on regeneration of palatable trees through direct predation (Crawley et al. 1995), but also indirectly through consumption of the young stages of the protective thorny shrubs, leaving young Oaks vulnerable to grazing by cattle. Young plants of thorny shrubs gradually develop their physical defences during their first year, leaving them vulnerable to predation at young age (Rackham 1980). Historical expansion patterns of Blackthorn shrubs in Junner Koeland showed a strong negative correlation with rabbit densities suggesting that rabbits can inhibit clonal expansion of Blackthorn shrubs. Cattle stocking rate showed no relation with shrub expansion, showing that expansion is possible under cattle grazing at various densities. These results are in line with studies of Buttenschøn & Buttenschøn (1985). They studied dynamics of woody species in Danish grasslands grazed by cattle (0.4 ha^{-1}) and sheep (0.8 ha^{-1}) and found that Blackthorn and Penduculate Oaks expanded under cattle grazing whereas sheep decreased cover of Blackthorn and Oaks (no rabbits were present in the area) (Buttenschøn and Buttenschøn 1985; Buttenschøn 1988; Buttenschøn and Buttenschøn 1990). They attribute this to the higher part of browse in the sheep diet, but also the smaller muzzle size that enables

sheep to forage more selectively. Under cattle grazing Oaks were mostly found in *Juniperus* and Blackthorn shrubs.

Summarizing, Oaks could better regenerate outside a woodland, i.e. in the forest edge or in grassland. For Oaks, several advantages are associated with regeneration in grassland. There is enough light and since Oaks possess a taproot that grows rapidly in the first years, they are relatively resistant to drought compared to other tree species (Danner and Knapp 2001). Insect herbivores and diseases as mildew, that are associated with the parent tree, are absent or less severe in grassland (Rackham 1980). Also seed predation by rodents is reduced in open grazed grassland compared to woodland (Jensen and Nielsen 1986; Hubbard and McPherson 1999), although typical grassland rodents, as several species of voles, can severely reduce tree establishment by grazing on saplings (Gill and Marks 1991; Manson et al. 2001).

Dispersal may be a limiting factor for shrub- and tree regeneration in grassland (Herrera and Jordano 1994). In the case of Oaks acorns are distributed by woodmice and jays (Bossema 1979; Jensen et al. 1986). In our study site woodmice were mostly restricted to the woodland, but jays did frequently bury acorns in open grassland (E.S. Bakker unpubl. data). Jays have been observed to bury acorns especially at the fringes of thorny scrub and at the base of thorny and non-thorny shrubs (Chettleburgh 1952; Bossema 1979; Vullmer and Hanstein 1995; Rousset et al. 2000). Seedling establishment of tree species is found to be inhibited through a large litter layer or competition from grasses and herbs (Facelli 1994; Siebel and Bouwma 1998). Therefore large grazers may even facilitate tree establishment through preventing the formation of a litter layer or by preferentially grazing on neighbouring plants. They can also reduce the abundance of predatory voles, that prefer a high vegetation (Jacob et al. 2000; Pusenius and Ostfeld 2000). Jays are found to bury acorns more frequently in short than tall grassland vegetation (Kollmann and Schill 1996), suggesting that grazing may increase the amount of acorns that are buried in a grassland patch. In fire-dominated systems, herbivores can reduce the fuel load through grazing, thereby reducing the intensity of the fires and enhancing the survival of woody species (Roques et al. 2001; Briggs et al. 2002).

The notion that light demanding trees regenerate better outside than in the forest (Vera 2000; Kuiters et al. 2002) has major implications for landscape patterns. Large grazers may induce a shifting mosaic of grassland, shrubs and woodland (Vera 1997; Bokdam 1998; Olff et al. 1999; Vera 2000; Gillet et al. 2002).

Riverine floodplains may be a habitat where this shifting mosaic vegetation may have naturally operated under grazing by large herbivores. Herbivores prefer nutrient rich vegetation in relatively dry habitats (WallisDeVries and Schippers 1994; Olff et al. 2002) and these areas contain a large amount of thorny shrub species (fig. 7.7), that may be a response to naturally high grazing pressures (Myers 1987). Most of these shrubs are light demanding species, nowadays these communities are found in extensive grazed grassland or in forest clearances or forest edges (Stortelder et al. 1999). Also in savannas most species of spiny shrubs and trees are found on fertile soils (Campbell 1986; Owen-Smith and Cooper 1987; Milton 1991). Spines on shrubs are generally assumed to be anti-herbivore defences and have been shown to
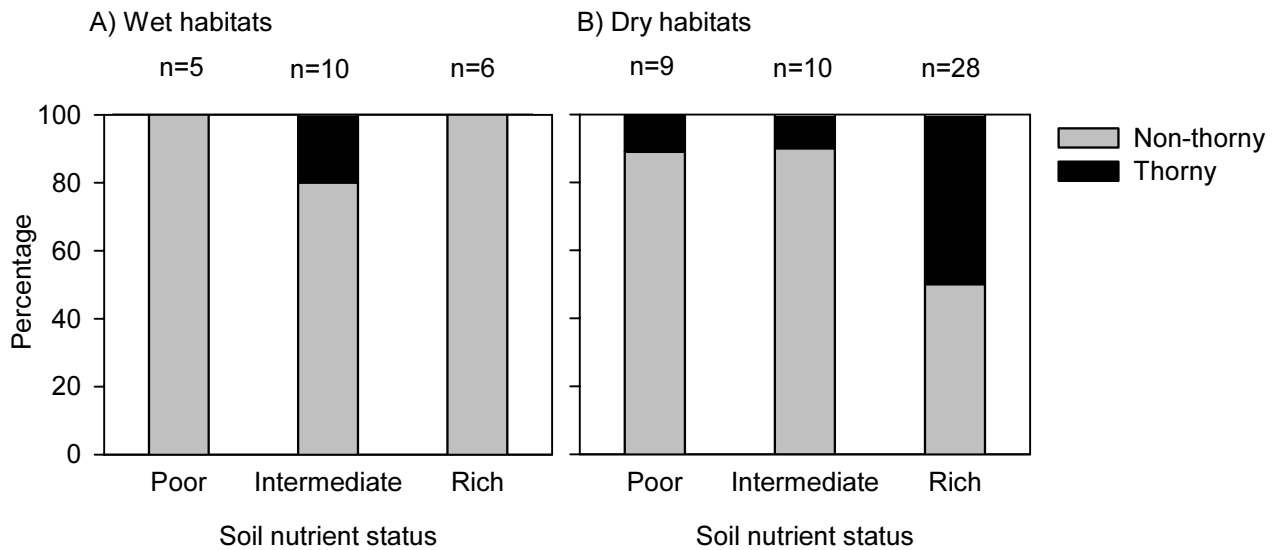
Figure 7.7 Percentage thorny species of shrubs in wet and dry habitats separated per habitat nutrient status. Per category the number of shrub species is indicated on top of the bars. Data based on Stortelder et al. (1999). See table 7.4 for details on the categories.

Table 7.4 Classification of shrub species per vegetation community in The Netherlands. The vegetation communities considered are all forest- and shrub communities treated in Stortelder et al. (1999). Only shrub species that were classified as woody species by Stortelder et al. (1999) were included in this table. This excludes *Rubus* sp., but includes *Rosa* sp. We distinguised shrubs from trees by size. From the genus *Salix* only *S. aurita*, *S. cinerea* and *S. repens* were considered shrubs. A shrub was considered thorny when it has spines or thorns on stem or branches with the exception of Ilex aquifolium, that has spines on its leaves that are commonly regarded as anti-herbivore defence and therefore we considered it thorny. Classification of soil nutrients and moisture is based on the accompanying habitat descriptions in Stortelder et al. (1999), but not on measured criteria.

Vegetation community[1]	No. of non-thorny shrub species	No. of thorny species	Total no. of shrub species	Soil nutrient status	Moisture
Alnetea glutinosae	6	0	6	rich	wet
Franguletea	7	1	8	intermediate	wet
Lonicero-Rubetea plicati	9	1	10	intermediate	dry
Salicetea purpureae	2	1	3	intermediate	wet
Quercetea robori-petraeae	7	1	8	poor	dry
Querco-Fagetea	13	8	21	rich	dry
Rhamno-Prunetea	7	13	20	rich	dry
Vaccinio-Betuletea pubescentis	5	0	5	poor	wet
Vaccinio- Piceetea	4	0	4	poor	dry

[1]Nomenclature follows Stortelder et al. (1999).

effectively reduce herbivore intake rate (Cooper and Owen-Smith 1986; Belovsky et al. 1991; Milewski et al. 1991). The question is against which herbivores these thorns were developed. The taxa *Prunus*, *Crataegus* and *Quercus* are known to have been present in Europe as far back as the Tertiair (Tallis 1991). From pollenanalysis and macrofossils it is known that *Prunus spinosa*, *Crataegus* and *Quercus* occurred together with large Pleistocene fauna like the elephant *Palaeoloxodon antiquus* (Gao et al. 2000). The interaction between Blackthorn, Oaks and large herbivores could therefore already have been shaped in concert with grazing by the Pleistocene fauna or earlier. In the Pleistocene vast areas of grassland interspersed with trees or bushes have existed. During the Holocene more trees appeared in the landscape; discussion remains whether this resulted in a closed canopy forest (Prins 1998; Svenning 2002) or an open park like landscape (Vera 1997, 2000). There is agreement however, that the areas along rivers have remained relatively open and contained at least some grassland that could have grazed by aurochs that may have preferred marshy areas before man introduced cattle (Van Vuure 2002). The interaction between cattle, thorny shrubs and light-demanding trees may therefore represent very old natural conditions. The mosaic of grassland, shrubs and trees often harbours many species due to the frequent transitions between structure types (Kollmann et al. 1999). Currently only a few remnants of this rich system remain scattered over northwestern Europe, which are mostly nature reserves. The most natural management of these areas may be the introduction of cattle or horses. Since these floodplains were naturally dynamic systems, a dynamic grazing pressure and natural river fluctuations belong with the system.

Acknowledgements

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Chapter 8

Synthesis and general discussion

Introduction

In the previous chapters of this thesis I showed that herbivores affect different aspects of grassland ecology, i.e. plant species richness, grassland plant community dynamics, nutrient turnover and establishment of woody species. These effects cannot be generalised: different herbivore species had different effects. In this synthesis I try to connect the observations that were made and explore how soil-plant-herbivore interactions shape the floodplain grasslands that I studied.

Determinants of plant species richness

The effects of different herbivore assemblages on plant species richness were not clear-cut (Ch. 3, 4). Excluding cattle resulted in decreased species richness in both exclosure experiments, but this effect was only significant in some years. Logically, this suggests that the different herbivore assemblages had little effect, if any, on plant species richness. Is that true? From chapter 3 we know that plant species richness was correlated with light penetration to the soil and even better with vegetation height, that presumably captured the heterogeneity in light availability better at a plot scale and is a more stable, integrating measure. Plotting vegetation height versus plant species richness for both exclosure experiments using all treatment plots available (fig. 8.1) showed that in both experiments species richness was negatively related to vegetation height. Although significant, variation exists around the relation, and it is non-linear. This graph shows why the average species richness is not very different between the grazing treatments. Nevertheless, this analysis yields an important conclusion regarding diversity. The most diverse plots are found at a low mean vegetation height, mostly in the cattle grazed plots. The vegetation height of 15 cm seems to be a critical threshold: I observed no plots with more than 15 species per $4m^2$ with over 15 cm height and no plot with more than 10 species per $1m^2$ with a vegetation of more than 15 cm height (fig. 8.1). Within short vegetation however, also plots with low species richness were found, causing considerable variation in diversity at this height range. For the exclosures established in 1994 I identified several factors important for species richness, but these factors were not necessarily measured in the same year. I assumed that environmental factors as light penetration and soil total nitrogen levels would be similar between years and correlated these parameters with plant species richness (tab. 8.1). Because the measurements were taken at different spatial scales and replication, I expressed the relation at the plot level, with n=90 and at the block level with n=15 (tab. 8.1). Apart from light penetration, the soil nitrogen mineralization rate was the only other significant variable: more available nitrogen coincided with less species in a plot. I did not measure nitrogen mineralization in every vegetation plot and can therefore not calculate the relation within the cattle grazed area. However, adding nutrients did not cause any changes in the vegetation under cattle grazing, due to compensatory
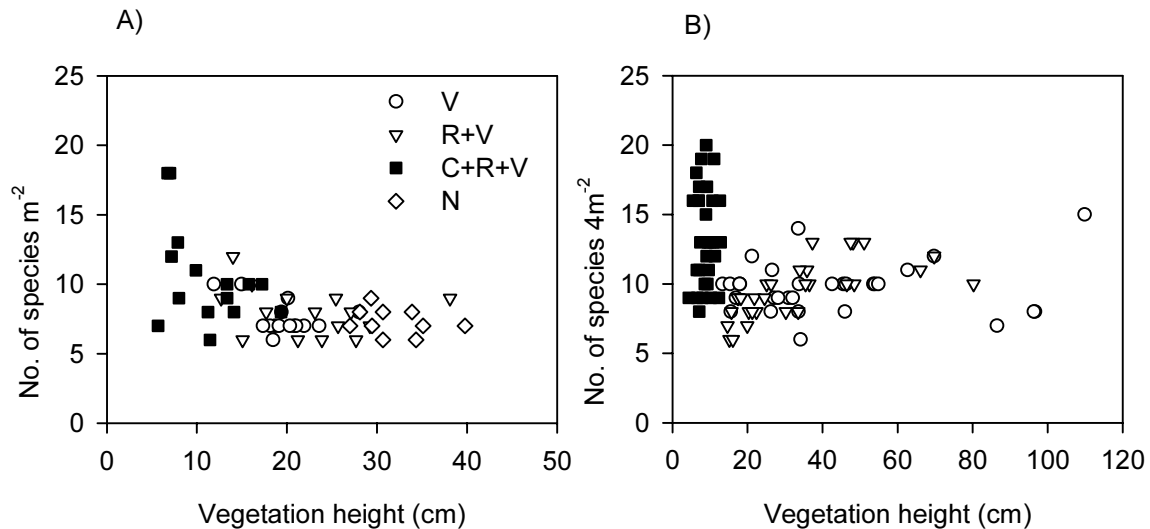
Figure 8.1 Relation between mean vegetation height (cm) and plant species richness. N: no vertebrate herbivores, V: voles, R+V: rabbits + voles, C+R+V: cattle + rabbits + voles.
A. Data from 2001 from the exclosures that were built in 1998, including the no vertebrate herbivore treatment, plot sizes are $1m^2$ (n=55, logarithmic regression, r^2=0.27, p<0.001).
B. Data from 2000 from the exclosures built in 1994, plot size is $4m^2$ (n=90, r^2=0.11, p=0.001).

grazing in the fertilized plots (Ch. 4). Therefore I do not expect nitrogen availability to explain the variation in plant species richness within the cattle grazed area. Also light availability was unrelated to plant species richness at this level of comparison (tab. 8.1). The amount of bare soil (as percentage of plot size) was highly related to plant species richness in the cattle grazed plots. Unfortunately these data are not available for the other grazing treatments. These results suggest that especially cattle are creating the conditions that potentially allow a high species richness.

High species richness is expected in a plot when many new species manage to colonize that location each year, compensating local extinctions through competition and chance. The creation of experimental gaps and sowing of subordinate herbs, the major part of the species pool, showed that these species were both dispersal limited and showed reduced seedling survival or did not germinate at all in grazed grassland patches without any further disturbance (Ch. 2). Suitable regeneration niches enhance the colonization rates by these species, such as bare soil patches. In addition, this may also expose the local soil seed bank. Indeed, a higher average species richness under cattle grazing in the 1998 exclosures was caused by increased colonization rates (Ch. 3: fig. 3.7). Cattle may create the conditions under which high species richness is possible, but rabbits were the main creators of bare soil patches (Ch. 2). The combination of cattle and rabbit grazing may therefore result in the highest species richness.

Table 8.1 Effects of different parameters on species richness in the 1994 exclosures, in 2000. All parameters are correlated (Pearson correlation) with species richness 4m^{-2} at the level that the parameter was measured. When measurements for all treatment plots were available, n=90 (6 plots * 5 replicates * 3 grazing treatments), when only one measurement per block was available, n=15.

Parameter	r	p
All treatment plots, n=90		
Light penetration to the soil	0.260	0.013
Mean vegetation height	-0.202	0.056
All treatments, n=15		
Light	0.414	0.125
Vegetation height	-0.274	0.323
N content of living biomass	0.052	0.855
N content of soil	-0.368	0.177
N mineralized year^{-1}	-0.600	0.030
Treatment grazed by cattle, rabbits and voles (C+R+V), n=30		
Light	0.038	0.841
Vegetation height	0.038	0.841
Average amount of rabbit droppings	-0.225	0.232
Average amount of bare soil	0.472	0.008

Diversity at different scales

Spatial scale is a critical determinant of species diversity (Ricklefs 1987; Glenn and Collins 1992). Species area relations may cross, causing conclusions on diversity to change with the scale at which these differences are evaluated. I compared species richness for the 1994-established exclosures in 2000 between three spatial scales: plot level (4 m^2), block level (6 plots = 24 m^2) and per grazing treatment (5 blocks = 120 m^2). In total 60 species were present in 2000 when all grazing treatments were combined. This analysis indeed shows that the effect of cattle grazing changes over scales (fig. 8.2). Compared with vole grazing, cattle grazing had a positive influence on species richness at smaller scales, while this effect disappeared and even became slightly negative at the largest scale of measurement. This might be due to two reasons: prolonged cattle grazing gradually excludes species from the local species pool that are intolerant to this type of grazing, therefore a pool of grazing tolerant species remains (Milchunas et al. 1988). Secondly, cattle can have a neutralizing effect on the vegetation composition. In chapter 4, I showed that cattle suppressed fluctuations in the dominant species, thereby stabilizing the vegetation. They also suppressed the expression of underlying soil heterogeneity in the vegetation composition, making the vegetation spatially more homogeneous. Also with respect to plant dispersal, cattle can decrease larger-scale differences since they disperse seeds over long distances and can spread species through the entire area, thus decreasing differences between local communities (Malo and Suarez 1995b). However, our largest scale was 120 m^2 that still lay within a rather intensively grazed vegetation. At larger scales large herbivores prefer certain vegetations over
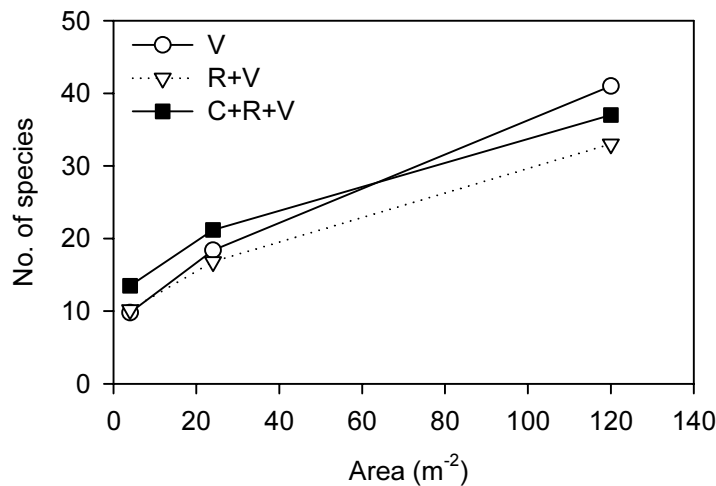
Figure 8.2 Plant species richness under grazing by different herbivore assemblages at different scales. V: voles, R+V: rabbits + voles, C+R+V: cattle + rabbits + voles. Data from the 1994 exclosures in 2000. In total 60 species were found in all grazing treatments together.

others, where they will graze more intensively (WallisDeVries et al. 1994). The intensity of grazing and the spatial grazing pressure determines whether grazing intolerant species will completely disappear or can survive in neglected patches or through association with unpalatable species (Callaway et al. 2000; Milchunas et al. 2002) (Ch. 7). At a stocking density of 1 heifer per 2.5 ha, there will be enough variation in grazing pressure to allow refuges for grazing intolerant species in a 100 ha area as our study site was (pers. obs.). The landscape mosaics that established under low intensity grazing by large herbivores are expected to promote diversity at a landscape scale (see Chapter 7).

Limiting factors for plant growth

Since the total amount of species is much larger than in the separate grazing treatments (60 species in total versus 40 species in the vole grazed treatment), I explored the differences in species composition between the cattle grazed and vole grazed treatments (app. 8.1). I used minimum plant height and the Ellenberg N-value (an index of soil fertility of their main habitat), both obtained from the Dutch botanical register (Botanisch Basis Register (CBS 1993)), to characterize the species growing in the different grazing treatments. The species in the cattle and vole grazed treatment differed significantly in average minimum height but not in N-value (app. 8.1). However, the species that were unique to these treatments did show such a pattern: in the vole grazed treatment more species indicating fertile conditions were found, whereas in the cattle grazed treatment especially shorter species were found (tab. 8.2). This is in agreement with the measurements of abiotic factors in the

Table 8.2 Characteristics of the V and C+R+V grazed treatment through plant species that grew exclusively in one of the treatments (in 2000). Minimum plant height and Ellenberg nitrogen value were obtained from the Dutch botanical register (Botanisch Basis Register (CBS 1993)). Data were tested with a One-way anova. Different letters indicate statistically different plant heights or Ellenberg N-values (p<0.05).

Plant characteristics	Species in V only	Species in C+R+V only	Species shared	F	P
Minimum plant height (cm)	40.0 ± 6.6^a	9.5 ± 2.0^b	25.4 ± 5.0^a	$F_{2,47}=7.035$	0.002
Ellenberg N-value	7.2 ± 0.5^a	4.9 ± 0.8^b	5.0 ± 0.4^b	$F_{2,37}=4.942$	0.013

Table 8.3 Shoot-root ratio (in August 2001) and abiotic parameters in the 1994 exclosures (light measurements from August 2000, mineralized nitrogen from 1999). V: voles, R+V: rabbits + voles, C+R+V: cattle + rabbits + voles.

Species	V	R+V	C+R+V	F	p
Shoot-root ratio	9.7 ± 3.1^a	6.5 ± 1.9^{ab}	2.1 ± 0.6^b	$F_{2,12}= 4.715$	0.031
Light to soil (%)	11.0 ± 3.9^b	1.8 ± 0.5^a	24.2 ± 4.9^c	$F_{2,42}= 18.472$	<0.001
Annual mineralized nitrogen (mg kg^{-1} soil)	189 ± 22^a	185 ± 22^b	121 ± 14^b	$F_{2,8}= 4.815$	0.042

different treatments (tab. 8.3), which showed reduced nitrogen mineralization in the cattle grazed treatment and reduced light availability in the vole grazed treatment. Together these observations of both plant species characteristics (app. 8.1) and abiotic parameters (Ch. 5) indicate that plants might be light limited in the vole grazed treatment and nitrogen limited in the cattle grazed area. Further evidence might come from the shoot-root ratios (tab. 8.3). The shoot-root ratios are very different; plants in the vole grazed treatment have over 4 times more of their biomass as aboveground standing crop than the cattle grazed plants. This is not purely an effect of removed aboveground biomass in the cattle grazed plots, since also absolutely more root mass is present in the cattle grazed plots (Ch. 5). Voles did consume mainly aboveground vegetation, therefore the reduced proportional root mass in the vole grazed treatment is not reflecting belowground vole herbivory (fig. 8.3). Although I will not draw strong conclusions because of a lack of root and aboveground biomass productivity data, these differences could well be interpreted as a physiological response to light limitation (more investment in shoots) in the vole grazed treatment and nitrogen limitation in the cattle grazed treatment (more investment in roots) (Tilman 1985; Olff 1992). This suggests that cattle grazing induces a shift from light limitation to nitrogen limitation for plant growth in the habitat I studied. Because light is a single resource and competition for light is asymmetric, the possibilities for coexistence between species are limited. When soil resources are limiting, more species can coexist (Tilman 1982) and, especially when combined with predation of the dominant species (Harper 1969), this may lead to reduced extinction and therefore more diverse vegetations. In addition, depletion zones due to limited mass flow and diffusion could facilitate further coexistence opportunities (Huston and DeAngelis 1994).
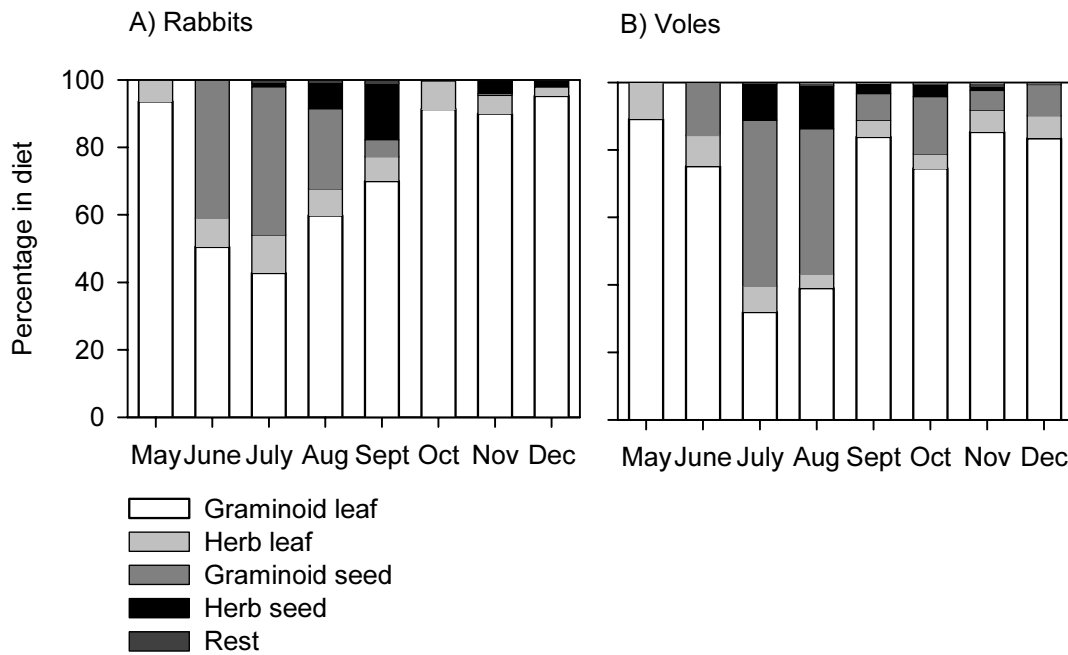
A) Rabbits   B) Voles

Figure 8.3 Diet of rabbits (A) and voles (B) in Junner Koeland in 2001, determined from faecal analysis by counting epidermis fragments. Droppings were collected around the 1994-exclosures.

In this study no effects of herbivores on extinction rates were found, but this may depend on the local grazing pressure by herbivores and their selectivity. In this site, all herbivores seem to focus on the same dominant species of grasses that are tolerant to grazing. Stronger effects on local extinction rates are expected in habitat where subordinate herbs are of much better quality than the dominants (e.g. sage brush landscapes) and/or in habitats with a short evolutionary history of grazing (Milchunas et al. 1988; Ritchie et al. 1999a).

Effects of separate herbivores on plant communities

The use of exclosure treatments in field experiments has some specific complications. Applying a treatment (excluding a species) usually means that other organisms respond at the same time. Such biotic treatments are not constant in time in magnitude and type. Originally, the 1994 exclosure study was designed to just separate the effects of rabbits and cattle on the vegetation. Soon after establishment of the exclosures, vole numbers strongly increased in the exclosures. Since many tracks of vole activity and grazing were present it was decided to start monitoring their density that became very high. Therefore, in the 1998 exclosure set a vole exclosure was included in the design. However, by excluding voles still herbivorous insects, as grasshoppers or belowground organisms, as nematodes, are present in our no-herbivore treatment that could redistribute themselves unequally over the vertebrate grazing treatments and can affect plant abundances (Ritchie and Tilman 1992; De Deyn et al. 2003). It is therefore not easy to study the separate effects of different species of herbivores on complex (natural) vegetations. It should be realised

153

that such confounding effects through the response of other trophic groups are most likely to occur in many other ecological field experiments (e.g. fertilizer application trials, plant diversity manipulations) but they are very seldom reported or even recognised.

In chapter 5 I compared the different densities of herbivores in terms of daily energy expenditure. With the aid of literature values I calculated the yearly offtake of biomass by the different herbivores in the different grazing treatments (tab. 8.4). This yields similar results as the energy expenditure calculations: the offtake of biomass in the different grazing treatments was similar for all assemblages at about 100 g m^{-2}. In 2001 I erected small exclosures within the vole grazed treatment from early May until late October. During this period voles had removed on average 90 g m^{-2} (unpubl. data), which is well in line with the calculated 104 g m^{-2} for a whole year (tab. 8.4). Also plant standing crop, measured in August 2001 yielded similar values in the three grazing treatments (Ch. 5). Therefore, I conclude that voles indeed exerted a grazing pressure in the same order of magnitude as cattle. Also, voles consumed mainly grass leaves, unless seeds were available, as shown in diet analyses (fig. 8.3a).

Table 8.4 Herbivore densities (no. of individuals ha^{-1}) and calculated plant biomass removal (g m^{-2}) in the grazing treatments in 1998. Data from the 1994 exclosures. The cattle densities were corrected for the time they were present in the area during spring and fall. * No data were available on vole densities in spring, therefore the average density between winter and summer was used. Cattle were assumed to consume 6.5 kg dry matter day^{-1} (WallisDeVries 1994), rabbits 80 g day^{-1} (Short 1985) and voles 5 g day^{-1} (Young Owl and Batzli 1998). The duration of each season was set to 90 days (3 months), winter started in December. Vole density was measured through live trapping, rabbit density through pellet counts and cattle density directly around the exclosures was not known, therefore the stocking density for the whole study area was used.

	Cattle	Rabbits		Voles			Total		
	C+R+V	C+R+V	R+V	C+R+V	R+V	V	C+R+V	R+V	V
Density (individuals ha^{-1})									
Winter	0	11 ± 1	7 ± 2	14 ± 14	0	28 ± 28			
Spring	0.2	9 ± 2	16 ± 1	7*	160*	430*			
Summer	0.4	14 ± 2	3 ± 2	0	319 ± 64	833 ± 64			
Autumn	0.2	19 ± 4	8 ± 2	278 ± 124	583 ± 261	1000 ± 447			
Consumption (g m^{-2})									
Winter	0	8	5	0.6	0	1	9	5	1
Spring	12	7	12	0.3	7	20	19	19	20
Summer	24	10	2	0	15	38	34	17	38
Autumn	12	14	6	13	27	46	38	32	46
Total	48	39	25	14	48	104	100	73	104

In the 1998 exclosures that included a vole exclosure, vole densities remained much lower than in the 1994 exclosures. The comparison of the vegetation in the vole exclosure and vole grazed plots reveals little effect of voles on the vegetation nor in biomass nor on plant species richness (Ch. 3) and also not on plant composition (unpubl. results). This can be due to the moderate vole density, but it also might be inherent to vole grazing. Several studies on vole herbivory in temperate grasslands have shown that voles have limited effects on the vegetation composition, despite moderate to high vole densities (Leutert 1983; Gibson et al. 1990; Norrdahl et al.

2002). Voles usually affect the abundance of a few plant species that they prefer (Batzli and Pitelka 1970; Leutert 1983; Hulme 1996; Norrdahl et al. 2002). The strongest effects of voles on plant communities are reported during the peak of vole cycles, especially when high densities persist over the winter (Hamback et al. 1998). Community level effects are more likely to occur through granivory (Howe and Brown 2001). In our study system about half of the vole diet consisted of seeds in summer and early autumn. However, most of these seeds were from the dominant grasses and herbs (unpubl. data), that were perennials and in the case of the grasses clonal. Also, the dominant plant species contained rather high nitrogen concentrations (tab. 8.5) and probably very low anti-herbivore defences, which might have caused voles to include mostly dominant grasses and herbs in their diet, that reduces their potential to affect community composition. Also the mismatch in timing between the highest vole densities and the plant growing season (Ch. 4), may have contributed to the reduced effects of vole herbivory (Norrdahl et al. 2002). A last reason for limited effects of voles may be that plant growth was light limited in the vole grazed treatments and voles are too small to counter light limitation through grazing, as cattle and rabbits can (the latter only at high densities)(Ch. 4).

Table 8.5. Nitrogen content (mg N g^{-1} dry weight) of the dominant plant species in the study area, collected in August 2001 in the cattle grazed area, n=3. Data tested with a Oneway Anova, $F_{6,14}$= 19.090, p<0.001. Different letters indicate statistically significant different nitrogen contents p<0.05.

Species	Functional group	N mg g^{-1}
Carex hirta	Sedge	20.3 ± 0.8[a]
Luzula campestris	Graminoid	21.5 ± 0.9[a]
Festuca rubra	Grass	23.4 ± 1.2[ab]
Holcus lanatus	Grass	28.7 ± 0.9[bc]
Agrostis capillaris	Grass	29.6 ± 3.3[bc]
Rumex acetosa	Herb	36.7 ± 2.1[c]
Trifolium repens	Legume	37.5 ± 1.3[c]

As the effects of direct vole grazing may be limited, voles have the potential to affect nutrient cycling through burrowing, returning nutrients through excreta and collecting plants in below-ground caches. The effects of voles on nutrient dynamics are little studied, but first calculations show that they can potentially strongly enhance nutrient cycling (Pastor et al. 1996), (Ch. 5).

For rabbits, effects were either in concert with voles, or in concert with cattle in the exclosure design. Also rabbit densities showed a clear trend over the time (fig. 8.4a). There used to be a large population of rabbits, but since 1996 numbers declined steeply, presumably caused by the national outbreak of the rabbit haemorrhagic disease virus (RHD, also known as Rabbit Calicivirus Disease (RCD)) in the Netherlands in the early '90's. Therefore with progressing years of exclosure, also the absolute number of rabbits decreased which makes it hard to separate factors. When tested within a year, rabbits had a negative effect on the species richness in the rabbit + vole (R+V) grazed treatment in 2000 (Pearson correlation: r=-0.56,

p=0.001, n=30), but not in the cattle grazed plots (r=-0.225, p=0.232, n=30). Rabbits did consume dicots, but most of their diet consisted of the dominant grassland plants (fig. 8.3b). The effect of rabbits is complex, they can exert a positive effect through grazing a short sward, reducing light limitation, and through creating disturbances that are suitable regeneration niches and dispersing seeds (Malo et al. 1995a; Pakeman et al. 1998). However, the effects can also be negative through selective consumption of rare herbs or seedlings (Crawley 1990). Probably it is dependent on the population density what the size and direction of the effect of rabbits will be.

Rabbits had a strong inhibitory effect on establishment and encroachment of woody species (Ch. 7). This effect is found for several other small herbivores. Voles are found to be the major cause of inhibition of establishment of woody species in oldfields (Kollmann et al. 1996; Manson et al. 2001) and prairie dogs can have the same function in prairies (Weltzin et al. 1997). Similar inhibiting effects of small mammals on tree recruitment have been found in African savannas (Miller 1994).

Interactions between herbivores; determinants of herbivore distributions

Strong interactions between the herbivores were found: rabbits were generally facilitated by cattle, whereas voles showed a strong preference for the treatments where cattle were excluded. The occurrence of voles was strongly related to the height of the vegetation (unpubl. data), probably due to reduced perceived predation risk in higher vegetations (Jacob et al. 2000; Smit et al. 2001). Rabbits, on the other hand, were attracted by short vegetation (Ch. 3, 6), either because of enhanced food quality or increased cropping rates or both (Iason et al. 2002). The facilitation effect became stronger over years, probably due to a combination of vegetation changes in the treatment were cattle were excluded and decreasing rabbit numbers (fig. 8.4b).

Future research

The data presented in this thesis, result from a study at one site with a given set of herbivores. Therefore the interpretation of the results is limited by the prevailing soils and climate and specific characteristics of the herbivores and plant species involved in this study. The effects of the herbivores on the vegetation depend to a large extent on whether they consume the dominant or subordinate species. The palatability of the dominant species in turn depends on herbivore selectivity and food quality demands, but also on the habitat productivity (Milchunas et al. 1993; Olff et al. 1998). In unproductive habitats the dominant plant species contain high concentrations of secondary compounds, have physical defences (thorns) or are of poor nutritional quality, causing (small) herbivores to graze on palatable subordinate species and thereby decreasing diversity. First literature compilations (Proulx et al. 1998) and a field study (Osem et al. 2002) point in this direction. Also the effect of small versus large herbivores may be more different in these systems (Ritchie et al. 1999a). The effect of herbivores on other grassland system properties, as nutrient turnover may equally well differ over a nutrient gradient. The connection between factors that limit
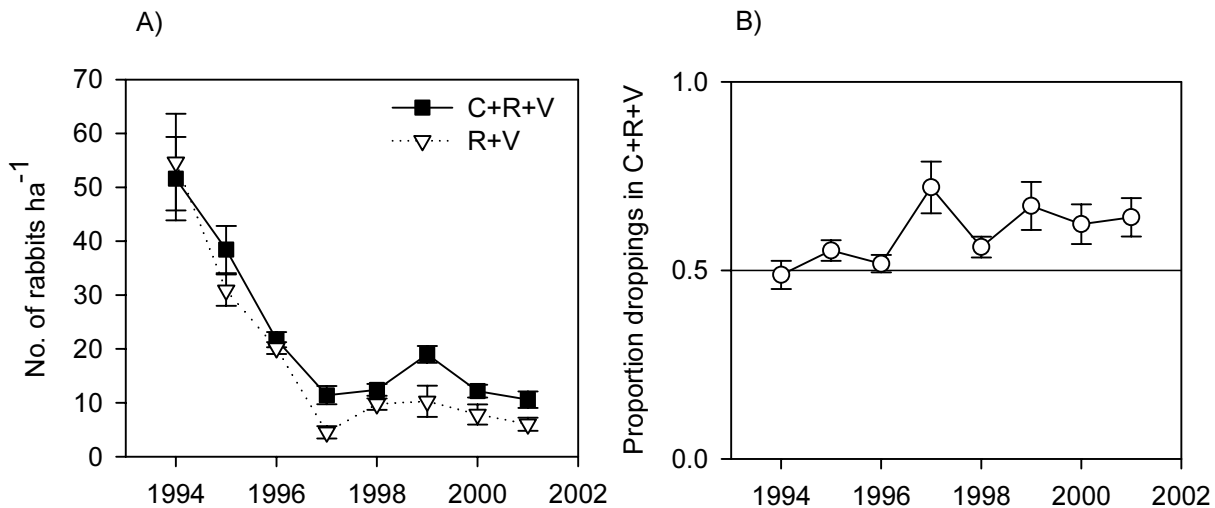
A)

B)

Figure 8.4 A. Rabbit density in the area over the years. Rabbit density is determined from the average amount of droppings counted each year, assuming a dropping rate of 400 droppings rabbit^{-1} day^{-1} (Lockley 1962). The amount of rabbits strongly decreased during the study, presumably due to the virus disease RHD.
B. Preference of rabbits for the cattle grazed plots expressed as the proportion of droppings found in the treatment grazed by cattle + rabbits + voles (C+R+V) relative to the total amount of droppings counted in the C+R+V and rabbit + voles only (R+V) plots.

plant growth and herbivore grazing pressure may help to understand and predict herbivore effects over a range of habitats (Olff et al. 1998; Huisman et al. 1999).

The connection between colonization and extinction processes through the approach of island biogeography (MacArthur and Wilson 1967) seems a promising method to summarize different herbivore effects in their final consequences for plant species richness (Glenn et al. 1992; Olff et al. 1998). Also the combination of experimental manipulation within different herbivore grazing treatments seems a useful way to study herbivore effects, whereas changes between different herbivore treatments are hard to interpret without additional experimentation.

The role of secretive herbivores, that are too small to be noticed by many people (as voles) or that are nocturnal, deserves more attention. These herbivores, especially the larger "small" herbivores as rabbits, hares and prairie dogs have been shown to have unexpected large effects on vegetation development, especially through their browsing capacities (Van der Wal et al. 2000). For many grasslands in the world, most about these herbivores remains unknown, although they are hypothesized to have a considerable effect on grassland vegetation (Keesing 2000). They may have effects on community structure and ecosystem functioning that are much stronger than expected from their abundance (see Power *et al*. (1996)).

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Appendix 8.1 Characteristics of species that occurred in the grazing treatments in 2000 (woody species were excluded from the analysis). Minimum plant height and Ellenberg nitrogen value were obtained from the Dutch botanical register (Botanisch Basis Register (CBS 1993)). The average minimum height and Ellenberg N-value were tested with a t-test comparing the species in the V and C+R+V grazed treatment. – means that no value was available.

Species	Grazing by voles (V)	Grazing by cattle, rabbits and voles (C+R+V)	Minimum height (cm)	Ellenberg N-value
Achillea millefolium	x	x	15	5
Agrostis cf. capillaris	x	x	10	4
Angelica sylvestris	x		90	-
Anthoxanthum odoratum	x	x	10	-
Arrhenatherum elatius	x		50	7
Barbarea stricta	x		30	8
Campanula rotundifolia	x	x	15	2
Carex hirta	x	x	30	5
Carex ovalis	x	x	20	3
Cerastium arvense	x	x	5	4
Cerastium fontanum	x	x	4	5
Cirsium arvense	x	x	60	7
Cirsium palustre	x	x	60	3
Cirsium vulgaris	x	x	60	8
Epilobium ciliatum	x	x	30	8
Festuca rubra	x	x	10	6
Galeopsis tetrahit	x		15	6
Galium aparine	x		60	8
Galium verum	x	x	5	3
Holcus lanatus	x		30	4
Juncus effusus	x		20	4
Luzula campestris	x	x	5	2
Phleum pratense	x	x	40	6
Plantago lanceolata		x	5	-
Poa annua		x	5	8
Poa pratensis	x	x	10	6
Poa trivialis	x	x	40	7
Potentilla erecta	x	x	15	2
Prunella vulgaris		x	7	-
Ranunculus acris		x	30	-
Ranunculus bulbosus		x	15	3
Ranunculus repens		x	10	-
Rumex acetosa	x	x	50	6
Rumex acetosella		x	10	2
Rumex crispus	x	x	100	5
Rumex obtusifolius	x		80	9
Sagina procumbens		x	2	6
Senecio sylvaticus	x		5	8
Sonchus asper	x		30	7
Sonchus oleaceus	x		30	8
Stachys palustris	x		30	6
Stellaria graminea	x	x	10	3
Taraxacum cf. officinale	x	x	5	-
Trifolium pratense		x	15	-
Trifolium repens		x	5	6
Urtica dioica	x		30	8
Veronica chamaedrys	x	x	10	-
Veronica officinalis		x	10	4
Veronica serpyllifolia		x	5	-
Mean minimum species height (cm ± se)	30.9 ± 4.1	19.7 ± 3.5	t-value=2.098	p=0.040
Mean nitrogen value (Ellenberg N-value ± se)	5.7 ± 0.4	5.0 ± 0.4	t-value=1.318	p=0.192

161

Summary

Most grasslands are, or have historically been, grazed by assemblages of vertebrate herbivores. Vertebrate herbivores are key components of grassland ecosystems with strong impacts on plant community structure and ecosystem functioning. Large domestic grazers are used in nature conservation management to maintain or restore grassland biodiversity. However, in field experiments contrasting effects of herbivores on plant diversity are found. Herbivores mostly enhance plant diversity, but also cases were herbivores decrease diversity, or have a neutral effect, are frequently reported. These conflicting results are also found in studies that investigate the effect of herbivores on ecosystem properties. Again herbivores are shown to both enhance and decrease nitrogen mineralization and turnover in different grasslands and field experiments. Herbivores can have a whole range of direct and indirect effects that can lead to an increase but equally well a decrease in plant production, depending on which effects are most important. Recent work proposed that these conflicting effects of herbivores may depend on habitat productivity, herbivore density or herbivore type. In this thesis I explore the impact of different-sized herbivores on their environment at different levels ranging from plant species richness, vegetation dynamics and nutrient dynamics to grassland-woodland transitions. I studied an assemblage of cattle, European rabbits (*Oryctolagus caniculus*) and common voles (*Microtus arvalis*) grazing in a moderately nutrient rich floodplain grassland in The Netherlands (Junner Koeland). The Junner Koeland is managed as a nature reserve where cattle are used as a management tool. Cattle graze at a stocking density of 0.4 heifer ha^{-1}, there are roughly 15 rabbits ha^{-1} and 100 voles ha^{-1}.

The vegetation consists of a matrix vegetation of two co-dominant grass species, *Festuca rubra* and *Agrostis capillaris*. In between this matrix several other graminoids, but mainly a large number of subordinate herbs occur infrequently. During seven years of measurements species richness (between 8 and 20 species 4 m^{-2}) was largely determined by the frequency of occurrence of this long list of subordinate herbs, which experienced high colonization and extinction rates in 4 m^{2} plots. Therefore re-colonization opportunities are of major importance to maintain a speciesrich vegetation. Sowing experiments with four subordinate herb species revealed that germination success was considerably improved in artificially created bare soil patches. Two of the four sown species managed to realize some germination in a control undisturbed grassland patch; the others did not germinate there at all. In the undisturbed vegetation, rabbits were the creators of most of the bare soil patches that were naturally present. Cattle did disperse many more seeds via their dung than rabbits, but seedlings established poorly on cattle dung patches. An experiment with artificially created dung patches, made from paper, revealed that the nutrients in the dung made the vegetation grow too tall, thereby outshading the seedlings. Therefore cattle had the highest potential as seed dispersers, whereas rabbits created the best regeneration sites. Plant species richness was positively related to the amount of bare soil present in the vegetation.

Plant species richness at a patch is determined by local colonization and extinction rates. Herbivores can therefore affect species richness through changing colonization or extinction rates or both. Species richness in grasslands has been shown to depend on the amount of plant biomass available, but also a litter layer of dead plants is reported to inhibit seedling establishment and therefore to cause a decline in plant species richness. Through grazing, herbivores remove living biomass, but also prevent that this biomass becomes litter after the death of the plants. By removing biomass and thus preventing litter formation herbivores may therefore promote seedling establishment and increase plant species richness. In a three-year experiment I excluded herbivores (cattle, rabbits and voles) stepwise from large to small and manipulated litter accumulation and biomass removal through raking and clipping. In the absence of cattle grazing plant litter accumulated over the years. The density of rabbits and voles on this site was relatively low; therefore grazing by rabbits and voles had little impact. Plant species richness was strongly negatively correlated with total plant biomass (including litter) and average vegetation height and strongly positively with light penetration through the vegetation. Litter had an additive effect with standing living and dead plant biomass and no separate unique effects of its own. The vegetation grazed by cattle, rabbits and voles was already relatively tall, lowering the vegetation height through experimental clipping strongly increased species richness in the plots grazed by all herbivores. This increase was due to increased species colonization rates whereas extinction rates were similar to the unclipped plots. An analysis according to island biogeography theory confirmed these experimental results for the undisturbed vegetation: grazing by all herbivores significantly increased colonization rates for plots with low species richness compared to ungrazed vegetation whereas extinction rates were unaffected by grazing. Therefore the equilibrium number of species was higher in grazed (8 m^{-2}) than in ungrazed (6 m^{-2}) vegetation.

Without directly affecting the number of species, herbivores can exert a strong influence on community composition and dynamics of individual species. I experimentally investigated the effects of different-sized herbivores on the spatio-temporal stability of grassland community composition and biomass by stepwise exclusion of cattle, rabbits and voles from large to small body size at two nutrient levels. This experiment was performed in another set of exclosures than the litter experiment mentioned earlier. I found that large herbivores had a strong stabilizing effect on plant community composition, whereas excluding them (leaving only small herbivores) induced strong variability in plant species composition, both in space and time. Seven years of nutrient addition did not change the vegetation in the presence of large herbivores, due to selective grazing in the fertilized plots. Small herbivores in contrast, did not counteract the effects of nutrient addition through enhanced grazing, resulting in strong compositional changes in the vegetation. Furthermore, large herbivores suppressed the expression of small-scale soil nutrient heterogeneity in the vegetation, resulting in homogeneous vegetation, while the presence of small herbivores enhanced vegetation heterogeneity. The temporal stability of the vegetation composition increased towards higher plant species richness, and large herbivores increased plant species richness more than small herbivores.

In the same exclosure set I also examined the effect of different-sized herbivores on nitrogen cycling by sampling vegetation and soil and monitoring herbivore densities five to seven years after the start of the exclosure experiment. Exclusion of cattle lead to an increase in vole numbers and a 1.5 increase in net annual nitrogen mineralization, while total herbivore densities (corrected to metabolic weight) were similar in the grazer assemblages. Also potential nitrogen mineralization rates measured under standardized laboratory conditions were the same for all three grazing treatments as well as all soil parameters, plant biomass and plant nutrient content measured in the field. Microclimatic differences (i.e. soil temperature) may have caused part of the measured differences in field mineralization rates, although we did not measure it. Timing and height of the mineralization peak in spring was the same in all treatments, but mineralization in the vole grazed treatment showed a peak in autumn, when mineralization had already declined under cattle grazing. The mineralization peak in autumn coincides with a peak in vole density and high levels of nitrogen input through vole faeces at a fine-scale distribution, whereas under cattle grazing only a few patches receive all nitrogen and most experience net nutrient removal. This different pattern of nutrient re-distribution may also be a cause of the lower nitrogen mineralization under cattle grazing.

Small herbivores as rabbits forage in a gradient from their burrows or cover into a meadow, thereby creating a gradient in grazing pressure. This gradient is hypothesized to result from a combination of increased predation risk further from cover and increased energy return when herbivores stay close to their burrows and therefore spend little travelling time. We tested the relative importance of food availability and predation risk on rabbit foraging behaviour in an experiment. Dropping counts revealed that rabbits grazed in a gradient from their burrows into the meadow, thereby creating a gradient in increasing vegetation height, biomass and decreasing plant nutrient content. When nitrogen content was experimentally increased with 150% through fertilizing and mowing, rabbits visited these plots four times more frequent than the untreated control plots. Rabbits preferred fertilized plots more strongly with increasing distance from burrows. Addition of predator scent in the form of mink pellets did not result in different visitation rates by rabbits. The combined addition of fertilizer and mink pellets yielded the same results as addition of fertilizer alone. However, the mink pellets did tremendously change the activity pattern of rabbits as measured with infrared detectors. Rabbits were predominantly nocturnal but shifted their activities to the day when mink pellets were added, resulting in equal activities during night and day. This shift in activity pattern towards the day may be because rabbits associated the mink pellets with a nocturnal predator as their natural mustelid predator, the polecat, is. We conclude that rabbits are sensitive to perceived predation risk, but that this does not influence their spatial grazing pressure. A selection for the highest food quality or intake rates on the other hand can explain the observed natural rabbit grazing gradient. Food quality was highest close to the burrows, therefore rabbits selecting for high quality food should forage most intensely close to the burrows and only move further away for higher quality items or when the vegetation close to their burrows is depleted. Through intensive grazing close to the

burrows rabbits facilitated for themselves either through stimulating fresh protein rich re-growth or via the return of nutrients through faeces or both.

If grassland is not grazed it will become a forest through vegetation succession. However, also under grazing shrub encroachment and tree establishment may be possible. I tested the hypothesis that grazing induces shifting mosaics of grassland, shrub thickets and woodlands through the key process of associational resistance: the protection of palatable young trees by thorny shrubs. A cross-site comparison of four floodplain woodlands in north-western Europe showed that Oak can regenerate in the presence of large herbivores through spatial association with Blackthorn, a clonal thorny shrub. I performed an exclosure experiment with transplanted Oak seedlings to test the importance of protection from grazing and landscape position in a cattle grazed area. Oaks grew best in grassland exclosures and on the edge of thorny shrub thickets that received most light. Oak survival was severely reduced in Oak woodland. The high success of young Oaks among young thorny shrubs thus reflects an optimal balance between sufficient protection from herbivores and sufficient light availability. However, in two of the four studied floodplains, no young trees could be found. These sites both experienced a high rabbit density over the past ten years. Rabbits graze both on young ramets of Blackthorn as well as on young Oaks. In a survey with aerial photographs of 50 years of shrub and tree expansion on one of the sites we found that expansion of Blackthorn shrubs and the establishment of Oaks coincided with rabbit abundance and not with livestock density. Therefore we conclude that the process of associational resistance did not work against rabbits, because they both consume the Oak seedlings as well as the thorny shrubs that have no thorns when they are young. Under grazing by large herbivores, a shifting mosaic of grassland, shrubs and trees may develop that has high conservation values due to the positive correlation between heterogeneity in vegetation structure and biodiversity of e.g. insects and birds. The interaction between thorny shrubs, light-demanding trees and large grazers may be a relict of the time when native large grazers were still present in north-western Europe.

Samenvatting

De meeste graslanden zijn, of waren vroeger, begraasd door verschillende soorten herbivoren. Herbivoren kunnen een sleutelrol vervullen in grasland ecosystemen door een sterke invloed uit te oefenen op de plantensamenstelling en de nutriëntenkringloop. Grote grazers worden in het natuurbeheer gebruikt om de biodiversiteit van graslanden te vergroten of te behouden. Het blijkt echter dat in veld experimenten tegenstrijdige resultaten worden gevonden wanneer het effect van grazers op de diversiteit van planten wordt gemeten. Meestal hebben de grazers een positief effect op de diversiteit, maar het komt ook voor dat er negatieve of helemaal geen effecten worden gevonden. Deze tegenstrijdige resultaten worden ook gerapporteerd in studies naar het effect van herbivoren op de nutriëntenkringloop. Ook hier kunnen grazers de nutriëntenkringloop zowel versnellen als vertragen. Herbivoren kunnen zeer uiteenlopende directe en indirecte effecten op de vegetatie hebben, wat zowel tot een stimulans van de plantengroei als tot een remming kan leiden, afhankelijk van welke effecten de overhand hebben. Recentelijk is het idee geopperd dat deze tegenstrijdige resultaten misschien het gevolg zijn van verschillen in bodemvruchtbaarheid, graasdruk of type grazers tussen de studies. In dit proefschrift onderzoek ik wat het effect van grazers van verschillende grootte op hun omgeving is en of dit effect anders is voor grote en kleine herbivoren. Ik heb verschillende soorten effecten van herbivoren onderzocht: effecten op de vegetatie samenstelling, de nutriëntenkringloop, de vorming van struweel en de vestiging van bomen in grasland.

Ik heb de effecten van begrazing door koeien, konijnen en veldmuizen bestudeerd in een matig voedselrijke rivieruiterwaard, het Junner Koeland. Het Junner Koeland is een natuurreservaat van Staatsbosbeheer waar koeien worden ingezet om het gebied te beheren. De veedichtheid bedraagt 1 pink per 2,5 hectare (0.4 per ha), er zitten naar schatting 15 konijnen per ha en grofweg 100 veldmuizen per ha.

De vegetatie bestaat uit een grasmat met twee dominante soorten, Roodzwenkgras en Gewoon struisgras. Daartussen vind je een paar andere grassoorten, maar vooral veel soorten kruiden, die elk maar hier en daar voorkomen. Uit een meetreeks van zeven jaar bleek dat de soortenrijkdom van planten variëerde van 8 tot 20 per vier vierkante meter, waarbij het totaal aantal soorten vooral afhing van het aantal kruiden. De kruidensoorten verdwenen en verschenen snel in de vier vierkante meter proefvlakken gedurende de zeven jaar en vertoonden dus een hoge omloopsnelheid. Daarom is het voor het behoud van een soortenrijke vegetatie belangrijk dat er genoeg her-vestigings mogelijkheden zijn als een soort ergens lokaal verdwijnt. Zaai experimenten met vier kruidensoorten laten zien dat het kiemingssucces van deze soorten aanzienlijk verbeterde in kunstmatig gecreëerde open plekken met kale grond. Twee van de vier soorten konden nog enigszins kiemen in de gesloten grasmat, maar de andere twee kiemden daar helemaal niet. De meeste natuurlijke kale plekken waren gemaakt door het graafwerk van konijnen en mollen. Koeien transporteerden veel meer zaden via mest dan konijnen, maar kiemplanten deden

het slecht op koeievlaaien. Een experiment waarbij koeievlaaien werden nagemaakt met papier mache, liet zien dat de vegetatie rond koeievlaaien te hoog werd door de extra nutriëntenstoot, waardoor de kiemplanten te veel in de schaduw stonden. Koeien zijn daarom de beste zaadverspreiders, maar konijnen maakten de beste vestigingsplekken. De soortenrijkdom in de vegetatie was hoger in proefvlakken met meer oppervlak kale bodem. Konijnen droegen dus met hun graafactiviteietn aanzienlijk bij aan de planten soortenrijkdom.

De soortenrijkdom van planten op een bepaalde plek wordt bepaald door de plaatselijke uitsterf- en vestigingssnelheden. Herbivoren kunnen de soortenrijkdom dus vergroten door de vestigings mogelijkheden toe te laten nemen, de uitsterfkans te verkleinen of allebei tegelijk. Eerder is aangetoond dat de soortenrijkdom in graslanden samenhangt met de hoeveelheid gewas die er staat (veel gewas komt overeen met weinig soorten) en ook dat een laag dood strooisel de vestiging van kiemplanten kan remmen en zo de soortenrijkdom kan doen afnemen. Door te grazen verwijderen herbivoren biomassa en zo voorkomen ze dat dode planten zich ophopen. Op die manier zouden ze de vestiging van kiemplanten kunnen bevorderen en zo de soortenrijkdom doen toenemen. Gedurende een drie-jarig experiment heb ik de grazers (koeien, konijnen en veldmuizen) stapsgewijs van groot naar klein uitgerasterd. Binnen de begrazingsbehandelingen heb ik de hoeveelheid strooisel gemanipuleerd door het weg te harken en de hoeveelheid gewas door vlakken kort te knippen. Waar alledrie de herbivoren soorten buitengesloten waren hoopte het strooisel zich in drie jaar flink op, terwijl onder begrazing door alledrie de soorten veel minder strooisel was te vinden. Dit was voornamelijk te danken aan koeiebegrazing omdat de dichtheid aan konijnen en veldmuizen op deze plek relatief laag was. Het wegharken van strooisel had echter geen effect op de soortenrijkdom. De planten soortenrijkdom was sterk negatief gecorreleerd met de hoeveelheid gewas, inclusief strooisel en ook met de hoogte van de vegetatie, maar positief met de hoeveelheid licht die tot de bodem door kon dringen. Er was teveel gewas en daardoor te weinig licht waar herbivoren uitgesloten waren en zelfs de vegetatie die begraasd werd door koeien, konijnen en veldmuizen samen was al vrij hoog voor een hoge soortenrijkdom. Dat bleek uit het lager maken van de vegetatie door extra te knippen, wat zorgde voor een sterke toename van de soortenrijkdom. Dit werd veroorzaakt door een toename in het aantal vestigende soorten, terwijl de verdwijnsnelheden niet anders waren. Dit gold ook voor de niet-geknipte vegetatie: een analyse gebaseerd op de eiland-biogeografie liet zien dat het aantal zich vestigende soorten toenam onder begrazing door alle herbivoren samen ten opzichte van de onbegraasde vegetatie, terwijl de verdwijnsnelheden niet verschillend waren. Daardoor was het aantal soorten dat zich onder evenwichtscondities kan handhaven, groter in begraasde (8 per vierkante meter) dan onbegraasde vegetaties (6 per vierkante meter). Herbivoren (vnl. de koeien), zorgden dus voor een hogere soortenrijkdom op de plekken waar zij de vegetatie kort genoeg graasden om voldoende licht toe te laten, waardoor meer soorten zich konden vestigen.

Herbivoren kunnen zonder dat ze direct het aantal soorten veranderen, ook grote invloed uitoefenen op de vegetatie samenstelling en de dynamiek van individuele

soorten. Ik heb de effecten van herbivoren van verschillende grootte op de stabiliteit van de vegetatie samenstelling onderzocht door ze van groot naar klein een voor een uit te rasteren en daarnaast de vegetatie te manipuleren door extra nutriënten toe te dienen. Het bleek dat de koeien een sterk stabiliserend effect hadden op vegetatie samenstelling, terwijl het uitsluiten van koeien, waardoor veldmuizen overbleven, een sterke variatie in samenstelling induceerde, zowel in ruimte als tijd. Na zeven jaar nutriënten te hebben toegediend, was de vegetatie nog steeds hetzelfde onder begrazing door koeien, doordat ze extra in de nutriëntrijke proefvlakken graasden. De veldmuizen daarentegen waren niet in staat om het effect van de nutriëntengift te nivelleren door er extra te grazen, waardoor er sterke veranderingen optraden in de vegetatie samenstelling. Begrazing door koeien zorgde er ook voor dat onderliggende verschillen in voedselrijkdom van de bodem niet to uiting kwamen in de vegetatie, waardoor de vegetatie relatief homogeen was, terwijl begrazing door veldmuizen juist voor extra heterogeniteit zorgde. De stabiliteit van de vegetatie over de tijd was groter naarmate de soortenrijkdom groter was, en begrazing door koeien leidde tot een hogere soortenrijkdom dan begrazing door veldmuizen.

In dezelfde experimentele opzet heb ik ook onderzocht of de verschillende combinaties van herbivoren verschillende effecten hadden op de nutriëntenkringloop door het nutriëntengehalte van de vegetatie en bodem te meten nadat het experiment vijf jaar had gelopen. Het uitsluiten van koeien leidde tot een toename van veldmuizen en een anderhalf keer zo grootte hoeveelheid gemineraliseerde stikstof. De biomassa aan herbivoren, gecorrigeerd voor metabolisch gewicht, was vergelijkbaar tussen de herbivoren combinaties. Ook de potentiële mineralisatie, gemeten onder gecontroleerde lab omstandigheden, was gelijk tussen de begrazingsbehandelingen, alsmede de bodem nutriënten, vegetatie biomassa en nutriënten inhoud van de planten gemeten in het veld. Verschillen in microklimaat, zoals bodemtemperatuur, zouden een deel van de verschillen kunnen veroorzaken, hoewel we deze parameters niet gemeten hebben. De timing en hoogte van de mineralisatie piek in het voorjaar was hetzelfde voor alle begrazingsbehandelingen, maar er was een extra mineralisatiepiek in de behandeling waar de meeste veldmuizen graasden in de herfst, terwijl dan de mineralisatie in het koebegraasde deel al flink verminderd was. Deze mineralisatie piek in de herfst onder veldmuis begrazing komt overeen met de periode waarin zij hun hoogste dichtheden bereiken. Muizen zorgen voor een flinke nutriënten input via hun keutels, die ze op een fijne schaal verspreiden, terwijl koeien ervoor zorgen dat slechts enkele plekken heel veel nutriënten ontvangen, terwijl het grootste deel van de vegetatie een netto verlies aan nutriënten ondergaat door verwijdering van biomassa. Dit verschil in patroon van nutriënten verspreiding kan er ook voor zorgen dat de gemiddelde mineralisatie in het koebegraasde deel lager is.

Kleine grazers, zoals konijnen, foerageren in een gradiënt vanaf hun burchten in het veld, waarbij ze een gradiënt in graasdruk veroorzaken. Deze gradiënt wordt beschouwd als het resultaat van het gecombineerde effect van een hoger predatie risico verder van hun holen, en een verhoogde energiewinst als ze dichtbij hun holen blijven en dus weinig hoeven te lopen. Wij hebben het relatieve belang van

voedselbeschikbaarheid en veiligheid voor het fourageergedrag van konijnen gemeten in een experiment. Tellingen van konijnenkeutels wezen uit dat de konijnen inderdaad in een gradiënt graasden vanaf hun holen het veld in, waarbij ze een gradiënt van toenemende vegetatie hoogte, biomassa en afnemende plant nutrienten concentraties veroorzaakten.

Wanneer we de stikstof concentratie in de planten met 150% lieten toenemen door bemesting en maaien, bezochten konijnen deze proefvlakken vier keer zoveel als de onbehandelde controleplots. De konijnen hadden een grotere voorkeur voor deze proefvlakken naarmate ze verder van hun holen gingen. Het kunstmatig verhogen van het gevoel van onveiligheid door het toedienen van nertsenmest had geen effect op het aantal bezoeken van de konijnen aan de proefvlakken. Toch veranderde de nertsenmest het fourageergedrag van de konijnen aanzienlijk. Met infrarood apparatuur konden we meten dat de konijnen voornamelijk 's nachts actief waren onder ongemanipuleerde omstandigheden, terwijl de konijnen meer dagactief werden wanneer nertsenmest werd toegediend. Deze verandering kan verklaard worden wanneer konijnen de nertsenmest associëerden met een marterachtige, zoals de bunzing, hun natuurlijk predator. We concluderen dat konijnen gevoelig zijn voor een mogelijk verhoogde aanwezigheid van een predator, maar dat dit geen effect heeft op hun keuze waar ze grazen. Een selectie voor de hoogste voedselkwaliteit daarentegen kan het waargenomen natuurlijk graaspatroon van de konijnen verklaren. De nutriënten concentratie was het hoogste in de planten het dichtst bij de holen, waardoor konijnen die de hoogste voedselkwaliteit selecteren dicht bij de holen kunnen blijven en alleen verder weg gaan als de kwaliteit daar beter is, of als het voedsel bij de holen op raakt. Waarschijnlijk maken de konijnen de vegetatie geschikter voor zichzelf door intensief te grazen rond de holen, wat nutriëntrijke hergroei stimuleert of doordat ze de vegetatie bemesten met keutels, of een combinatie van beide.

Als graslanden niet begraasd worden ontstaat uiteindelijk bos door vegetatie successie. Struweel en bomen kunnen zich echter ook vestigen in begraasde graslanden. Ik heb de hypothese getest dat onder begrazing een mozaïek van grasland, struweel en bos kan ontstaan wanneer jonge smakelijke boompjes beschermd worden door stekelige struiken, een proces wat associational resistance wordt genoemd. Een vergelijkende studie tussen vier rivieruiterwaarden in noordwest Europa liet zien dat eiken zich kunnen verjongen in de aanwezigheid van grote grazers doordat ze opslaan in sleedoorns, een klonale stekelstruik. Ik heb een exclosure experiment uitgevoerd waarbij jonge eikjes geplant werden op diverse plekken in het landschap met en zonder bescherming tegen grazers. De eikjes overleefden en groeiden het best in grasland exclosures en in de rand van een sleedoornstruweel (zowel met als zonder exclosure) waar het meeste licht aanwezig was. De overleving van de eikjes was ernstig beperkt wanneer ze geplant waren in een eikenbos. Het succes van de eikjes in de struweelrand weerspiegelt waarschijnlijk een optimale balans tussen bescherming tegen begrazing en toch voldoende licht. In twee van de vier onderzochte gebieden konden we echter geen jonge boompjes vinden. Deze gebieden herbergden beiden een grote konijnenpopulatie gedurende de afgelopen tien jaar. Konijnen begrazen zowel de

jonge sleedoornuitlopers als de eikjes. Met behulp van luchtfoto's over de afgelopen 50 jaar van één van de gebieden konden we vaststellen dat de uitbreiding van het sleedoornstruweel en de vestiging van eikjes overeenkwam met de talrijkheid van konijnen en niet met de veedichtheid in het gebied. We concluderen dat het proces van associational resistance niet werkt tegen konijnen, omdat ze zowel de eikjes als het beschermende struweel opeten, waarvan de jonge uitlopers nog geen stekels hebben. Onder begrazing door grote grazers (als koeien) kan zich wel een mozaïek van grasland, struweel en bos vormen dat van belang is voor natuurbescherming, doordat veel overgangen in het landschap gerelateerd is aan een hogere diversiteit van bijvoorbeeld vogels en insecten. De interactie tussen stekelstruweel, lichtbehoevende bomen en grote grazers zou een overblijfsel kunnen zijn van de tijd waarin er nog natuurlijke grote grazers in noordwest Europa voorkwamen.

Samenvattend, herbivoren kunnen een grote invloed uitoefenen op hun omgeving, maar het effect is niet voor elke herbivoor hetzelfde: verschillende herbivoren hebben verschillend potentiëel dat kan worden verklaard door verschillen in lichaamsgrootte en soortverschillen in leefwijze (wel of niet gravend) en diëet selectie (wel of geen houtigen).

Nawoord

Op de voorgaande pagina's staat weergegeven wat ik de afgelopen vijf jaar heb gedaan. En toch, hoewel het aardig wat pagina's zijn, geeft het maar een zeer beperkt beeld van de voorafgaande jaren. Want hoe vang je de eerste bloeiende voorjaarszegge op een mierenbult, een ree met twee kalfjes op een grafheuvel 's ochtends vroeg of vier grote zilverreigers langs de westarm van de Vecht in een wetenschappelijke publicatie? Niet dus. Om nog maar te zwijgen van de uren knippen (van gewas), tellen (van keutels), meten (van boomdiameters), graven (naar mineralisatiezakjes), knutselen (van exclosures en knipframes), hameren (van palen), sjouwen (van materiaal), rapen (van keutels), verzamelen (van koeievlaaien en zaden), schatten (van plant bedekkingen), legen (van muizevallen) en rijden (Wageningen-Ommen vv). Gelukkig hoefde ik het knippen, tellen, meten etc. niet alleen te doen. Voor het verwerken van het verzamelde materiaal konden we een beroep doen op de mensen van unifarm, die ook paraat waren voor het betere graafwerk, het uitlenen van schrikdraad en, op diverse locaties, de verzorging van de planten in de kas op zich namen. Ook ons eigen labteam waagde zich sporadisch buiten de grenzen van het Wageningse, Jan, Frans en Henk hielpen met de installatie en/of afbraak van de muizenexclosures ("ik ga liever wat vroeger weg als we maar wel op tijd thuis zijn") en diverse kleinere klussen op het koeland als konijnenkeutels tellen ("ik vertel thuis nooit wat ik overdag doe"). Maar eerlijk is eerlijk, Frans is altijd te raadplegen voor een goede praktische oplossing en ik denk dat ik niet snel een lab zal vinden dat beter draait dan het TON-lab onder leiding van Jan, waar veel van mijn monsters verwerkt zijn.

Daarnaast zijn er nog vele studenten, oude bekenden, vrienden en familie geweest die eens een dagje meegingen en daarbij uiteraard te werk werden gesteld bij één van de vele lopende projecten. Allen bedankt voor het harde werk en het aangenaam gezelschap. Dank aan mijn ouders voor het doorbrengen van een vakantie in Ommen in het laatste projectjaar waarbij het vakantiehuisje goed onderdak bood aan de veldwerkers en de vakantievierders nuttige veldassistenten bleken.

Door het werk op het Junner Koeland kwamen theorie en praktijk regelmatig samen, meestal in de vorm van overleg en excursies met de mensen van Staatsbosbeheer Ommen. Bij deze wil ik Staatsbosbeheer bedanken voor het openstellen van enkele van hun terreinen voor onderzoek en tevens voor het openstaan voor de processen waardoor grazers het grasland beïnvloeden. Ik heb de samenwerking altijd erg prettig en leerzaam gevonden. Ook het gebruik van de woning "Eerderveld" als uitvalsbasis tijdens het eerste jaar heeft ons erg geholpen.

Behalve met SBB waren er ook excursies met het BON (Begrazings Overleg Nederland) op het koeland of in andere begraasde terreinen in Nederland of België. Deze excursies waren erg inspirerend en bovendien een goede gelegenheid om een beeld te krijgen van wat er gaande is op begrazingsgebied. De jaarlijkse aio-dagen van de onderzoeksschool Functionele Ecologie hebben mij de gelegenheid geboden mijn werk te presenteren en commentaar te krijgen op wat ik deed, als ook een overzicht van wat anderen deden. Dit was vaak erg inspirerend.

Ommense wereld, het landschap, de historie, de soorten en de mensen, waardoor het een hele goede tijd is geweest. Jouw betrokkenheid bij het onderzoek ging ver en je was altijd in om iets nieuws te proberen. Ik denk niet dat ik snel weer een assistent zal vinden die blijmoedig om 12 uur 's nachts de klimcapaciteiten van een pas gevangen dwergmuis kan aanschouwen na een week muizen vangen. En zo hebben we vele mooie dingen gezien. Grote klasse en dank. O ja, en ik ga al die gegevens van de eindeloze wortelspoelbeurten serieus nog uitwerken en opschrijven.

Wouter, toen ik begon aan dit project kende ik je nauwelijks en nu zijn we getrouwd en zit jij achter mij de figuren te lay-outen. In de afgelopen jaren heb je aardig in het project meegedraaid. Aan het begin zei je dat je alles een keer meegedaan wilde hebben en ik dacht toen: dat moet ik nog zien. Maar je bent ver gekomen en het was erg leuk om samen in het veld te zijn. Je hebt nu zelfs het leeuwedeel van de lay-out gedaan, niet zozeer omdat je dat zo leuk vindt, maar omdat het dan sneller af is en we samen naar buiten kunnen. Een redenering die mij prima bevalt.

Tot slot, mijn dank aan Jean Knops, voor zijn geduld om me het proefschrift te laten afmaken en manuscripten te bewerken en naar tijdschriften te sturen onder het motto: I don't care what you do, as long as you're productive.

Liesbeth, Lincoln 27 juli 2003

Curriculum vitae

Elisabeth (Liesbeth) Suzanna Bakker was born on March 31st in 1973 in Haren (NL). She attended Grammarschool 'Willem Lodewijk Gymnasium' in Groningen. Next to school she was an active member of the Nederlandse Jeugdbond voor Natuurstudie (NJN), a nature organization for members aged 12-25, with whom she made many excursions and fieldcamps in the Dutch nature and adjacent Belgium and Germany.

Subsequently she choose to study Biology at the University of Groningen. During her undergraduate studies she became interested in the study of plant-animal interactions that perfectly combined her interest in both plant and animal ecology.

For her masters she studied pollination ecology in fragmented populations of *Scabiosa columbaria* in the east and south of the Netherlands. She then studied the foraging ecology of Greylag geese feeding on *Spartina angelica* and *Scirpus maritimus* on the Waddensea island of Schiermonnikoog. For her third project she moved to Canada to study foraging ecology of Pikas (*Ochtona princeps*) in Jasper National Park in the Canadian Rockies through Simon Fraser University in Burnaby. During her masters she followed the course introduction in tropical ecosystems in Naivasha, Kenya, organized by the Tropical Biology Association (TBA). She spent three months on Öland, Sweden, to study the effects of juniper shrub encroachment on plant diversity. She worked for one month as a fieldassistant with Oxford University where she made vegetation relevées in floodplain meadows with different management histories. In August 1997 she graduated cum laude with ecology as specialization.

Liesbeth has watched birds since the age of 8. However, her experiences in Kenya and Canada first raised her interest in mammals. Therefore she was happy to start a position as a PhD-student (0.8 FTE) with the Nature Conservation and Plant Ecology Group at Wageningen University (NL) in October 1997, with the task to study the interaction between mammalian herbivores and the vegetation. In January 2002 she was appointed a staff member on a half-time basis. She contributed to the course Landscape Ecology, Systems Ecology and Nature Conservation. In May 2003 she left Wageningen University for a postdoc position at the University of Nebraska (USA). Here she studies the impact of herbivores on nutrient dynamics over a productivity gradient in cooperation with the University of Syracuse, Colorado State University and the University of Groningen. She obtained a 'talent'-stipendium from the Netherlands Organisation for Scientific Research (NWO) for a one-year postdoc to study foraging behaviour of Collared Pikas (*Ochtona collaris*) in the Yukon (Canada) with the University of Alberta, which she hopes to start in May 2004.

Liesbeth is married to Wouter van Steenis.

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