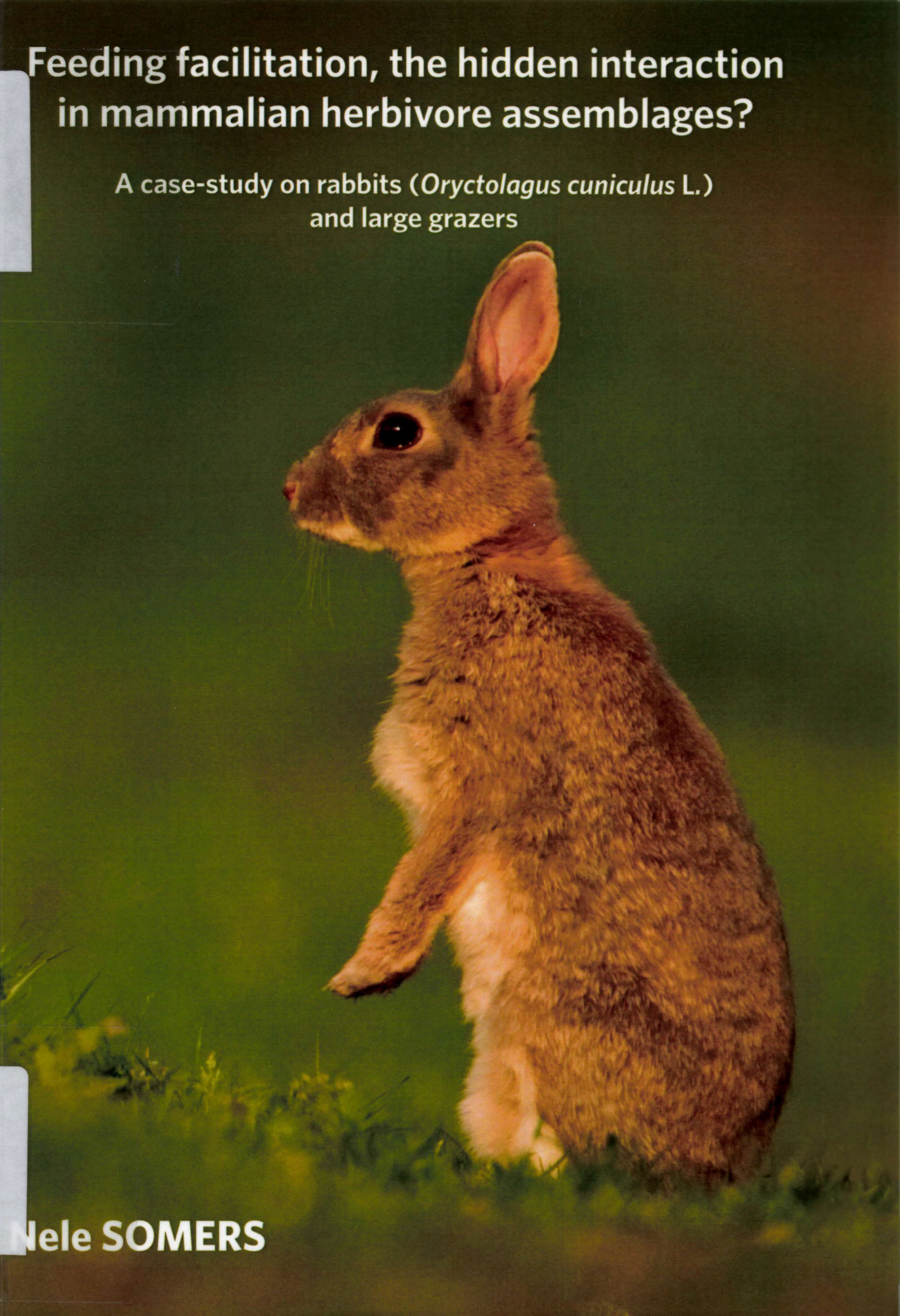
Feeding facilitation, the hidden interaction in mammalian herbivore assemblages?

A case-study on rabbits (*Oryctolagus cuniculus* L.) and large grazers



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CHAPTER 1

General introduction

Feeding facilitation in herbivore assemblages

Coexistence of different terrestrial grazer species is an important topic in ecology, leading to the question: how can species using the same or a similar limiting resource live together (e.g. PRINS & OLFF, 1998; FARNSWORTH *et al.*, 2002)? In this context, the concept of competition and niche differentiation has been extensively investigated (for a general overview: see BEGON *et al.*, 1990). However, positive interactions are far less extensively studied and deserve greater attention from ecologists (KRUEGER, 1986; ARSENAULT & OWEN-SMITH, 2002). This thesis is focussing on the positive interspecific interaction called 'facilitation'. Facilitation between animal species is a process or action, undertaken by one species, by which "something" is facilitated for the other species. This positive interaction has been suggested to play an important role in understanding coexistence of grazers with different body mass (PRINS & OLFF, 1998): smaller grazer species may benefit from the grazing impacts of larger species that modify the vegetation (ARSENAULT & OWEN-SMITH, 2002). This is called 'feeding facilitation'.

Feeding facilitation may arise for several reasons. When grazing by one species stimulates grass re-growth, it may thereby enhance the nutritional quality of forage for another species (ARSENAULT & OWEN-SMITH, 2002): the creation of low, high-productive and high-qualitative vegetation (MCNAUGHTON, 1984). Secondly, feeding facilitation may arise when grazing by one species makes more forage accessible to another species (ARSENAULT & OWEN-SMITH, 2002). For example, a tall sward might be more difficult to handle (FRYXELL, 1991; VAN DE KOPPEL *et al.*, 1996; VAN DER GRAAF *et al.*, 2002). A third form of feeding facilitation was mentioned by KUIJPER *et al.* (2008): in the long term, large herbivores may not only alter vegetation structure (and eventually forage quality) but also alter plant species composition. This could lead to a long term form of feeding facilitation: smaller grazers would be facilitated by large grazers because other plant species emerge, which was shown in a herbivore assemblage of cattle and hares (KUIJPER *et al.*, 2008). As far as feeding facilitation is concerned, we should further consider the option that medium-sized herbivores are able to maintain favourable grazing swards themselves, without the help of large herbivores. This is the concept of self facilitation (ARSENAULT & OWEN-SMITH, 2002) which might interfere with interspecific feeding facilitation.

Additionally, there might be a lower cost of vigilance in short grazed swards, because of increased predator detection capacity (VAN DE KOPPEL *et al.*, 1996; IASON *et al.*, 2002; VAN DER GRAAF *et al.*, 2002). Finally, more difficult locomotion might be at hand too (VAN DER GRAAF *et al.*, 2002): a low sward makes it easier for smaller herbivores to flee from predators (BAKKER *et al.*, 2009) as in the case of rabbits that are bound to burrows. Adversely, a higher sward gives better hiding opportunities for still smaller herbivores like voles (BAKKER *et al.*, 2009).

This multitude of facilitation mechanisms makes it clear that this herbivore interaction is not yet completely understood. The first type of feeding facilitation (facilitation by enhancement of forage quality) has been considered one of the most likely facilitation phenomena (ARSENAULT & OWEN-SMITH, 2002). However, more recognition should be given to the effective mechanisms through which feeding facilitation operates (ARSENAULT & OWEN-SMITH, 2002). This thesis focuses on the mechanisms of feeding facilitation by the creation of short, supposedly high quality swards.

Feeding facilitation by the creation of short, high quality swards

Grazing optimization hypothesis

How large grazers create nutritionally advantageous swards was first described in the Serengeti ecosystem (MCNAUGHTON, 1979; 1984): moderate grazing by large herbivores not only reduced the height of the vegetation, but also stimulated re-growth. Therefore, biomass production and nitrogen concentration were enhanced. This was due to several plant compensatory re-growth mechanisms that occur after defoliation (for an overview: see MCNAUGHTON, 1983). Grazing might maintain the phenologically young plant stages leading to a high nutrient concentration in leaf material; re-growth may be stimulated by grazing leading to increased biomass; tillering might be promoted, increasing shoot density; grazing might reduce standing dead biomass (VAN DER GRAAF *et al.*, 2005; TAHMASEBI KOHYANI *et al.*, 2008). The low, high-productive and high-qualitative vegetation resulting from this process has been named 'grazing lawns'; the hypothesis predicting increased production and quality of plants grazed at intermediate grazing pressures is called the 'grazing optimization hypothesis' (e.g. VAN DER GRAAF *et al.*, 2005). Although the positive effects of grazing or grazing simulation on forage quality (COPPOCK *et al.*, 1983; RUESS, 1984; CHENG & RITCHIE, 2006) and forage availability (VAN DER GRAAF *et al.*, 2005) have been observed, this hypothesis has been doubted by BELSKY (1986). At least, many factors may influence the effect of grazing on plant productivity and quality, e.g. soil nutrient availability, shading, intensity and frequency of defoliation (MILCHUNAS *et al.*, 1995). Also, simulated grazing might result in positive effects on only certain plant parts and the frequency of grazing or clipping influences the outcome (FOX *et al.*, 1998).

Quality threshold hypothesis

By creating grazing lawns, large grazers promote the availability of high-quality forage for other (smaller) herbivores. For allowing feeding facilitation of the type that we studied, these other herbivores should be sensitive for these short, high quality swards and should preferentially forage in this type of vegetation (OLFF *et al.*, 1997). These quality-sensitive herbivores are typically smaller animals, as larger grazers tolerate lower plant nutrient concentration (but require greater plant abundance (OLFF *et al.*, 2002)) and thus are not very quality-sensitive. This phenomenon is expounded by DEMMENT & VAN SOEST (1984): the proportion of the metabolic needs and the capacity of the digestive system of larger herbivores is very low. As a result, these grazers can have relatively long retention times, and consequently digest low quality food very slowly, in order to acquire sufficient nutrients. Some large herbivores even developed the very efficient system of being ruminant. Small herbivores have a very low capacity of the digestive system, and a very high metabolic need. As food is passing very quickly through their digestive tract, they should primarily feed on high quality plants to fulfil their metabolic needs.

Quality-sensitive herbivores would therefore not forage in vegetation patches where biomass intake is maximized as was traditionally predicted by the Type II-functional response (classical 'exploitation theory' - OKSANEN *et al.*, 1981; LUNDBERG, 1988; LUNDBERG & ÅSTRÖM, 1990; GROSS *et al.*, 1993). Instead, the medium-sized herbivores should forage according to a unimodal, dome-shaped Type IV functional response (BOS *et al.*, 2002a; DURANT *et al.*, 2003; DEKKER & VAN LANGEVELDE, 2007; VAN LANGEVELDE *et al.*, 2008): as a vegetation with a high standing crop would decrease in forage quality (see above), gross daily food intake should decrease at higher vegetation biomass (PRINS & OLFF, 1998). The 'quality threshold hypothesis' (OLFF *et al.*, 1997; KUIJPER, 2004) predicts that the medium-sized herbivore populations are rather controlled by bottom-up effects than by top-down effects (predation - VAN DE KOPPEL *et al.*, 1996; KUIJPER, 2004). The 'green world' hypothesis (HAIRSTON *et al.*, 1960) predicts that terrestrial grazing herbivore populations are not limited by their food supply and are generally controlled by predators. In contrast, the quality threshold hypothesis fits better in the 'optimal foraging theory' (BELOVSKY *et al.*, 1999), which predicts animals to forage in order to optimize their fitness by a maximal net nutritional intake, thereby coping with several constraints.

Factors influencing the occurrence of facilitation

Several factors may influence the occurrence of facilitation by the creation of grazing lawns and its balance with competition in mammalian grazer assemblages. There might be a temporal (seasonal) trade-off between facilitation and competition (ARSENAULT & OWEN-SMITH, 2002, RUEDA *et al.*, 2008) as forage maturation especially occurs during the growing season (FRYXELL, 1991). Whether facilitation does occur or not also depends on the density of the large grazers and of the facilitated grazers (BAKKER *et al.*, 2009). There should be an increase of the importance of facilitation at declining density of the smaller grazers. Also, there should be an optimal difference between the body mass of the herbivores involved (PRINS & OLFF, 1998): when the difference is too large, smaller species are not expected to benefit (e.g. KEESING, 1998; BAKKER *et al.*, 2009), while resource competition occurs when the difference is too small. This was especially shown in African grazers assemblages (PRINS & OLFF, 1998). In this case, a grazer with a large body mass was per definition a grazer with (relatively) lower energy and protein requirements and a higher capacity to ingest and digest the vegetation. We could however doubt whether this relationship between body mass and the occurrence of facilitation is universal, as feeding facilitation has been detected in assemblages of bison (*Bison bison* L.) and prairie dog (*Cynomys ludovicianus* ORD) (large difference in body size - KRUEGER, 1986), Barnacle geese (*Branta leucopsis* BECHSTEIN) and hare (*Lepus europaeus* PALLAS) (small difference in body mass - STAHL *et al.*, 2006). Finally, habitat productivity may be important for the balance between facilitation and competition in herbivore assemblages (CHENG & RITCHIE, 2006): facilitation by vegetation modification is more likely in productive habitats (DEKKER & VAN LANGEVELDE, 2007).

Examples of feeding facilitation in grazer assemblages

Feeding facilitation by limiting forage maturation has been mentioned to occur in some terrestrial herbivore assemblages. The most famous example is that of the migratory wildebeest (*Connochaetes taurinus* BURCHELL) and Thomson's gazelle (*Eudorcas thomsonii* GÜNTHER) in the Serengeti-Mara-ecosystem in Tanzania and Kenya (MCNAUGHTON, 1976; but see SINCLAIR & NORTON-GRIFFITHS, 1982). Other examples in assemblages consisting only of wild herbivores are bison (*Bison bison* L.) and prairie dog (*Cynomys ludovicianus* ORD) (KRUEGER, 1986), Barnacle geese (*Branta leucopsis* BECHSTEIN) and hare (*Lepus europaeus* PALLAS) (STAHL *et al.*, 2006)). In North-Western Europe, the wild large grazers have mostly been replaced by livestock. Although the communities of domestic and wild herbivores have not been co-evolving for a long time (as in the savannah-ecosystem (McNaughton, 1979)), feeding facilitation between domestic herbivores and wild herbivores has been observed as well. Some examples are red deer (*Cervus elaphus* L.) and cattle (Gordon, 1988), geese and cattle (Bos *et al.*, 2002b), hares and cattle (Kuijper, 2004), rodents and sheep (Austrheim *et al.*, 2007) and alpine reindeer (*Rangifer tarandus* L.) and sheep (Mysterud & Austrheim, 2008).

Also wild rabbits (*Oryctolagus cuniculus* L.) have been widely assumed to be facilitated by larger domestic grazers (e.g. Williams *et al.*, 1974; Wallage-Drees, 1982; Oosterveld, 1983; Drees, 1989; Drees, 1998). However, supporting evidence is mainly anecdotic or indirect. Descriptive field studies indicate a preference for swards of medium plant standing crop (van de Koppel *et al.*, 1996) or find no consistent effect of large herbivores on rabbits (Olff & Boersma, 1998); more recent (field-)experimental studies at least suggest the preference of the rabbit for shorter swards (Iason *et al.*, 2002; Bakker *et al.*, 2009).

Aims of this research

In this thesis, the assemblage of wild rabbits and introduced large grazers serve as a model to gain insights in the occurrence and mechanisms of feeding facilitation by the creation of grazing lawns. We focused on a limited number of aspects concerning feeding facilitation in this grazer assemblage. First, we studied the effect of large herbivores and rabbits on vegetation (effects on vegetation structure, on vegetation composition and on food quality for rabbits). Second, we studied some factors possibly determining the food and foraging choice of rabbits, with a focus on food quality and vegetation height.

The main hypothesis of the research is that introduced large herbivores facilitate rabbits (medium-sized herbivores) by modification of the vegetation. This modification involves creating short swards, creating denser (more productive) swards, creating swards that have a high food quality for rabbits and influencing vegetation composition. We expect that these modifications are not completely independent from each other: shorter swards are expected to have a higher food quality and to be more productive. This main hypothesis has been subdivided into several partial hypotheses:

- Large herbivores modify vegetation structure by grazing, thereby creating short and dense swards.
- Medium-sized herbivores (represented by rabbits) preferentially forage in short swards.
- These short swards are of a higher nutritional quality (for rabbits) than ungrazed swards.

- When foraging, food quality is an important factor determining the diet choices of the rabbit.
- Rabbits prefer the short swards created by large herbivores because of the higher nutritional value of these swards.
- Rabbits are able to create short and high qualitative swards, thereby facilitating themselves.
- Large herbivores can modify vegetation composition. This has an influence on rabbits as they prefer certain plants species above others when foraging.

We therefore performed several research projects with different levels of environmental control. We performed field observations in two coastal dune areas (IJzermonding and Dune Fossile de Ghyvelde - see "*Study areas and large herbivores*"). Here, herbivore assemblages consisting of rabbits and large grazers were studied to obtain information about:

  - the possibility of vegetation modification (vegetation structure, vegetation composition, food quality for rabbits) by the large herbivores
  - the occurrence of facilitation in these communities
  - plants species selection by rabbits (when foraging)

As these dune areas are structurally very heterogeneous, there was a need for another study area were a semi-controlled field experiment could be performed. This study area was Puyenbroeck (see "*Study areas and large herbivores*"). In this area, it was possible to experimentally study preferences of rabbits for certain sward heights, because of:

  - the straight forest borders
  - the relatively flat relief
  - the large surface of a homogeneous and relatively simple vegetation composition and structure
  - the presence of large rabbit populations
  - the possibility to easily control vegetation height by mowing machines.

Finally, also entirely controlled experiments in laboratory conditions were performed to study diet preferences of rabbits when controlling for vegetation height, but not for food quality.

Outline of the thesis

The following chapters of this thesis describe the research that was conducted in order to test the hypotheses or parts of the hypotheses mentioned above, including one methodological chapter.

Chapter 2 describes the results of a six-year exclosure study in the coastal dune nature reserves the IJzermonding and Ghyvelde. The exclosures had three treatments: accessibility for all herbivores (large grazers included), accessibility for rabbit but not for large grazers, and no accessibility to rabbits and large grazers. Several vegetation characteristics were measured in subsequent periods, in order to learn more about the influence of the different herbivore combinations on vegetation structure and vegetation composition. The possible importance of different grazer combinations for these dune grasslands will be discussed.

Chapter 3 examines the expected preference of rabbits for short swards. A mowing experiment, simulating extreme large grazer impact on vegetation sward, was conducted in two flat, monotonous lawns in the Flemish Provincial Domain 'Puyenbroeck' (Wachtebeke, Belgium). Short and high sward strips were created by differential mowing. We evaluated the use of the short and high strips by the wild rabbits in this study area. The second part of this chapter tests the preference of rabbits in Ghyvelde and IJzermonding for plots that had been grazed by the large herbivores in this area. Vegetation height and vegetation quality of grazed and ungrazed plots was measured.

Chapter 4 experimentally tests whether rabbits prefer high-quality forage. As sward height and food quality can be correlated under natural conditions, we experimentally eliminated differences in sward height between swards of different quality and offered the plants to some rabbits during a controlled feeding trial. Originally, we planned a full factorial experiment in which also swards of different height, but with equal food quality, were offered to the rabbits. However, as the short and long swards did not prove to have a similar food quality, these data could not be used for statistical analysis. Therefore, this part of the experiment was omitted from Chapter 4.

Chapter 5 is a methodological study, checking the use of epidermal plant fragments in rabbit pellets to determine the diet composition of rabbits. The method has several advantages, but some limitations have been mentioned in the past. So we performed a feeding trial with rabbits to compare the known diet with the results obtained by faecal analysis. The main goal of the study was to find out whether the diet composition can be reliably derived from faecal analysis (despite problems of differential digestion). The method of faecal analysis was used for the research described in Chapter 6.

Chapter 6 is a field study that compares the diet of wild rabbits in Ghyvelde (by using faecal analysis) with the availability of food plants in the study area. We tested whether food preferences of rabbits would occur on the plant species level. Plant quality was considered as a possible explanatory variable for diet preference.

Chapter 7 synthesizes the results of the previous chapters. The results are commented within the framework of feeding facilitation. The hypotheses of the thesis are confronted with the field observations and experimental results.

Description of the ecological model systems used in this research

The wild rabbit

The wild rabbit is one of the approximately 40 contemporary living species of the family Leporidae. This family comprises all rabbit and hare species. The Leporidae belong to the order of Lagomorpha, which is part of the class of Mammalia (CORBET, 1994).

After the last glacial, rabbits were withdrawn to Spain and South-Western France (TACK *et al.*, 1993). The Romans introduced the rabbits in Italy, but especially abbeys and convents are responsible for the large-scale distribution in Western-Europe during the Middle Ages (TACK *et al.*, 1993). The monks domesticated the animals (ROGERS *et al.*, 1994), and from the 2nd half of the Middle Ages (10th-11th century), they were kept in large parks, also in Flanders (DREES *et al.*, 2007) and Northern French coastal dune areas (TERMOTE, 1992). Their populations expanded, partly caused by destruction of their predators by hunters (ROGERS *et al.*, 1994). This expansion was stopped in the 1950's when the disease myxomatosis was successfully introduced in Europe (LOCKLEY, 1976). Originally, 99% of the animals died from the disease; nowadays, only 50% of the ill animals die (PROVOOST & BONTE, 2004). However, the rabbit populations now suffer from the outbreak of RHD (Rabbit Haemorrhagic Disease - VANDEKERCHOVE & PEETERS, 2002) and are generally supposed to be small nowadays (JANSSEN, 2004; DREES *et al.*, 2007).

Rabbits are medium-sized hindgut fermenters (DEMMENT & VAN SOEST, 1984); their medium-sized statue forces them to feed on high qualitative, quickly digestible forage. As a consequence of the quick digestion of these animals, some nutrients are lost through their faeces, which is partly compensated by caecotrophy (DEMMENT & VAN SOEST, 1984): rabbits are known to re-ingest soft faeces, directly after excretion and directly from the anus

(HIRAKAWA, 2001). The two types of pellets (hard and soft faeces) are formed by a separation mechanism in the proximal colon. During hard faeces excretion, water-soluble substances and fine particles (including micro-organisms) from the colon are brought back to the caecum by means of antiperistaltic movements. During soft faeces formation, the motility of the caecal base and proximal colon decrease and the caecal contents are covered by a mucous envelope (CARABAÑO & PIQUER, 1998). So the difference between the soft and hard faeces is not due to the food having passed once or twice (HIRAKAWA, 2001), as is sometimes misinterpreted.

Study areas and large herbivores

The field work of this thesis was conducted in two coastal dune areas (Figure 1): the Flemish Nature Reserve 'IJzermonding' (Nieuwpoort, Belgium; 51°9'4" N, 2°43'57" E; managed by the Agency for Nature and Forest (ANB) of the Flemish Government) and the French Nature Reserve 'Dune Fossile de Ghyvelde' (Ghyvelde, France, 51°2'48" N, 2°33'02" E; managed by Conservatoire du Littoral); they are ca. 25 km apart. The nearness of the North Sea mitigates climatological extremes in these areas (mild winters and mild summers). In addition, relief, exposition and the oligotrophic, porous and quickly warming sandy substrate create microclimatological differentiation, resulting in a wide variety of abiotic conditions and communities. The area of the original open coastal dune landscape has been reduced drastically by man (PROVOOST & BONTE, 2004). Since the abandonment of agro-pastoral use of the coastal landscape since the mid 20th century, a general encroachment of scrub and monospecific rough grasslands is taking place, leading to a general loss of biodiversity of blond dune, grey dune and dry dune grassland communities. One of the nature management measures taken for this reason was large-scale cutting of scrubs and the consecutive introduction of large herbivores. Grazing by large grazers in the study areas started between 1996 and 1999, in order to prevent further encroachment of shrubs and competitive grass species. A large variety of herbivore species were used for this purpose and at different sites: at the two study sites, these are sheep (Mergelland sheep, *Ovis aries* L.) in the IJzermonding and horses (Haflinger pony, *Equus caballus* L.) in Dune Fossile de Ghyvelde (Table 1). Sheep are ruminant grazers, having a very efficient digestion, enabling them to forage on lower quality plants (DEMMENT & VAN SOEST, 1984). The horses can be considered large bulk feeders. They are not ruminants, but large hindgut fermenters, with a long retention time, allowing them to feed on low quality food (DEMMENT & VAN SOEST, 1984).

Besides, wild rabbits are the most important "natural" grazers in the study sites, although they appear at different densities at the two study sites.

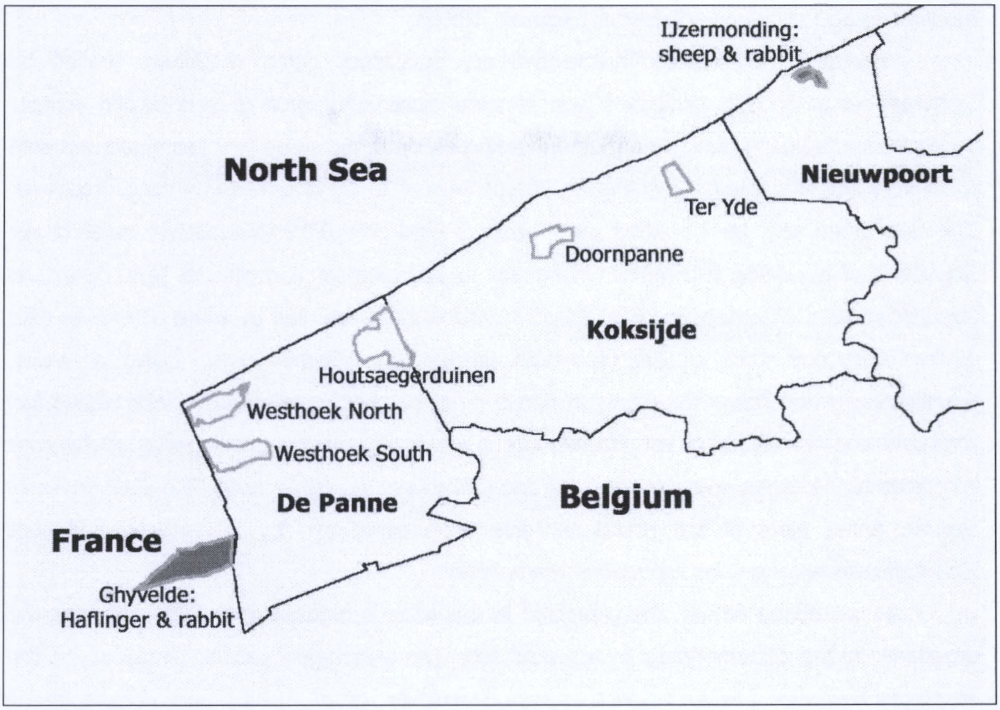

Figure 1: This map indicates the position of the two coastal dune study sites (IJzermonding and Dune Fossile de Ghyvelde) and a few other important (grazed) nature reserves in the Belgian and northern French coastal dune area.

Area	Large herbivores	Introduced in	Grazed surface	Grazing regime
IJzermonding	5-25 Mergelland sheep until 2003 accompanied by 3-4 goats	1999	5-10 ha	winter grazing (August - March)
Dune Fossile de Ghyvelde	10-15 Haflinger horses	1996	75 ha	year round grazing

Table 1: Introduction of large herbivores in the study areas (according to HOFFMANN *et al.*, 2005) with indications on grazer densities and grazing regime.

In each of these areas, we selected a dry dune grassland, neighbouring a scrub vegetation of *Hippophae rhamnoides* (IJzermonding) or *Ligustrum vulgare* (Ghyvelde). These grasslands have a sandy substrate that is originally calcareous. This is still the case in the relatively young dunes of the IJzermonding that are situated very close to the coast. In Dune Fossile de Ghyvelde, situated at ca. 3 km from the coast line and of older age (BAETEMAN, 2001) compared to the IJzermonding, local decalcification of the substrate resulted in moderately acid conditions (TAHMASEBI KOHYANI, 2008).

Vegetation composition in the two dry grasslands differ as follows (based on HOFFMANN *et al.* (2005), combined with personal observations). A grey dune-like pioneer vegetation on a young dune area that was artificially flattened some fifty years ago and with a still very limited organic top soil layer, characterizes the dry IJzermonding dune grassland. The vegetation can be classified as a *Tortulo-Koelerion (Phleo-Tortuletum ruraliformis,* SCHAMINÉE *et al.*, 1996). It contains several annual and biennial plant species (e.g. *Cerastium semidecandrum, Phleum arenarium, Crepis capillaris*), accompanied by some perennials (like *Sedum acre*) and some smaller graminoid species (e.g. *Festuca rubra, Carex arenaria*). Stabilisation of the substrate results in dense moss patches, dominated by *Homalothecium lutescens* and *Tortula ruralis* var. *ruraliformis*, in which also other annual species as *Myosotis ramosissima, Veronica arvensis, Arenaria serpyllifolia* and perennial herbs like *Galium verum* appear. Some parts of the grassland have been overgrown by increasingly dominant *Calamagrostis epigejos* and *Hippophae rhamnoides*.

As mentioned before, the grassland in Ghyvelde is fundamentally different from the grassland in the IJzermonding by its acid soil. The vegetation can be assigned to the *Plantagini-Festucion* (*Festuco-Galietum veri,* SCHAMINÉE *et al.*, 1996) and *Corynephorion canescentis (Violo-Corynephoretum,* SCHAMINÉE *et al.*, 1996). More or less acidophytic moss species (e.g. *Dicranum scoparium, Polytrichum juniperinum*) and lichens dominate large surfaces. *Carex arenaria, Anthoxanthum odoratum, Luzula campestris* and *Rumex acetosella* are the more important herb and grass species, accompanied by, for example, *Viola curtisii* and *Mibora minima*. The dominant shrub species here is *Ligustrum vulgare*.

The semi-controlled mowing experiment was carried out in another study area (see above): 'Puyenbroeck'. The Flemish Provincial Domain Puyenbroeck (Wachtebeke, Belgium, 51°9'11" N, 3°52'43" E) is managed by the Provincial Government East-Flanders. The area is situated ca. 75 km W from Nieuwpoort. At this site, wild rabbits are the dominant natural grazers (absence of large grazers). We selected two flat, monotonous lawns in the study area Puyenbroeck, both bordered by a Poplar plantation and mown at regular intervals.

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Mergelland sheep (IJzermonding, 2005)

Exclosure (IJzermonding, 2001)

Exclosure (Dune Fossile de Ghyvelde, 2005)

Haflinger horses (Dune Fossile de Ghyvelde, 2004)

(Photographs by N. Somers)

CHAPTER 2

The effect of rabbits (*Oryctolagus cuniculus* L.) and large herbivores on dry dune grassland structure and composition

Nele Somers, Katrien De Maeyer, Beatrijs Bossuyt, Luc Lens & Maurice Hoffmann

Abstract

The wild rabbit (*Oryctolagus cuniculus* L.) has often been mentioned as an important species for vegetation structure and composition in European coastal dune landscapes, through consumption of plants, digging, latrines, zoochory and effects on below-ground biota. This study focuses on grazing and digging effects of rabbits, when in combination with large herbivores. An exclosure experiment was carried out to measure the effect of rabbits and the combination of large herbivores and rabbits on vegetation structure and composition in two different coastal dune areas. We hypothesized that progressively excluding grazers would lead to a more rough vegetation structure (higher vegetation, higher litter cover, higher bare soil cover, expansion of shrubs and competitive graminoids, leading to a decline of annual plant species and mosses). We expected these structural differences to result in changes in vegetation composition, due to competitive effects and changing possibilities for germination. In all cases, the combination of rabbits and large grazers as well as rabbits without large grazers were able to diminish vegetation height and litter cover. Additionally, rabbits created bare soil patches, giving extra structural diversity. Through these structural impacts, grazer activity maintained plant species diversity in only one of the study areas. The effect of rabbits on vegetation was smaller than the combined effect of rabbits and large grazers. Therefore, it is concluded that rabbit presence is not sufficient to stop succession of the open, species rich dune grassland to rough grass or shrub dominated, species poor grassland. Because of the added effect of digging activity of rabbits, a combination of grazing by large herbivores and rabbits is the best grazing management option for these coastal dune areas.

Introduction

Since maintaining plant diversity is a central goal in the management of biodiversity throughout the world (OLFF & RITCHIE, 1998), the effect of herbivores on the vegetation deserves our attention, even when it concerns medium-sized herbivores. Different herbivore species may have different effects and assemblages of different herbivores may have compensatory or additive effects (RITCHIE & OLFF, 1997). In this study we will specifically focus on the effect of grazing and digging activity of the rabbit (*Oryctolagus cuniculus* L.), both separately and in combination with large herbivore grazing.

The wild rabbit is often considered to be an important species for vegetation (LEES & BELL, 2008): DREES (1989, 1998), DE BRUIJN (1991) and VAN DER HAGEN (1994) stated that rabbits would be necessary to preserve the general structural and compositional diversity of coastal dune vegetation in the Netherlands. Variation in rabbit density in space and in time is an important environmental factor in a dune area (ZEEVALKING & FRESCO, 1977). Also in Flanders, moderate rabbit grazing was shown to be positive for dune grassland conservation (VAN STEERTEGEM, 1982). Rabbits influence vegetation by different mechanisms, of which grazing is the most direct mechanism. Herbivores are generally thought to enhance plant diversity by direct consumption of competitively dominant plant species, causing diminished competition for less competitive species (OLFF & RITCHIE, 1998). The second mechanism is digging: soil disturbances can be created, thereby enhancing possibilities for (ruderal, annual) plants to colonize and establish themselves (OLFF & RITCHIE, 1998). Third, grazing at selected sites and dunging at others causes nutrient re-distribution: rabbit pellets have been shown to locally fertilize the vegetation via latrines (WILLOT *et al.*, 2000). Latrines make significant localised contributions to soil fertility and may therefore be important in establishing and maintaining plant cover. Zoochory is a fourth mechanism: seeds can survive the rabbit gut, hence enabling plants to be dispersed by the rabbits (PAKEMAN *et al.*, 2002; COSYNS *et al.*, 2005). Finally, it has been described that rabbits can structure below-ground biota. WEARN & GANGE (2007) showed that moderate grazing by rabbits had a rapid and persistent positive effect on mycorrhizal colonization of the roots of three grass species.

Rabbit grazing was found to diminish vegetation height (THOMAS, 1960; RANWELL, 1960; VAN STEERTEGEM, 1982), resulting in a decreasing the cover of litter (accumulation of dead plant biomass - VAN STEERTEGEM, 1982; BAKKER *et al.*, 2003b). Litter accumulation in ungrazed situations was observed to lead to decreased moss cover (TEN HARKEL & VAN DER MEULEN, 1995) and a decrease of the cover of annual grasses and forbs (TEN HARKEL & VAN DER MEULEN, 1995). Shrub enhancement can be retarded by rabbit grazing (WATT, 1957;

THOMAS, 1960; BAKKER *et al.*, 2004b). Also, rabbits create bare soil patches (VAN STEERTEGEM, 1982; TEN HARKEL & VAN DER MEULEN, 1995; BAKKER & OLFF, 2003). These structural changes in vegetation caused by rabbits, combined with their selective grazing behaviour (Chapter 6) could lead to alterations in species composition of the grasslands. Grazing can cause a shift in plant composition towards an annual life history and an increasing relative abundance of forbs and annual grasses in dune grasslands (TAHMASEBI KOHYANI, 2008). This is partially depending on the individual plant tolerance to herbivory and on competitive relations between competitive plant species and subordinate species: small annual plants can be released from competition when highly competitive grasses are suppressed by grazing or digging. An enhancement of thick grass layers when excluding rabbits has indeed been observed (WATT, 1957; VAN DER HAGEN, 1994), as well as negative changes in species composition (decreasing diversity, ZEEVALKING & FRESCO, 1977; VAN STEERTEGEM, 1982). These trends are not universal: the variation in rabbit density (ZEEVALKING & FRESCO, 1977; VAN STEERTEGEM, 1982), soil conditions (WATT, 1962; TAHMASEBI KOHYANI, 2008) and productivity (BAKKER *et al.*, 2006) can also be critical factors in an ecosystem, affecting the extent of the effect of grazing on vegetation.

After the collapse of the rabbit populations following the introduction of myxomatosis in 1952 (LOCKLEY, 1976), there was a lot of interest in studying the effects of rabbits on vegetation in Western Europe. WATT (1957; 1962) studied the impact of rabbit grazing by using exclosures; ZEEVALKING & FRESCO (1977) compared plots with variable degrees of rabbit grazing; several authors (RANWELL, 1960; THOMAS, 1960; WHITE, 1961; THOMAS, 1963) used the advent of myxomatosis as a kind of "natural experiment", analysing the changes in the vegetation after the disease caused a major crash of the rabbit populations. Little is known about whether rabbits nowadays are still important in dune grasslands. The rabbit populations are generally supposed to be small nowadays, partly as a consequence of the outbreak of RHD (Rabbit Haemorrhagic Disease - VANDEKERCHOVE & PEETERS, 2002; JANSSEN, 2004). Also, there is a recent trend of introducing large herbivores in nature reserves (HOFFMANN *et al.*, 2005) and the question is raised whether the presence of rabbits has become superfluous for the conservation of the dune grassland structure and composition. Recent studies of BAKKER (2003) in the Netherlands suggest that this is not the case in an alluvial plain grassland situation in the Netherlands: a combination of cattle and rabbits would be the best grazer combination to enhance species as well as structural diversity.

This paper describes a similar study, carried out in two different coastal dune areas along the Belgian and Northern French coast, which are grazed by rabbits on the one hand, and sheep or horses on the other. The areas differ significantly in soil pH (largely due to differences in calcium concentration of the soil) and associated age, leading to different kinds of grassland types, belonging to respectively *Tortulo-Koelerion (Phleo-Tortuletum ruraliformis,* SCHAMINÉE *et al.*, 1996*)* and *Plantagini-Festucion* (*Festuco-Galietum veri,* SCHAMINÉE *et al.*, 1996*)*. We hypothesize that the rabbit is a crucial species for the vegetation in these dry sand dune areas: rabbit grazing is expected to cause a lower vegetation height, a higher bare soil cover, a decrease in shrub cover and a decrease of litter cover. We hypothesize that these effects lead to a shift in vegetation composition, whereby perennial grasses cover decreases, thereby promoting annual plant species and moss cover, which would eventually lead to a higher number of species in the vegetation. We further hypothesize that rabbit impact is quite similar to the combined impact of large grazers and rabbits and that rabbit impact is largely similar in both areas, despite their large soil conditional differences and different accompanying large herbivores.

Materials & Methods

Study area

Exclosure experiments were conducted in coastal dune grasslands at the Flemish Nature Reserve 'IJzermonding' (Nieuwpoort, Belgium; 51°9'4" N, 2°43'57" E; managed by ANB, Flemish Government) and the French Nature Reserve 'Dune Fossile de Ghyvelde' (Ghyvelde, France, 51°2'48" N, 2°33'02" E; managed by Conservatoire du Littoral). Both sites were located ca. 25 km apart from each other. At both sites, wild rabbits comprise the dominant natural grazers while large domestic herbivores are used for grazing management (IJzermonding: Mergelland sheep, *Ovis aries* L.; Ghyvelde: Haflinger pony, *Equus caballus* L.). More information about these dune areas and the introduced large grazers can be found in Chapter 1.

Exclosure-experiment

Between August 1999 - April 2000, a total of five exclosures (each consisting of three treatments of 350 x 350 cm) were set up along shrub-grassland edges in coastal dune grasslands at IJzermonding and Ghyvelde (Figure 1). Within each exclosure treatment, six 75 x 75 cm permanent quadrates (pqs) were delineated and assigned to one of the three treatments: (i) larger herbivores, rabbits and smaller herbivores allowed (L+R+S); (ii) larger herbivores excluded (R+S); (iii) larger herbivores and rabbits excluded (S). L+R+S treatments were not fenced, R+S treatments were fenced with non rabbit-proof wire, S treatments were fenced with rabbit-proof wire.

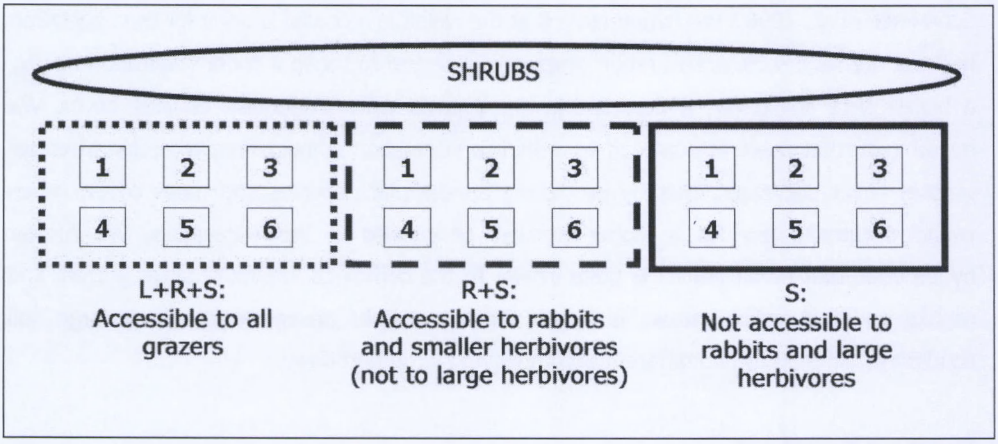


Figure 1: Schematic presentation of one exclosure-group. Each group is positioned near shrubs and consists of one L+R+S-treatment (accessible to all herbivores), one R+S-treatment (accessible to rabbits and smaller herbivores, not to larger herbivores) and one S-treatment (accessible to small herbivores but not to rabbits and larger herbivores). Six pqs of 75 cm by 75 cm are marked off within each treatment.

Vegetation measurements in all pqs were carried out four times: spring 2000 (April 2000), summer 2000 (June – September 2000), summer 2001 (July – September 2001) and summer 2005 (August – September 2005). As horses did enter the S treatment in exclosures 3 and 5 in Dune Fossile de Ghyvelde in 2005, no measurements were done in this pqs in summer 2005.

Vegetation height was measured at 25 fixed points in each pq by lowering down a disc (diameter 15 cm) with a central slot around a vertically held ruler, measuring the height at which the disc touched the vegetation first. This method is a combination of the "drop disc method" and "sward stick method", both described and evaluated in STEWART *et al.* (2001). The 25 measurements were averaged, resulting in one average vegetation height for each pq.

In each pq, all plant species (including mosses and lichens) were determined (following HENNIPMAN (1978), VAN DOBBEN & KOEMAN (1978), LANDWEHR (1984), TOUW & RUBERS (1989), VAN DER MEIJDEN (1996), LAMBINON *et al.* (1998) and VAN DORT *et al.* (1998) – Appendix 11 and 12) and their cover was estimated using the Londo scale (LONDO 1975). We also estimated the percentage of bare soil cover, cover of woody plants (shrubs), cover of non-woody plants, cover of graminoid species (Poaceae, Cyperaceae and Juncaceae together), cover of mosses (including lichens) and cover of litter.

Between April-September 2000 and August 2001- September 2005, monthly or bi-monthly counts of rabbit pellets were performed in L+R+S and R+S pqs. To estimate the rate of pellet decay, 100 freshly collected pellets were placed in the S treatment within each exclosure and the remaining pellets were counted during the following visit. To estimate local rabbit densities, we used the method of Taylor & Williams (1956), taking into account daily production of pellets per individual, rate of pellet decay, and time interval between consecutive counts:

$$\text{number of rabbits/ha} = \frac{m2.k1 - m1.k2}{g.(k1 - k2)} \, . \, \frac{\ln(k1 / k2)}{(t2 - t1)}$$

in which

m1, m2: mean number of pellets per plot during the first (1) and second (2) count, at the level of the study site; as pellets were removed from each plot after each count, m1=0; t2-t1: time interval between two consecutive counts; k1, k2: rate of pellet decay based on samples of 100 pellets exposed away from rabbits; g: number of pellets produced per rabbit and per day, estimated at 333 which was the average of 360 pellets/ind/day reported by Lockley (1962; 1976) and 305 pellets/ind/day based on nine days' counts of pellets of six domestic rabbits (Chapter 5). Local rabbit densities were calculated per counting date and study area.

Statistical analysis

As the data did not meet the assumptions for parametric analysis, non-parametric analyses (Kruskal-Wallis-tests) were carried out to test whether the grazing treatments resulted in significant differences in vegetation structure and composition. All tests were performed per season and per study area with TREATMENT as the independent variable. Various response variables were tested: mean vegetation height, the percentage of bare soil cover, cover of woody plants (shrubs), cover of non-woody plants, percentage of graminoid species cover in the non-woody cover, cover of mosses, cover of litter, number of species per plot, percentage of annual plant species cover in the non-woody cover and the cover of plant species that were present in 120 or more plots per year and over the four seasons (except for the shrub species which would be very strongly correlated with woody plant cover and some less clearly identifiable species). Also, Simpson's index of diversity was calculated and tested statistically the same way. Simpson's index of diversity was calculated as:

$$1 - \frac{\Sigma n\ (n-1)}{N\ (N-1)}$$

in which n = the total cover of a particular species and N = the total cover of all species (after Simpson, 1949). Sequential Bonferroni correction was applied for every four tests of a dependent variable per study area. For example, Bonferroni correction was applied for the four tests of mean vegetation height in IJzermonding in spring 2000, summer 2000, summer 2001 and summer 2005. The correction was applied sequential, which means that for example the most significant p-value was multiplied by 4, while the least significant p-value was multiplied by 1.

Non-parametric Spearman correlations were performed to test the dependence of species richness (total number of species) or species diversity (Simpson's index of diversity) and bare soil cover (global analysis per study area).

Species composition and abundance data were also analysed with multivariate ordination techniques. DCA was used when the length of gradient was larger than 2.3; PCA was used in the other cases (Jongman *et al.*, 1987). Differences between the scores of the pqs along the first ordination axis per exclosure group and per season were tested by Kruskal-Wallis-test, with TREATMENT as the independent variable. As differences between exclosure groups, resulting from the ordinations, were larger than differences between treatments within an exclosure group, these analyses were performed separately for each exclosure group. PC-ord 4 was used for ordinations. The non-parametric analyses were performed using SAS 9.1. Sequential Bonferroni correction was applied for every five tests of a dependent variable per study area and per period (e.g. the five tests for the five exclosures in IJzermonding in summer 2000).

The analyses from spring 2000 were shown separately in Appendix 1, 2, 3, 4 and 5, as these were considered to represent more or less the original state of the vegetation (comparable to the situation before the establishment of exclosures).

Results

Rabbit activity

The rabbit activity in IJzermonding and Dune Fossile de Ghyvelde shows a cyclical course, generally with peaks in spring and summer months (Figure 2). From April 2000 until the summer of 2002, rabbit activity is higher in IJzermonding compared to Dune Fossile de Ghyvelde, but since the beginning of 2003, the pattern is reversed with higher rabbit activity in Ghyvelde than in the IJzermonding. This pattern remains stable until the last counts in 2005.

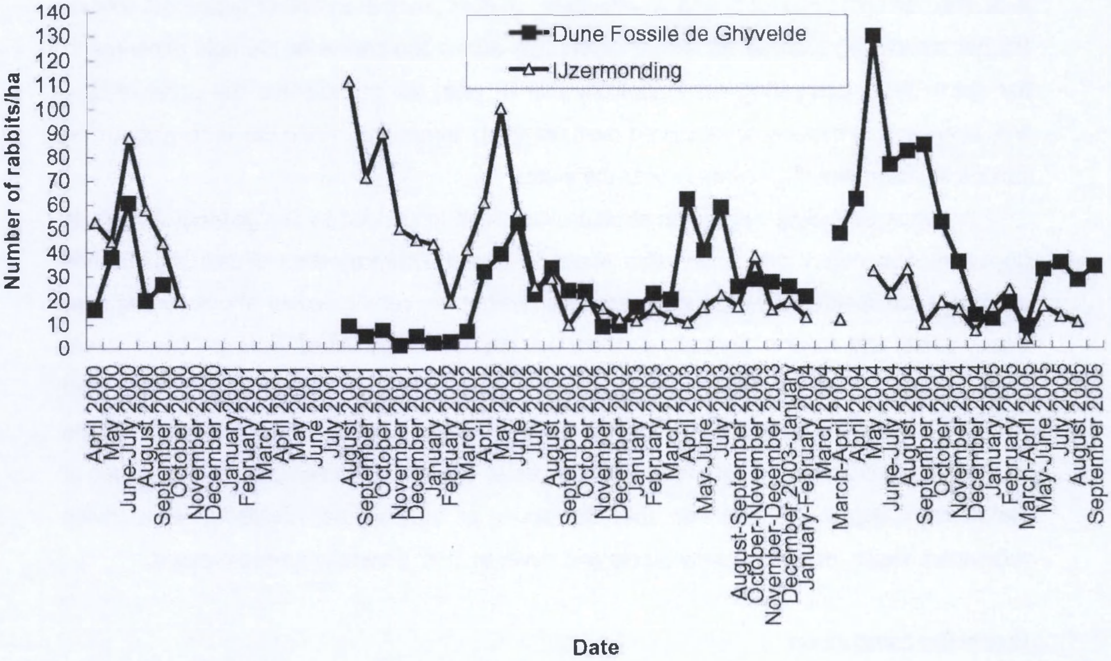


Figure 2: Presence of rabbits in the two study areas, expressed as number of rabbits/ha, calculated by the formula of TAYLOR & WILLIAMS (1956). No data are available for November 2000 until July 2001.

Vegetation structure

Several characteristics of the vegetation structure in IJzermonding have been influenced by the grazing treatment (Figure 3, Appendix 6 and 8, see also Appendix 1 and 3). As woody plant cover and moss cover are already significantly different in spring 2000, we cannot be certain that these differences were due to the grazing treatment and we will not draw conclusions from these results. Trends originating during the course of the experiment and still visible in summer 2005 are: i) significant differences in vegetation height (highest in vegetation in S, lowest in L+R+S); ii) a significantly different bare soil cover percentage between the treatments (lower percentage in S); iii) significant differences in annual plant species cover (with a different pattern in different years); iv) a significantly higher litter cover in S. The percentage cover of non-woody plants (higher cover in S) and of graminoid species (higher cover in S) showed significant differences during the course of the field experiment, but not in 2005. Comparing averages from year to year, we can see that the cover of bare soil, annuals and mosses has declined over the years in general, while the other vegetation variables have generally increased over the years.

Also in Ghyvelde, vegetation structure has been influenced by the grazing treatments (Figure 4, Appendix 7 and 9, see also Appendix 2 and 4). Parameters already significantly differing since spring 2000 are mean vegetation height, percentage cover of woody and non-woody plants and cover of annuals, so these are not further discussed. Bare soil cover, cover of graminoids and cover of litter are significantly different between treatments in 2005: bare soil cover in 2005 is highest in R+S, while graminoid and litter cover are highest in S. The cover of mosses shows no significant differences at all. When we compare the averages of the different years, we can see that the cover of annuals has declined, while mean vegetation height, cover of woody plants and cover of litter generally have increased.

Vegetation composition

Overall differences in plant species composition and abundance (Appendix 10, see also Appendix 5) indicate that significant differences between the three treatments in both areas are already present since spring 2000, in the IJzermonding as well as in Ghyvelde. However, the differences between treatments in spring 2000 in the IJzermonding are smaller than the differences in the subsequent periods (Figure 5), indicating that differences between the treatments are not only spatial differences, but could be related to the treatments. This is not the case in Ghyvelde. Therefore, it is hard to attribute the general plant species composition differences in Ghyvelde really to the treatments. Although there are differences between two consecutive grazing treatments (L+R+S versus R+S, or R+S versus S), these differences are not consistent. Contrary, in almost all the cases, the L+R+S treatment is significantly different from the S treatment.

The total number of species per plot in the IJzermonding significantly differs between almost all treatments since summer 2001 (Figure 3, Appendix 6 and 8, see also Appendix 1 and 3). The number of species is generally lowest in S, and higher in L+R+S and R+S. The number of species per plot has declined over the years. Simpson's index of diversity show a significantly lower species diversity in S. In Ghyvelde (Figure 4, Appendix 7 and 9, see also Appendix 2 and 4), the average number of species is statistically different between treatments only in summer 2005, when comparing L+R+S and S, with the highest number of species in L+R+S and the lowest number of species in S. Also here, the average number of species per plots has been declining. Simpson's index of diversity was not significantly influenced by the grazing treatments in summer 2005 (but see summer 2001). The Spearman correlation showed a positive relation between number of species and bare soil cover percentage in IJzermonding (Rho = 0.21, $p < 0.0001$), but not in Ghyvelde (Rho = 0.08, $p = 0.16$). The correlation between bare soil cover and Simpson's index of diversity was not significant in IJzermonding (Rho = -0.10, $p = 0.06$), but was significant in Ghyvelde (Rho = 0.13, $p = 0.0006$).

Qualitative plant species composition has changed during the subsequent years: certain species have disappeared; others have appeared (Appendix 13). For example, in IJzermonding, some annuals (*Erodium cicutarium, Vulpia* sp.) disappeared from S, while the perennial grass species *Arrhenatherum elatius* appeared in S and expanded to R+S. Also, quantitative differences are observed. In IJzermonding (Appendix 6 and 8), several species show significant differences between treatments in summer 2005: *Arenaria serpyllifolia, Bromus hordeaceus* subsp. *thominei, Calamagrostis epigejos, Hypochaeris radicata, Lotus corniculatus, Phleum arenarium, Poa pratensis, Sedum acre, Senecio jacobaea, Tortula ruralis* var. *ruraliformis* and *Veronica arvensis*; some of them already earlier (*Carex arenaria* in summer 2000, *Crepis capillaris* and *Leontodon* species in summer 2001). A global trend is detectable for the majority of these significantly different species: the significant differences are noticed when comparing L+R+S and S, or R+S and S, but seldom when comparing L+R+S and R+S. Most of these species have the lowest percentage of cover in S and a higher percentage of cover in L+R+S and R+S in the period of significant differences, often intermediate in R+S. During the course of the study, especially shrub cover (*Hippophae rhamnoides*) has increased, which was apparently at the expense of annual and perennial

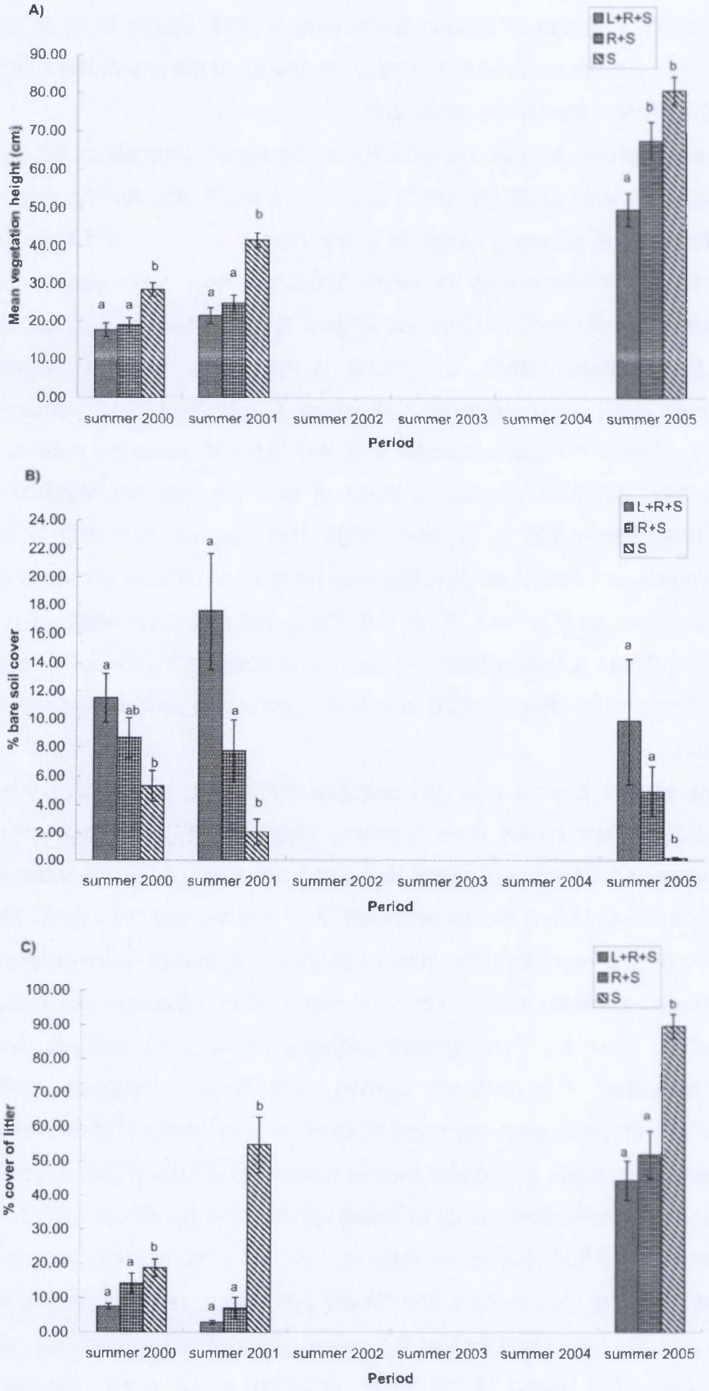


Figure 3 (part I)

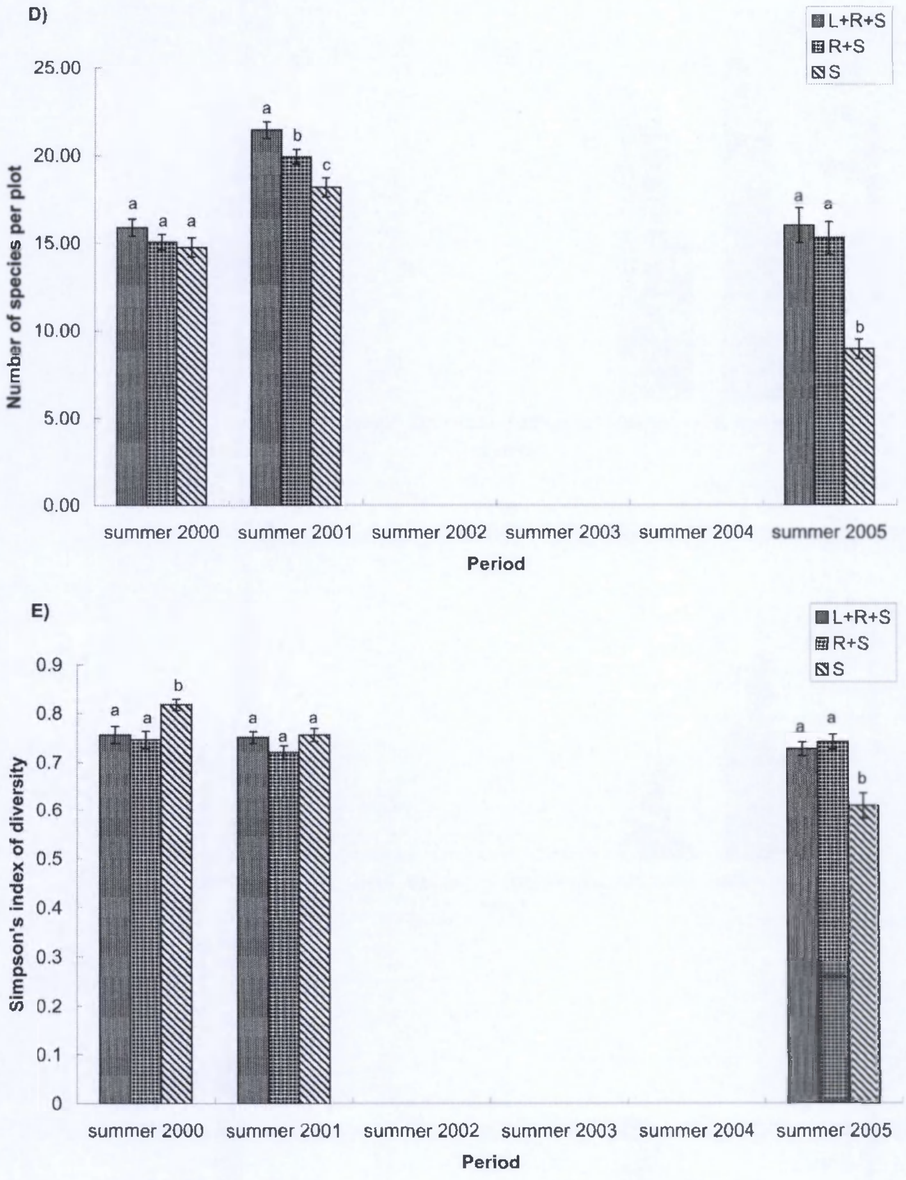


Figure 3 (part II): Mean and standard error of mean vegetation height, percentage of bare soil cover, percentage of litter cover, number of species per plot and Simpson's index of diversity in the exclosures in IJzermonding, per treatment and per season. L+R+S = all grazers. R+S = rabbits and smaller herbivores, but no larger grazers. S = only herbivores that are smaller than rabbits. Standard errors are shown. Different letters stand for significant differences.

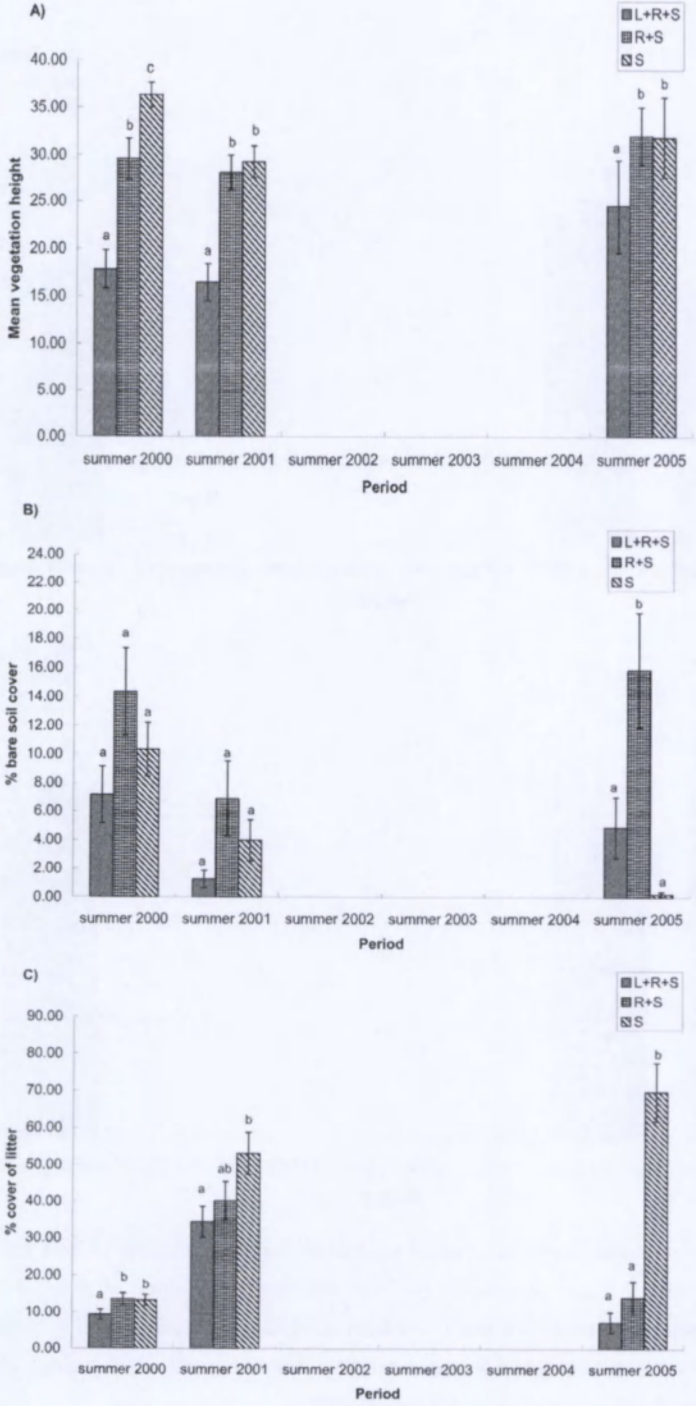


Figure 4 (part I)

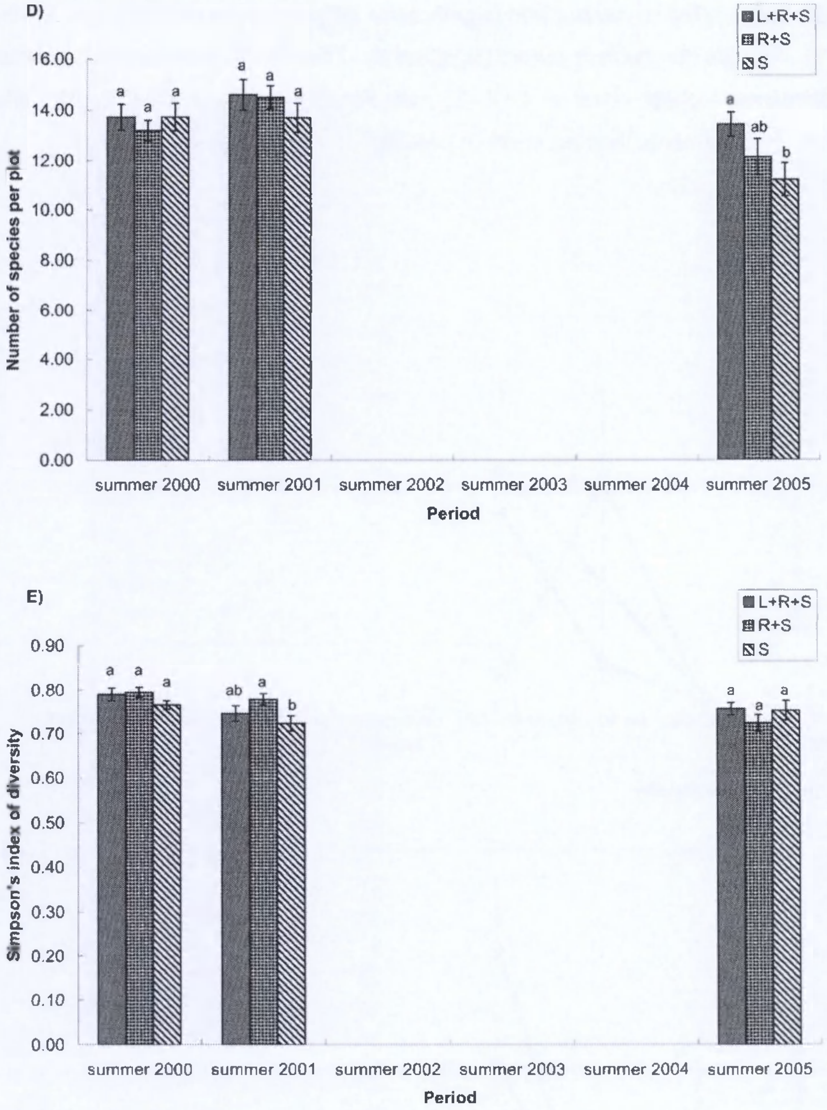
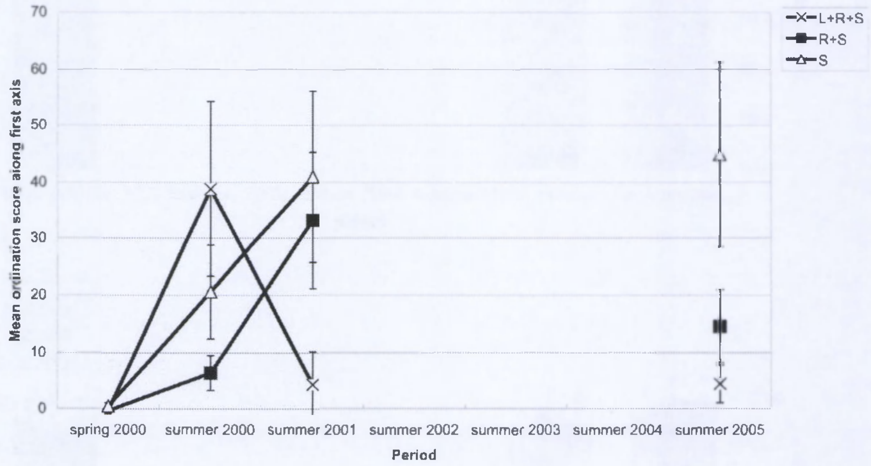


Figure 4 (part II): Mean and standard error of mean vegetation height, percentage of bare soil cover, percentage of litter cover, number of species per plot and Simpson's index of diversity in the exclosures in Dune Fossile de Ghyvelde, per treatment and per season. L+R+S = all grazers. R+S = rabbits and smaller herbivores, but no larger grazers. S = only herbivores that are smaller than rabbits. Standard errors are shown. Different letters stand for significant differences.

non-woody plant species cover. In Ghyvelde (Appendix 7 and 9), species differing significantly between treatments after spring 2000 are: *Carex arenaria* (not significantly different in 2005), *Plagiomnium affine* (significantly different between R+S and S, highest cover in S), *Pseudoscleropodium purum* (significantly different between L+R+S and the two other treatments, highest cover in L+R+S) and *Senecio jacobaea* (significantly different between all the treatments, highest cover in L+R+S).

IJzermonding



Dune Fossile de Ghyvelde

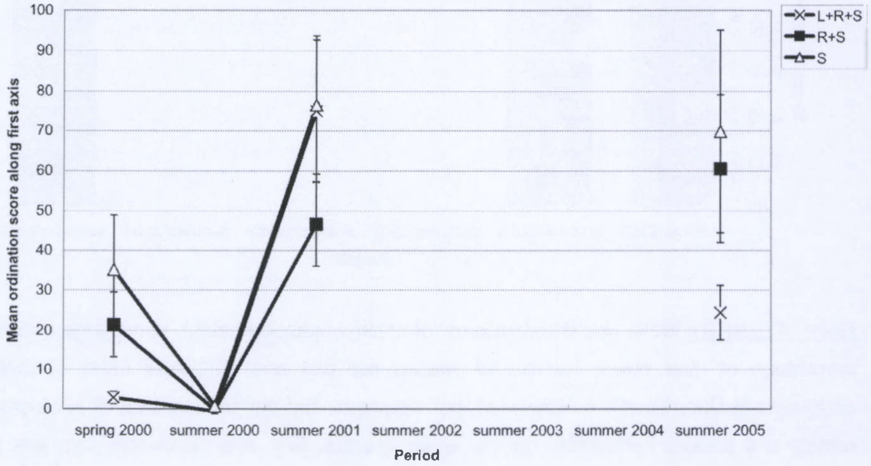


Figure 5: The scores along the first DCA-axis of all relevés per study area. The scores were averaged per season and per treatment. Standard errors are shown.

Discussion

Vegetation structure

The exclosure experiment in the IJzermonding and Ghyvelde reveals that different grazing treatments result in a different vegetation structure: effect on vegetation height, bare soil cover and litter cover. These three parameters globally are intermediate in the grazing treatment with only rabbits and smaller grazers.

The intermediate vegetation height in R+S in the IJzermonding is varying: sometimes very close to L+R+S, sometimes more similar with S. In Ghyvelde, the differences were already significant in spring 2000 and they increased in the subsequent periods. Although it is not possible to prove that these were caused by the grazing treatment alone, this hypothesis seems nonetheless very probable because of the latter observation. So rabbits at the given densities are able to reduce vegetation height significantly, confirming earlier findings of RANWELL (1960), THOMAS (1960), CRAWLEY (1990), CRAWLEY & WEINER (1991), VAN DER HAGEN (1994) and BAKKER *et al.* (2003a). However, their impact on vegetation height is less important than in combination with larger herbivores.

Related to this increase of vegetation height by excluding rabbits and larger herbivores, there is also an increase in litter accumulation. This effect was also observed by BAKKER *et al.* (2003b) in a much more nutrient rich alluvial plain grassland in the Netherlands. Although the effect of R+S is intermediate between L+R+S and S, R+S resembles L+R+S more, indicating that rabbits play an important role in diminishing litter quantity. This is true for the calcareous, dry pioneer grassland vegetation in the IJzermonding as well as for the acidophytic dry grassland in Ghyvelde. Litter accumulation is however much higher in IJzermonding (compared to Ghyvelde), which was probably due to the high litter accumulation in the expanding *Hippophae*-layer in IJzermonding. The shrub-layer in Ghyvelde had a lower cover percentage and was expanding more slowly.

The third important significant difference between the treatments concerns the bare soil cover percentage. In the IJzermonding, bare soil cover percentage is clearly lower in S; while in Ghyvelde, especially R+S has a high bare soil cover percentage in 2005. The absence of rabbits and larger herbivores makes the amount of bare soil almost disappear. In IJzermonding, it is not very clear whether the rabbits or the larger herbivores are responsible for the bare soil patches, but the results of Ghyvelde make clear that rabbits are able to create them, which is also observed in the studies of TEN HARKEL & VAN DER MEULEN (1995), BAKKER & OLFF (2003) and BAKKER *et al.* (2004a). BAKKER & OLFF (2003) observed that almost half of the bare soil patches in their study area were created by rabbits, slightly 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.

It was expected that the increasing vegetation height and litter cover would be disadvantageous for mosses (competition for light and space). The cover of mosses seems to be influenced by the grazing treatments in IJzermonding, but since the significant differences are already observed in spring 2000, this cannot be proven. In Ghyvelde, where mosses are an important part of plant diversity, moss cover was not influenced by the treatments. Therefore, it is not possible to draw straightforward conclusions about the effect of grazing both by rabbits and large grazers on moss cover. Although it should be mentioned that TEN HARKEL & VAN DER MEULEN (1995) observed a decrease of mosses after excluding rabbits, the effect of rabbit grazing on mosses is disputable: moss cover increased in some areas after the advent of myxomatosis (THOMAS, 1960) and WHITE (1961) shows that the effect of rabbits on moss cover largely depends on the moss species under consideration. To understand the effect of rabbits on moss vegetation, an analysis at species level is necessary.

It is hard to come to a conclusion about the effect of the treatments on the cover of woody plants, non-woody plants, graminoid species and annual species in our study areas. The results are either already significant at the start of the experiment, or not very consistent in time. Only in Ghyvelde, grasses become more dominant in the non-woody layer of the S treatment, compared to L+R+S and R+S. Other authors noticed effects of rabbit grazing on woody plants (ZEEVALKING & FRESCO, 1977; BAKKER *et al.*, 2004b) and an increase in the frequency of perennial graminoids and a decrease in the frequency of annual graminoids and herbs (TEN HARKEL & VAN DER MEULEN, 1995).

Vegetation composition

Although not all vegetation structure parameters are significantly different between the treatments, the fact that vegetation height, litter cover and bare soil cover are influenced, has possible implications for plant species composition, abundance and diversity. The most diverse vegetation is found at a low vegetation height. Light limitation related to increased living biomass negatively affects plant species richness due to reduced local colonization (BAKKER *et al.*, 2003b). According to LAMB (2008), litter is the primary mechanism structuring grassland diversity, with both richness and evenness declining with increasing litter cover. In dry habitats, litter may have several effects on species richness, i.e. through microclimate effects or providing nutrients (BAKKER *et al.*, 2003b). Finally, by creating burrows, rabbits are loosening up the soil, increasing aeration (BAKKER *et al.*, 2004a) and creating patches were germination success is strongly improved (compared to an undisturbed vegetation or to dung pats of large herbivores). As a result, a significant positive correlation between the occurrence of bare soil and plant species richness has been proven (BAKKER & OLFF, 2003). Our results only partially confirm this finding, but when a significant correlation was found, it was indicated that bare soil cover and number of species/species diversity are positively correlated. Therefore, we could expect that plant species diversity, composition and abundance would be dependent on the grazing treatments in the IJzermonding and Ghyvelde.

In IJzermonding, plant diversity has indeed decreased in S. R+S is again intermediate in position. This fact confirms the importance of rabbits in maintaining the diversity of dune grasslands, which was already mentioned by e.g. ZEEVALKING & FRESCO (1977) and VAN STEERTEGEM (1982), who stated that species diversity is highest in the plots with moderate rabbit grazing; lowest in species number were the plots without rabbit grazing. In Ghyvelde, the number of species per plot has been influenced only since 2005. It is possible that the importance of the grazers for this ecosystem will become only clear after a longer period of time, but by now, the effect of vegetation structure differences in IJzermonding and Ghyvelde does not lead to the same effect on diversity.

Grazing seemed to have influenced plant species composition and abundance in IJzermonding (Figure 5). These effects seemed to be really due to the grazer treatments, as the spatial differences in species composition present in spring 2000 had become larger over the years. As the vegetation became more rough when progressively excluding herbivores, we expected perennial graminoids cover to increase in S and also in R+S in a lesser extent, causing annual plant species to decrease in these treatments due to competitive effects. Also, bare soil cover creation by rabbits in L+R+S and R+S should have offered more germinating possibilities to annuals. During the course of the experiment, several plant species, of which the abundance in the three treatments was quite similar in 2000, declined in S, while staying more abundant in R+S and certainly in L+R+S. Several of them (e.g. *Arenaria serpyllifolia, Bromus hordeaceus* subsp. *thominei, Phleum arenarium, Veronica arvensis*) were indeed annuals. Some typical plants of the *Tortulo-Koelerion (Phleo-Tortuletum ruraliformis,* SCHAMINÉE *et al.*, 1996), like *Phleum arenarium, Sedum acre, Tortula ruralis* var. *ruraliformis* and *Arenaria serpyllifolia* decreased in S or even disappeared from S (*Erodium cicutarium*), suggesting that the maintenance of this vegetation type was dependent on the indirect effects of grazing and digging of the herbivores. Also the perennial grass species *Calamagrostis epigejos* had decreased in S, probably due to increasing competition from the expanding shrub layer. Although this rough grass had a higher cover in L+R+S than in S, the typical vegetation of the grassland (see above) is globally best preserved in the L+R+S treatment, intermediately in R+S, although this last treatment has a reasonably similar vegetation composition as L+R+S. This situation is equivalent to the situation observed by RANWELL (1960) after the disappearance of the rabbits by myxomatosis and indicates the importance of grazing for the typical dune vegetation.

As mentioned before, in Ghyvelde results about species composition and abundance should be approached with caution as many differences were already present in spring 2000 (Figure 5). In this area, only three species differ significantly between treatments (differences which were not already present in 2000). One of them is *Senecio jacobaea*, which is decreasing in S. This phenomenon has been confirmed by other authors (THOMAS, 1960; WHITE, 1961) and was mentioned as one of the most noticeable changes after the advent of myxomatosis. The vegetation structure in S is rougher than in the other treatments: the higher graminoid cover probably outcompeted *Senecio jacobaea*. This plant was however favoured in L+R+S (and intermediately in R+S) as vegetation structure was more open in these treatments, and the plant species was probably hardly touched by the herbivores (unpalatable plant species). *Plagiomnium affine* seems to increase after the exclusion of rabbits and large grazers. The opposite was the case for *Pseudoscleropodium purum,* which is contrary to the observations of RANWELL (1960). Although plant species composition and abundance are influenced by the grazing treatments, the extent of this influence is smaller in the closed acidophytic dune grasslands of Ghyvelde than in the open pioneer lime-rich grasslands of the IJzermonding. It should be noted that studies in areas similar to Ghyvelde did find significant effects of the exclusion or disappearance of rabbits. CRAWLEY (1990) did find an increase of *Anthoxanthum odoratum*, *Rumex acetosella* and *Luzula campestris* in rabbit-grazed grassland and a decrease of *Festuca rubra* and *Agrostis capillaris.* In his case, there was only little change in the total number of plant species with grazing. Excluding rabbits in the study area of VAN DER HAGEN (1994) resulted in an increase of *Luzula campestris* (contrary to CRAWLEY (1990)) and a decrease of *Cladonia furcata*, *Aira praecox* and *Rumex acetosella.* In his study, species diversity declined in the rabbit-proof exclosures.

The question remains why the importance of grazing for plant diversity, composition and abundance is different between both study areas. In a global study, BAKKER *et al.* (2006) stress the importance of habitat productivity in predicting the importance of mammalian herbivores on grassland diversity. However, when studying only small herbivores, the effect of grazing on plant species richness was not consistent. Also OLFF & RITCHIE (1998) indicate the importance of the soil fertility, combined with other factors (e.g. precipitation): contrasting results of grazing studies may be dependent on several factors. Our two study areas have a different soil acidity, which may be the critical difference between the two areas. It was shown that the mechanisms underlying vegetation responses to grazing may be primarily determined by edaphic factors (SASAKI *et al.*, 2008). Also, IJzermonding has an open pioneer vegetation which is different from the more or less stable closed-canopy grassland in Ghyvelde (Chapter 1). Another factor possibly causing differences between the two areas is the number of grazers. The rabbit populations in the two areas evolved differently. The large rabbit population in IJzermonding in 2001 and 2002 crashed, so that their numbers were quite low from autumn 2002 until the end of the experiment. The population in Ghyvelde was quite low in 2001, but expanded and came to a climax in 2004. The importance of the number of grazers and severity of the grazing pressure can be derived from the studies of ZEEVALKING & FRESCO (1977), BOWERS (1993) and ALBON *et al.* (2007). Moreover, the large grazers involved in the two areas and their grazing regimes were very different (winter grazing of sheep in IJzermonding, year round grazing of horses in Ghyvelde). All these factors and the possible interactions between them make it impossible to find a straightforward explanation for the differences between both areas.

Conclusions

Different grazing treatments in our two study areas lead to significant differences in certain aspects of vegetation structure (vegetation height, litter cover, bare soil cover). Generally, we can conclude that progressively excluding herbivore species leads towards a more rough vegetation. We expected these structural differences to influence plant species diversity, composition and abundance. This was indeed the case in IJzermonding, but only very limited in Ghyvelde. In IJzermonding, grazing seemed to be necessary to preserve the typical dune vegetation composition.

In most cases the influence of the rabbits is weaker than the combination with the impact of larger grazers. This intermediate position is similar to the findings of BAKKER *et al.* (2003a), and indicates that the rabbit populations in our study areas during the study period are not able to maintain the characteristic dune grassland vegetation. It seems that the introduction of large herbivores in these areas was indeed necessary to preserve vegetation diversity and structure.

However, effects of herbivores on different aspects of grassland ecology cannot be generalised: different herbivore species have different effects (BAKKER, 2003). The creation of bare soil patches is a property of rabbits, much more than of non-burrowing large grazers: large herbivores may create the conditions under which high species richness is possible, but rabbits are the main creators of bare soil patches (BAKKER, 2003): the combination of large herbivores and rabbit grazing will probably result in a higher structural diversity and sometimes also higher species richness in dry dune grasslands, than in a system with large grazers but without rabbits. Therefore, the rabbit still has the potential to be an important species in coastal dune succession, although not being able to preserve the dry dune grassland vegetation composition and structure on its own.

Acknowledgements

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Appendices

IJzermonding	spring 2000		
Treatment	L+R+S	R+S	S
	Mean ± SE	**Mean ± SE**	**Mean ± SE**
mean vegetation height (cm)	4.90 ± 0.47	4.78 ± 0.42	5.36 ± 0.34
% bare soil cover	8.83 ± 1.99	8.83 ± 1.91	6.83 ± 1.00
% cover of woody plants	3.50 ± 1.46	2.73 ± 0.79	4.87 ± 1.05
% cover of non-woody plants	51.00 ± 2.62	62.17 ± 4.81	62.83 ± 4.59
% graminoid species cover in the non-woody cover	35.87 ± 2.77	39.76 ± 3.81	41.42 ± 3.93
% annual plant species cover in the non-woody cover	28.48 ± 2.72	26.07 ± 2.57	21.67 ± 2.37
% cover of mosses	86.00 ± 2.51	70.00 ± 4.84	67.50 ± 4.12
% cover of litter	2.30 ± 0.27	2.07 ± 0.37	2.17 ± 0.29
number of species per plot	20.73 ± 0.51	19.23 ± 0.54	20.63 ± 0.65
Simpson's index of diversity	0.78 ± 0.02	0.78 ± 0.02	0.81 ± 0.02
Arenaria serpyllifolia	5.47 ± 0.81	3.83 ± 0.53	3.83 ± 0.53
*Bromus hordeaceu s*subsp. *thominei*	4.63 ± 0.65	5.40 ± 0.73	5.83 ± 0.81
Calamagrostis epigejos	7.97 ± 1.79	3.70 ± 0.71	3.10 ± 0.65
Carex arenaria	3.10 ± 0.49	4.03 ± 0.57	4.37 ± 0.53
Cladonia furcata	1.87 ± 0.52	0.50 ± 0.13	0.97 ± 0.40
Crepis capillaris	0.03 ± 0.03	0.17 ± 0.08	0.00 ± 0.00
Elymus repens	1.17 ± 0.38	0.07 ± 0.05	1.37 ± 0.53
Erodium cicutarium	5.90 ± 0.81	3.53 ± 0.68	3.53 ± 0.68
Festuca species	3.70 ± 0.71	7.27 ± 1.53	9.40 ± 1.87
Galium verum	0.97 ± 0.36	1.47 ± 0.48	2.43 ± 0.66
Geranium molle	7.13 ± 0.80	8.60 ± 0.80	8.40 ± 0.88
Hypochaeris radicata	0.23 ± 0.11	1.20 ± 0.45	1.37 ± 0.49
Leontodon species	3.93 ± 0.90	4.73 ± 1.15	5.00 ± 0.87
Lotus corniculatus	1.13 ± 0.44	1.10 ± 0.49	1.67 ± 0.56
Myosotis ramosissima	3.87 ± 0.49	4.40 ± 0.50	5.63 ± 0.51
Ononis repens	2.13 ± 0.70	3.67 ± 1.07	3.37 ± 1.06
Phleum arenarium	2.20 ± 0.52	1.57 ± 0.47	1.27 ± 0.39
Poa pratensis	5.00 ± 0.90	13.37 ± 4.02	11.80 ± 2.88
Sedum acre	8.17 ± 0.81	8.63 ± 0.95	9.53 ± 1.15
Senecio jacobaea	2.80 ± 0.63	5.43 ± 1.32	6.50 ± 1.12
Tortula ruralis var. *ruraliformis*	61.67 ± 5.23	51.83 ± 6.01	55.67 ± 5.07
Veronica arvensis	2.70 ± 0.53	2.90 ± 0.48	2.70 ± 0.43

Appendix 1: Mean and standard error (SE) of the cover of some plant species and some other vegetation parameters in the exclosures in IJzermonding, per treatment in spring 2000. L+R+S = all grazers. R+S = rabbits and smaller herbivores, but no larger grazers. S = only herbivores that are smaller than rabbits.

Dune Fossile de Ghyvelde	**spring 2000**		
Treatment	L+R+S	R+S	S
	Mean ± SE	**Mean ± SE**	**Mean ± SE**
mean vegetation height (cm)	7.89 ± 3.60	4.74 ± 0.16	8.45 ± 0.47
% bare soil cover	9.50 ± 2.87	8.17 ± 2.01	5.33 ± 1.31
% cover of woody plants	0.53 ± 0.16	0.07 ± 0.05	0.10 ± 0.06
% cover of non-woody plants	70.33 ± 2.94	77.00 ± 3.11	84.50 ± 2.08
% graminoid species cover in the non-woody cover	72.45 ± 3.91	56.80 ± 4.89	62.40 ± 4.55
% annual plant species cover in the non-woody cover	46.10 ± 5.69	61.61 ± 5.11	64.40 ± 5.18
% cover of mosses	61.83 ± 4.72	49.83 ± 5.58	43.33 ± 4.61
% cover of litter	8.70 ± 2.63	9.40 ± 1.42	8.00 ± 1.17
number of species per plot	15.73 ± 0.59	15.37 ± 0.38	17.17 ± 0.58
Simpson's index of diversity	0.80 ± 0.01	0.80 ± 0.01	0.83 ± 0.01
Agrostis capillaris	23.73 ± 2.88	15.97 ± 3.64	19.57 ± 3.53
Aira praecox	9.80 ± 3.34	12.07 ± 4.24	3.70 ± 0.70
Carex arenaria	8.53 ± 0.83	10.33 ± 1.00	13.23 ± 1.81
Cladonia furcata	2.00 ± 0.58	2.50 ± 0.56	1.67 ± 0.44
Dicranum scoparium	17.57 ± 4.56	12.37 ± 3.91	7.03 ± 2.07
Festuca species	3.83 ± 0.80	4.73 ± 1.28	10.20 ± 1.83
Galium verum	0.73 ± 0.19	0.63 ± 0.17	0.73 ± 0.16
Geranium molle	7.27 ± 1.41	7.60 ± 1.92	15.20 ± 3.79
Hypnum cupressiforme	26.67 ± 5.85	24.47 ± 5.37	22.17 ± 4.58
Luzula campestris	11.00 ± 1.88	4.53 ± 1.28	8.63 ± 1.95
Plagiomnium affine	2.90 ± 1.12	2.83 ± 1.02	6.67 ± 2.39
Pseudoscleropodium purum	14.00 ± 4.89	9.90 ± 4.26	3.37 ± 1.42
Rumex acetosella	2.97 ± 0.51	4.13 ± 0.58	3.07 ± 0.47
Senecio jacobaea	2.80 ± 0.89	4.10 ± 1.28	3.10 ± 0.86
Veronica arvensis	4.13 ± 0.58	4.03 ± 0.59	3.47 ± 0.47

Appendix 2: Mean and standard error (SE) of the cover of some plant species and some other vegetation parameters in the exclosures in Dune Fossile de Ghyvelde, per treatment in spring 2000. L+R+S = all grazers. R+S = rabbits and smaller herbivores, but no larger grazers. S = only herbivores that are smaller than rabbits.

IJzermonding	**All treatments**			**L+R+S versus R+S**			**L+R+S versus S**			**R+S versus S**		
	Chi-square	**DF**	**P**	**Chi-square**	**DF**	**P**	**Chi-square**	**DF**	**P**	**Chi-square**	**DF**	**P**
mean vegetation height (cm)	2.81	2	0.25	0.01	1	0.94	1.91	1	0.17	2.27	1	0.13
% bare soil cover	0.00	2	1.00	0.00	1	0.99	0.00	1	1.00	0.00	1	0.98
% cover of woody plants	6.50	2	**0.04**	0.00	1	0.97	4.77	1	**0.03**	4.82	1	**0.03**
% cover of non-woody plants	5.50	2	0.06	3.99	1	**0.05**	4.24	1	**0.04**	0.01	1	0.92
% graminoid species cover in the non-woody cover	0.42	2	0.81	0.02	1	0.89	0.46	1	0.50	0.16	1	0.69
% annual plant species cover in the non-woody cover	2.34	2	0.31	1.64	1	0.20	1.85	1	0.17	0.02	1	0.88
% cover of mosses	16.43	2	**0.00** *	9.51	1	**0.00** *	14.36	1	**0.00** *	0.80	1	0.37
% cover of litter	1.83	2	0.40	1.90	1	0.17	0.36	1	0.55	0.48	1	0.49
number of species per plot	5.02	2	0.08	4.36	1	**0.04**	0.01	1	0.92	3.18	1	0.07
Simpson's index of diversity	3.29	2	0.19	0.00	1	0.98	2.36	1	0.12	2.55	1	0.11
Arenaria serpyllifolia	2.13	2	0.34	1.63	1	0.20	1.53	1	0.22	0.00	1	0.96
Bromus hordeaceus subsp. *thominei*	1.00	2	0.61	0.38	1	0.54	1.00	1	0.32	0.12	1	0.73
Calamagrostis epigejos	5.17	2	0.08	3.82	1	0.05	3.81	1	0.05	0.01	1	0.93
Carex arenaria	2.64	2	0.27	1.03	1	0.31	2.65	1	0.10	0.24	1	0.62
Cladonia furcata	7.42	2	**0.02** *	6.00	1	**0.01** *	4.77	1	**0.03** *	0.02	1	0.88
Crepis capillaris	5.48	2	0.06	1.97	1	0.16	1.00	1	0.32	4.21	1	**0.04**
Elymus repens	11.70	2	**0.00** *	12.58	1	**0.00** *	1.78	1	0.18	3.72	1	0.05
Erodium cicutarium	6.59	2	**0.04**	5.44	1	**0.02**	4.32	1	**0.04**	0.10	1	0.75
Festuca species	7.93	2	**0.02** *	4.02	1	**0.05**	6.96	1	**0.01** *	0.86	1	0.35
Galium verum	2.81	2	0.25	0.19	1	0.66	2.48	1	0.12	1.40	1	0.24
Geranium molle	2.13	2	0.34	1.79	1	0.18	1.40	1	0.24	0.01	1	0.91
Hypochaeris radicata	4.92	2	0.09	4.34	1	**0.04**	3.76	1	0.05	0.01	1	0.94
Leontodon species	0.83	2	0.66	0.06	1	0.81	0.89	1	0.35	0.30	1	0.58
Lotus corniculatus	0.83	2	0.66	0.06	1	0.81	0.89	1	0.35	0.30	1	0.58
Myosotis ramosissima	6.09	2	**0.05**	0.38	1	0.54	5.64	1	**0.02** *	3.17	1	0.07
Ononis repens	1.10	2	0.58	1.09	1	0.30	0.38	1	0.54	0.18	1	0.67
Phleum arenarium	2.07	2	0.36	1.47	1	0.23	1.54	1	0.21	0.00	1	1.00
Poa pratensis	3.06	2	0.22	0.55	1	0.46	3.53	1	0.06	0.55	1	0.46
Sedum acre	0.62	2	0.73	0.19	1	0.66	0.56	1	0.45	0.18	1	0.68

Appendix 3 (part I)

Senecio jacobaea	3.73	2	0.15	0.25	1	0.62	4.60	1	0.03	0.86	1	0.35
Tortula ruralis var. *ruraliformis*	1.68	2	0.43	1.31	1	0.25	1.19	1	0.28	0.01	1	0.93
Veronica arvensis	0.37	2	0.83	0.24	1	0.62	0.31	1	0.58	0.00	1	0.96

Appendix 3 (part II): Results of Kruskal-Wallis-tests of the cover of some plant species and some other vegetation parameters in the exclosures in IJzermonding, with treatment as independent variable, in spring 2000. Chi-square = chi-square-value obtained by the test. DF = degrees of freedom. P = significance value obtained by the test. * indicates that P is still significant after sequential Bonferroni correction. L+R+S = all grazers. R+S = rabbits and smaller herbivores, but no larger grazers. S = only herbivores that are smaller than rabbits.

Dune Fossile de Ghyvelde	**All treatments**			**L+R+S versus R+S**			**L+R+S versus S**			**R+S versus S**		
	Chi-square	**DF**	**P**	**Chi-square**	**DF**	**P**	**Chi-square**	**DF**	**P**	**Chi-square**	**DF**	**P**
mean vegetation height (cm)	48.20	2	**<.0001** *	2.55	1	0.11	33.59	1	**<.0001** *	36.93	1	**<.0001** *
% bare soil cover	0.42	2	0.81	0.07	1	0.79	0.06	1	0.80	0.50	1	0.48
% cover of woody plants	11.55	2	**0.00** *	8.09	1	**0.00** *	6.24	1	**0.01** *	6.24	1	**0.01**
% cover of non-woody plants	11.24	2	**0.00** *	2.90	1	0.09	11.43	1	**0.00** *	2.47	1	0.12
% graminoid species cover in the non-woody cover	5.72	2	0.06	5.68	1	**0.02**	2.10	1	0.15	0.75	1	0.39
% annual plant species cover in the non-woody cover	6.55	2	**0.04**	4.26	1	**0.04**	5.32	1	**0.02**	0.21	1	0.65
% cover of mosses	5.97	2	0.05	1.53	1	0.22	7.35	1	**0.01** *	0.32	1	0.57
% cover of litter	3.51	2	0.17	2.48	1	0.12	2.73	1	0.10	0.09	1	0.76
number of species per plot	5.36	2	0.07	0.01	1	0.92	3.32	1	0.07	4.73	1	**0.03**
Simpson's index of diversity	4.60	2	0.10	0.01	1	0.91	3.67	1	0.06	3.20	1	0.07
Agrostis capillaris	6.41	2	**0.04**	6.69	1	**0.01** *	2.26	1	0.13	0.68	1	0.41
Aira praecox	0.14	2	0.93	0.00	1	0.97	0.00	1	0.96	0.28	1	0.60
Carex arenaria	4.07	2	0.13	3.15	1	0.08	3.00	1	0.08	0.07	1	0.79
Cladonia furcata	0.20	2	0.90	0.14	1	0.70	0.02	1	0.90	0.15	1	0.70
Dicranum scoparium	2.91	2	0.23	0.85	1	0.36	3.16	1	0.08	0.34	1	0.56
Festuca species	9.15	2	**0.01** *	0.00	1	0.97	7.38	1	**0.01** *	5.98	1	**0.01**
Galium verum	0.58	2	0.75	0.13	1	0.72	0.17	1	0.68	0.57	1	0.45
Geranium molle	2.51	2	0.29	0.07	1	0.79	1.54	1	0.21	2.10	1	0.15
Hypnum cupressiforme	0.25	2	0.88	0.23	1	0.63	0.15	1	0.70	0.01	1	0.92
Luzula campestris	10.63	2	**0.00** *	11.57	1	**0.00** *	2.25	1	0.13	2.08	1	0.15
Plagiomnium affine	2.77	2	0.25	0.04	1	0.84	1.79	1	0.18	2.14	1	0.14
Pseudoscleropodium purum	1.11	2	0.57	0.20	1	0.65	1.03	1	0.31	0.43	1	0.51
Rumex acetosella	2.32	2	0.31	2.01	1	0.16	0.41	1	0.52	1.08	1	0.30
Senecio jacobaea	1.13	2	0.57	1.11	1	0.29	0.57	1	0.45	0.03	1	0.85
Veronica arvensis	0.36	2	0.84	0.01	1	0.93	0.28	1	0.60	0.25	1	0.62

Appendix 4: Results of Kruskal-Wallis-tests of the cover of some plant species and some other vegetation parameters in the exclosures in Dune Fossile de Ghyvelde, with treatment as independent variable, in spring 2000. Chi-square = chi-square-value obtained by the test. DF = degrees of freedom. P = significance value obtained by the test. * indicates that P is still significant after sequential Bonferroni correction. L+R+S = all grazers. R+S = rabbits and smaller herbivores, but no larger grazers. S = only herbivores that are smaller than rabbits.

Study area	**Exclosure**	**ordination technique**	**All treatments**			**L+R+S versus R+S**			**L+R+S versus S**			**R+S versus S**		
			Chi-square	**DF**	**P**	**Chi-square**	**DF**	**P**	**Chi-square**	**DF**	**P**	**Chi-square**	**DF**	**P**
IJzermonding	Excl 1	PCA	10.05	2	**0.01** *	0.64	1	0.42	8.31	1	**0.00** *	5.77	1	**0.02**
	Excl 2	PCA	15.16	2	**0.00** *	8.31	1	**0.00** *	8.31	1	**0.00** *	8.31	1	**0.00** *
	Excl 3	PCA	7.52	2	**0.02** *	0.23	1	0.63	5.77	1	**0.02** *	5.03	1	**0.03**
	Excl 4	PCA	6.77	2	**0.03**	1.26	1	0.26	6.56	1	**0.01** *	2.08	1	0.15
	Excl 5	PCA	11.66	2	**0.00** *	8.31	1	**0.00** *	8.31	1	**0.00** *	0.64	1	0.42
Ghyvelde	Excl 1	PCA	8.57	2	**0.01** *	0.41	1	0.52	6.56	1	**0.01** *	5.77	1	**0.02** *
	Excl 2	PCA	13.05	2	**0.00** *	8.31	1	**0.00** *	3.69	1	0.05	8.31	1	**0.00** *
	Excl 3	PCA	7.52	2	**0.02** *	0.23	1	0.63	5.77	1	**0.02** *	5.03	1	**0.03** *
	Excl 4	PCA	11.66	2	**0.00** *	0.64	1	0.42	8.31	1	**0.00** *	8.31	1	**0.00** *
	Excl 5	DCA	14.00	2	**0.00** *	8.31	1	**0.00** *	8.31	1	**0.00** *	5.77	1	**0.02** *

Appendix 5: Results of Kruskal-Wallis-tests on the scores of the pqs on the first ordination axis, with treatment as independent variable, in spring 2000 per exclosure. Chi-square = chi-square-value obtained by the test. DF = degrees of freedom. P = significance value obtained by the test. * indicates that P is still significant after sequential Bonferroni correction. L+R+S = all grazers. R+S = rabbits and smaller herbivores, but no larger grazers. S = only herbivores that are smaller than rabbits.

IJzermonding	**summer 2000**			**summer 2001**			**summer 2005**		
Treatment	L+R+S	R+S	S	L+R+S	R+S	S	L+R+S	R+S	S
	Mean ± SE	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
mean vegetation height (cm)	17.71 ± 1.81	19.03 ± 1.95	28.45 ± 1.80	21.53 ± 2.11	24.88 ± 2.18	41.74 ± 1.92	49.71 ± 4.29	67.52 ± 4.94	80.60 ± 3.70
% bare soil cover	11.50 ± 1.75	8.67 ± 1.40	5.33 ± 1.04	17.60 ± 4.06	7.77 ± 2.17	2.10 ± 0.95	9.87 ± 4.41	4.97 ± 1.73	0.17 ± 0.10
% cover of woody plants	15.50 ± 2.91	13.20 ± 2.50	21.33 ± 3.16	17.10 ± 3.11	15.97 ± 3.21	33.83 ± 5.58	63.80 ± 5.86	63.50 ± 5.07	73.53 ± 4.59
% cover of non-woody plants	53.67 ± 3.83	64.50 ± 5.04	76.00 ± 3.04	48.40 ± 4.49	54.43 ± 4.97	74.90 ± 4.87	52.33 ± 2.92	60.17 ± 5.08	48.67 ± 5.33
% graminoid species cover in the non-woody cover	60.39 ± 3.41	69.04 ± 3.04	63.64 ± 3.23	69.01 ± 3.91	75.69 ± 3.30	83.47 ± 4.49	89.17 ± 2.44	87.37 ± 3.76	93.09 ± 2.67
% annual plant species cover in the non-woody cover	19.87 ± 4.31	18.35 ± 3.14	11.03 ± 2.03	22.71 ± 4.33	16.71 ± 2.60	10.19 ± 2.56	5.52 ± 1.06	8.79 ± 2.53	7.68 ± 3.16
% cover of mosses	79.00 ± 1.89	65.00 ± 5.70	47.90 ± 5.69	75.70 ± 4.11	70.13 ± 6.76	66.97 ± 6.48	52.43 ± 6.43	36.63 ± 6.64	9.87 ± 4.50
% cover of litter	7.50 ± 0.82	14.17 ± 2.96	18.67 ± 2.53	2.93 ± 0.55	7.03 ± 3.13	54.80 ± 8.03	44.43 ± 5.76	51.93 ± 6.87	89.77 ± 3.51
number of species per plot	15.90 ± 0.49	15.07 ± 0.44	14.77 ± 0.55	21.43 ± 0.46	19.93 ± 0.41	18.20 ± 0.55	16.03 ± 1.00	15.30 ± 0.93	8.97 ± 0.56
Simpson's index of diversity	0.76 ± 0.02	0.75 ± 0.02	0.82 ± 0.01	0.75 ± 0.01	0.72 ± 0.01	0.76 ± 0.01	0.73 ± 0.01	0.74 ± 0.02	0.61 ± 0.03
Arenaria serpyllifolia	1.67 ± 0.34	0.87 ± 0.09	1.20 ± 0.18	1.17 ± 0.11	0.97 ± 0.12	0.77 ± 0.12	0.60 ± 0.12	0.57 ± 0.09	0.03 ± 0.03
Bromus hordeaceus subsp. *thominei*	0.40 ± 0.24	1.97 ± 0.67	1.10 ± 0.38	0.90 ± 0.06	0.93 ± 0.07	1.00 ± 0.14	0.47 ± 0.09	0.60 ± 0.09	0.10 ± 0.06
Calamagrostis epigejos	10.10 ± 2.03	5.63 ± 1.41	6.67 ± 1.34	13.20 ± 2.68	11.87 ± 2.16	13.97 ± 3.28	15.07 ± 1.68	14.73 ± 2.35	9.63 ± 2.14
Carex arenaria	6.20 ± 0.84	14.00 ± 2.02	9.77 ± 1.32	7.83 ± 1.03	13.70 ± 1.55	13.23 ± 2.67	10.40 ± 1.61	14.60 ± 1.93	3.97 ± 1.20
Cladonia furcata	3.87 ± 0.84	0.87 ± 0.34	0.40 ± 0.24	1.93 ± 0.39	0.90 ± 0.27	0.53 ± 0.10	1.00 ± 0.34	0.40 ± 0.11	0.07 ± 0.05
Crepis capillaris	0.63 ± 0.15	0.93 ± 0.32	0.53 ± 0.25	1.43 ± 0.19	1.70 ± 0.29	0.63 ± 0.16	0.57 ± 0.10	0.57 ± 0.12	0.17 ± 0.07
Elymus repens	0.93 ± 0.51	0.03 ± 0.03	0.00 ± 0.00	2.03 ± 0.39	1.77 ± 0.70	6.23 ± 1.64	5.57 ± 1.37	12.03 ± 2.69	17.33 ± 4.51
Erodium cicutarium	2.70 ± 0.68	3.30 ± 0.74	1.43 ± 0.53	0.60 ± 0.13	0.67 ± 0.11	0.23 ± 0.09	0.13 ± 0.06	0.20 ± 0.07	0.00 ± 0.00
Festuca species	10.43 ± 1.80	17.67 ± 3.43	24.43 ± 2.84	17.80 ± 3.64	21.57 ± 4.07	37.53 ± 4.82	8.20 ± 1.50	6.17 ± 1.67	0.97 ± 0.40
Galium verum	1.97 ± 0.74	2.23 ± 0.76	8.00 ± 2.68	1.63 ± 0.73	5.90 ± 2.39	9.47 ± 3.75	0.90 ± 0.25	6.90 ± 2.75	4.33 ± 1.53
Geranium molle	0.43 ± 0.10	0.57 ± 0.09	0.50 ± 0.10	0.37 ± 0.09	0.53 ± 0.10	0.47 ± 0.10	0.33 ± 0.09	0.40 ± 0.09	0.37 ± 0.09
Hypochaeris radicata	1.00 ± 0.42	2.20 ± 0.65	0.63 ± 0.28	0.97 ± 0.14	1.63 ± 0.36	0.80 ± 0.21	0.40 ± 0.24	0.53 ± 0.18	0.07 ± 0.07
Leontodon species	4.70 ± 0.70	4.47 ± 0.66	4.10 ± 0.73	2.53 ± 0.41	3.10 ± 0.46	1.50 ± 0.32	1.10 ± 0.34	0.70 ± 0.20	0.10 ± 0.07
Lotus corniculatus	2.53 ± 0.70	2.17 ± 0.66	4.67 ± 1.39	1.47 ± 0.51	1.33 ± 0.40	3.43 ± 1.22	0.60 ± 0.17	2.57 ± 1.67	0.37 ± 0.25
Myosotis ramosissima	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.03	0.87 ± 0.06	0.90 ± 0.09	0.50 ± 0.10	0.47 ± 0.09	0.20 ± 0.07	0.07 ± 0.05
Ononis repens	5.93 ± 2.12	5.57 ± 1.65	9.33 ± 2.47	3.77 ± 1.79	4.30 ± 1.27	4.37 ± 1.49	0.90 ± 0.40	0.67 ± 0.37	0.13 ± 0.08
Phleum arenarium	1.43 ± 0.42	0.57 ± 0.10	0.43 ± 0.12	0.67 ± 0.10	0.63 ± 0.13	0.40 ± 0.12	0.23 ± 0.08	0.27 ± 0.08	0.03 ± 0.03

Appendix 6 (part I)

Poa pratensis	7.97 ± 1.67	7.33 ± 1.47	9.80 ± 1.89	1.67 ± 0.24	1.90 ± 0.30	6.00 ± 2.27	2.97 ± 0.99	2.53 ± 1.16	0.13 ± 0.06
Sedum acre	3.87 ± 0.57	3.40 ± 0.66	3.47 ± 0.61	4.20 ± 0.70	3.13 ± 0.52	2.50 ± 0.50	0.73 ± 0.14	0.77 ± 0.18	0.07 ± 0.07
Senecio jacobaea	5.70 ± 0.79	5.73 ± 0.89	8.67 ± 1.21	2.73 ± 0.33	4.07 ± 0.50	3.23 ± 0.53	7.40 ± 1.31	3.83 ± 0.61	1.03 ± 0.30
Tortula ruralis var. *ruraliformis*	52.93 ± 5.64	49.60 ± 6.50	41.07 ± 5.80	50.40 ± 5.48	54.40 ± 7.51	51.50 ± 7.53	21.77 ± 5.88	11.40 ± 4.56	0.67 ± 0.67
Veronica arvensis	0.03 ± 0.03	0.10 ± 0.06	0.07 ± 0.05	0.97 ± 0.03	0.93 ± 0.05	0.87 ± 0.06	0.20 ± 0.07	0.30 ± 0.09	0.03 ± 0.03

Appendix 6 (part II): Mean and standard error (SE) of the cover of some plant species and some other vegetation parameters in the exclosures in IJzermonding, per treatment and per season. L+R+S = all grazers. R+S = rabbits and smaller herbivores, but no larger grazers. S = only herbivores that are smaller than rabbits.

Dune Fossile de Ghyvelde	**summer 2000**			**summer 2001**			**summer 2005**		
Treatment	L+R+S	R+S	S	L+R+S	R+S	S	L+R+S	R+S	S
	Mean ± SE	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
mean vegetation height (cm)	17.82 ± 2.02	29.58 ± 2.18	36.32 ± 1.31	16.48 ± 1.94	28.11 ± 1.88	29.26 ± 1.71	24.56 ± 4.92	32.07 ± 3.02	31.89 ± 4.26
% bare soil cover	7.17 ± 1.99	14.33 ± 3.01	10.33 ± 1.87	1.27 ± 0.60	6.90 ± 2.63	3.97 ± 1.45	4.90 ± 2.14	15.87 ± 3.97	0.22 ± 0.17
% cover of woody plants	5.17 ± 1.56	4.00 ± 1.47	0.33 ± 0.23	4.47 ± 1.87	4.77 ± 1.58	0.67 ± 0.32	13.80 ± 4.38	20.43 ± 5.66	8.61 ± 3.72
% cover of non-woody plants	77.50 ± 2.28	70.83 ± 3.75	81.00 ± 2.47	68.13 ± 3.06	65.90 ± 4.11	68.63 ± 4.00	81.57 ± 4.18	68.77 ± 4.72	91.94 ± 3.43
% graminoid species cover in the non-woody cover	92.65 ± 1.18	91.77 ± 1.54	92.24 ± 1.37	74.19 ± 1.55	74.06 ± 1.61	76.96 ± 1.70	80.33 ± 2.53	80.98 ± 3.53	96.75 ± 1.43
% annual plant species cover in the non-woody cover	8.25 ± 1.20	9.52 ± 1.54	4.70 ± 0.73	2.26 ± 0.28	2.95 ± 0.51	4.40 ± 1.07	1.09 ± 0.20	1.34 ± 0.46	0.52 ± 0.15
% cover of mosses	67.83 ± 4.36	60.17 ± 4.74	61.50 ± 4.97	77.77 ± 4.02	69.33 ± 5.05	70.43 ± 4.92	59.07 ± 6.91	38.13 ± 7.15	60.22 ± 8.41
% cover of litter	9.37 ± 1.41	13.67 ± 1.61	13.33 ± 1.50	34.40 ± 4.31	40.23 ± 5.25	53.13 ± 5.71	7.27 ± 2.91	14.17 ± 4.32	69.78 ± 7.83
number of species per plot	13.73 ± 0.50	13.20 ± 0.40	13.73 ± 0.54	14.60 ± 0.60	14.50 ± 0.45	13.70 ± 0.57	13.43 ± 0.47	12.13 ± 0.73	11.22 ± 0.67
Simpson's index of diversity	0.79 ± 0.01	0.79 ± 0.01	0.77 ± 0.01	0.75 ± 0.02	0.78 ± 0.01	0.72 ± 0.02	0.76 ± 0.01	0.72 ± 0.02	0.75 ± 0.02
Agrostis capillaris	31.63 ± 4.65	23.20 ± 4.81	31.90 ± 5.16	31.50 ± 4.52	16.33 ± 3.60	29.57 ± 5.48	13.67 ± 2.76	8.30 ± 1.55	38.00 ± 5.43
Aira praecox	2.57 ± 0.63	3.97 ± 0.73	1.80 ± 0.54	0.43 ± 0.10	0.57 ± 0.12	0.53 ± 0.27	0.03 ± 0.03	0.00 ± 0.00	0.00 ± 0.00
Carex arenaria	26.93 ± 4.49	30.40 ± 4.64	28.60 ± 5.62	16.83 ± 3.28	24.67 ± 2.57	14.37 ± 2.41	31.47 ± 2.31	31.60 ± 3.91	27.50 ± 4.94
Cladonia furcata	2.40 ± 0.62	4.67 ± 1.17	2.03 ± 0.58	0.87 ± 0.16	1.27 ± 0.30	1.83 ± 0.54	0.60 ± 0.12	0.47 ± 0.13	0.28 ± 0.14
Dicranum scoparium	10.67 ± 4.18	4.57 ± 2.52	3.37 ± 1.42	8.13 ± 3.55	4.33 ± 2.63	5.23 ± 3.08	0.53 ± 0.27	0.57 ± 0.36	1.50 ± 1.12
Festuca species	8.53 ± 1.57	10.73 ± 2.19	21.90 ± 3.88	3.70 ± 0.80	8.13 ± 2.27	17.23 ± 4.26	0.33 ± 0.10	1.00 ± 0.66	4.61 ± 2.44
Galium verum	3.37 ± 0.71	3.33 ± 0.74	3.57 ± 0.69	2.30 ± 0.64	2.03 ± 0.55	2.83 ± 0.82	2.03 ± 0.59	1.83 ± 0.57	1.50 ± 0.58
Geranium molle	1.47 ± 0.42	0.73 ± 0.16	0.50 ± 0.10	0.97 ± 0.18	0.80 ± 0.22	2.37 ± 0.90	0.60 ± 0.13	0.27 ± 0.08	0.28 ± 0.11
Hypnum cupressiforme	29.90 ± 6.19	26.30 ± 5.51	24.67 ± 5.32	27.00 ± 6.66	26.53 ± 5.77	25.50 ± 5.97	10.10 ± 3.04	8.33 ± 3.33	11.89 ± 5.53
Luzula campestris	8.60 ± 2.29	2.10 ± 0.72	3.13 ± 0.76	10.87 ± 2.17	2.87 ± 1.07	3.27 ± 1.01	19.40 ± 2.80	6.00 ± 1.91	3.44 ± 1.80
Plagiomnium affine	5.97 ± 1.91	10.33 ± 3.50	19.20 ± 5.41	7.43 ± 2.97	9.37 ± 3.02	12.57 ± 4.23	5.13 ± 1.64	5.83 ± 2.36	22.56 ± 6.12
Pseudoscleropodium purum	17.90 ± 5.44	12.83 ± 4.58	7.77 ± 2.23	28.67 ± 6.48	16.80 ± 5.36	17.33 ± 5.59	41.37 ± 6.95	20.87 ± 5.55	12.61 ± 5.41
Rumex acetosella	4.23 ± 0.57	3.33 ± 0.56	4.43 ± 0.65	2.93 ± 0.47	2.97 ± 0.48	2.80 ± 0.40	8.07 ± 2.91	7.00 ± 1.92	3.11 ± 1.19
Senecio jacobaea	3.83 ± 1.01	6.60 ± 2.03	5.53 ± 1.41	2.93 ± 0.42	3.83 ± 0.84	2.97 ± 0.75	16.77 ± 2.98	7.93 ± 2.02	2.89 ± 1.37
Veronica arvensis	0.53 ± 0.09	0.53 ± 0.09	0.63 ± 0.09	0.00 ± 0.00	0.13 ± 0.06	0.03 ± 0.03	0.07 ± 0.05	0.03 ± 0.03	0.11 ± 0.08

Appendix 7: Mean and standard error (SE) of the cover of some plant species and some other vegetation parameters in the exclosures in Dune Fossile de Ghyvelde, per treatment and per season. L+R+S = all grazers. R+S = rabbits and smaller herbivores, but no larger grazers. S = only herbivores that are smaller than rabbits.

IJzermonding		**All treatments**				**L+R+S versus R+S**			**L+R+S versus S**				**R+S versus S**			
	Period	**Chi-square**	**DF**	**P**		**Chi-square**	**DF**	**P**	**Chi-square**	**DF**	**P**		**Chi-square**	**DF**	**P**	
mean vegetation height (cm)	summer 2000	16.80	2	**0.00**	*	0.52	1	0.47	14.16	1	**0.00**	*	10.39	1	**0.00**	*
	summer 2001	35.51	2	**<.0001**	*	1.30	1	0.25	29.76	1	**<.0001**	*	21.97	1	**<.0001**	*
	summer 2005	19.21	2	**<.0001**	*	6.21	1	**0.01**	19.15	1	**<.0001**	*	3.31	1	0.07	
% bare soil cover	summer 2000	7.17	2	**0.03**		0.87	1	0.35	7.08	1	**0.01**	*	2.83	1	0.09	
	summer 2001	23.60	2	**<.0001**	*	3.81	1	0.05	24.02	1	**<.0001**	*	7.78	1	**0.01**	*
	summer 2005	10.98	2	**0.00**	*	0.01	1	0.94	8.96	1	**0.00**	*	9.66	1	**0.00**	*
% cover of woody plants	summer 2000	5.14	2	0.08		0.39	1	0.53	2.91	1	0.09		4.38	1	**0.04**	
	summer 2001	6.85	2	**0.03**		0.16	1	0.69	4.90	1	**0.03**		5.19	1	**0.02**	
	summer 2005	1.95	2	0.38		0.01	1	0.92	0.97	1	0.32		1.97	1	0.16	
% cover of non-woody plants	summer 2000	14.78	2	**0.00**	*	3.34	1	0.07	16.13	1	**<.0001**	*	2.73	1	0.10	
	summer 2001	14.57	2	**0.00**	*	0.48	1	0.49	13.56	1	**0.00**	*	7.74	1	**0.01**	*
	summer 2005	3.41	2	0.18		1.56	1	0.21	0.69	1	0.41		2.89	1	0.09	
% graminoid species cover in the non-woody cover	summer 2000	3.43	2	0.18		3.09	1	0.08	0.44	1	0.51		1.63	1	0.20	
	summer 2001	8.44	2	**0.01**		2.19	1	0.14	5.64	1	**0.02**		5.47	1	**0.02**	
	summer 2005	4.02	2	0.13		0.31	1	0.58	3.41	1	0.06		2.58	1	0.11	
% annual plant species cover in the non-woody cover	summer 2000	2.30	2	0.32		0.00	1	0.95	1.58	1	0.21		1.85	1	0.17	
	summer 2001	13.93	2	**0.00**	*	0.51	1	0.47	12.71	1	**0.00**	*	7.57	1	**0.01**	*
	summer 2005	8.12	2	**0.02**		0.57	1	0.45	5.10	1	**0.02**		6.57	1	**0.01**	*
% cover of mosses	summer 2000	16.13	2	**0.00**	*	1.06	1	0.30	16.75	1	**<.0001**	*	6.19	1	**0.01**	
	summer 2001	0.06	2	0.97		0.14	1	0.71	0.00	1	0.97		0.01	1	0.92	
	summer 2005	18.93	2	**<.0001**	*	2.84	1	0.09	18.14	1	**<.0001**	*	7.56	1	**0.01**	*
% cover of litter	summer 2000	15.02	2	**0.00**	*	0.60	1	0.44	16.37	1	**<.0001**	*	5.53	1	**0.02**	*
	summer 2001	26.47	2	**<.0001**	*	2.33	1	0.13	21.96	1	**<.0001**	*	15.19	1	**<.0001**	*
	summer 2005	32.89	2	**<.0001**	*	0.73	1	0.39	30.50	1	**<.0001**	*	18.11	1	**<.0001**	*
number of species per plot	summer 2000	3.13	2	0.21		2.11	1	0.15	2.39	1	0.12		0.22	1	0.64	
	summer 2001	17.55	2	**0.00**		5.09	1	**0.02**	15.30	1	**<.0001**	*	6.03	1	**0.01**	*
	summer 2005	29.69	2	**<.0001**	*	0.19	1	0.66	24.13	1	**<.0001**	*	20.03	1	**<.0001**	*
Simpson's index of diversity	summer 2000	11.43	2	**0.00**	*	0.12	1	0.73	7.48	1	**0.01**	*	9.46	1	**0.00**	*
	summer 2001	4.18	2	0.12		2.41	1	0.12	0.25	1	0.62		3.58	1	0.06	
	summer 2005	18.76	2	**<.0001**	*	0.59	1	0.44	12.70	1	**0.00**	*	14.78	1	**0.00**	*

Appendix 8 (part I)

Arenaria serpyllifolia	summer 2000	4.37	2	0.11		4.92	1	**0.03**		0.46	1	0.50		1.63	1	0.20	
	summer 2001	5.56	2	0.06		1.44	1	0.23		5.53	1	**0.02**		1.35	1	0.25	
	summer 2005	21.11	2	**<.0001**	*	0.01	1	0.94		16.42	1	**<.0001**	*	19.98	1	**<.0001**	*
Bromus hordeaceus	summer 2000	5.29	2	0.07		2.18	1	0.14		5.80	1	**0.02**	*	0.30	1	0.58	
subsp. thominei	summer 2001	0.08	2	0.96		0.13	1	0.72		0.03	1	0.85		0.00	1	0.96	
	summer 2005	16.74	2	**0.00**	*	1.05	1	0.30		9.77	1	**0.00**	*	16.21	1	**<.0001**	*
Calamagrostis epigejos	summer 2000	2.25	2	0.32		2.05	1	0.15		0.42	1	0.51		0.90	1	0.34	
	summer 2001	0.17	2	0.92		0.08	1	0.77		0.17	1	0.68		0.00	1	0.98	
	summer 2005	8.09	2	**0.02**	*	0.58	1	0.45		7.65	1	**0.01**	*	3.88	1	**0.05**	
Carex arenaria	summer 2000	13.12	2	**0.00**	*	13.60	1	**0.00**	*	3.78	1	0.05		2.31	1	0.13	
	summer 2001	9.64	2	**0.01**	*	10.96	1	**0.00**	*	0.45	1	0.50		3.26	1	0.07	
	summer 2005	21.75	2	**<.0001**	*	2.17	1	0.14		12.08	1	**0.00**	*	18.57	1	**<.0001**	*
Cladonia furcata	summer 2000	32.21	2	**<.0001**	*	16.71	1	**<.0001**	*	26.97	1	**<.0001**	*	2.17	1	0.14	
	summer 2001	22.48	2	**<.0001**	*	12.31	1	**0.00**	*	20.85	1	**<.0001**	*	0.26	1	0.61	
	summer 2005	17.56	2	**0.00**	*	3.57	1	0.06		17.31	1	**<.0001**	*	6.73	1	**0.01**	*
Crepis capillaris	summer 2000	3.33	2	0.19		0.01	1	0.93		2.95	1	0.09		2.21	1	0.14	
	summer 2001	15.05	2	**0.00**	*	0.04	1	0.85		11.60	1	**0.00**	*	11.23	1	**0.00**	*
	summer 2005	9.72	2	**0.01**	*	0.05	1	0.83		8.91	1	**0.00**	*	6.70	1	**0.01**	*
Elymus repens	summer 2000	5.57	2	0.06		2.06	1	0.15		4.21	1	**0.04**		1.00	1	0.32	
	summer 2001	15.75	2	**0.00**	*	3.00	1	0.08		7.29	1	**0.01**	*	13.56	1	**0.00**	*
	summer 2005	2.84	2	0.24		2.52	1	0.11		1.77	1	0.18		0.00	1	0.98	
Erodium cicutarium	summer 2000	3.71	2	0.16		0.05	1	0.83		3.63	1	0.06		1.99	1	0.16	
	summer 2001	9.25	2	**0.01**	*	0.39	1	0.53		4.97	1	**0.03**		9.24	1	**0.00**	*
	summer 2005	6.23	2	**0.04**		0.47	1	0.49		4.21	1	**0.04**		6.56	1	**0.01**	*
Festuca species	summer 2000	17.87	2	**0.00**	*	2.15	1	0.14		18.90	1	**<.0001**	*	5.81	1	**0.02**	*
	summer 2001	10.28	2	**0.01**	*	0.62	1	0.43		9.59	1	**0.00**	*	5.13	1	**0.02**	*
	summer 2005	29.68	2	**<.0001**	*	3.29	1	0.07		28.00	1	**<.0001**	*	13.77	1	**0.00**	*
Galium verum	summer 2000	1.75	2	0.42		0.00	1	0.98		1.34	1	0.25		1.22	1	0.27	
	summer 2001	1.78	2	0.41		0.01	1	0.93		1.35	1	0.25		1.23	1	0.27	
	summer 2005	0.34	2	0.84		0.00	1	0.96		0.46	1	0.50		0.08	1	0.78	
Geranium molle	summer 2000	1.27	2	0.53		1.27	1	0.26		0.24	1	0.62		0.38	1	0.54	
	summer 2001	1.25	2	0.54		1.26	1	0.26		0.38	1	0.54		0.24	1	0.62	
	summer 2005	0.28	2	0.87		0.28	1	0.60		0.07	1	0.79		0.07	1	0.79	

Appendix 8 (part II)

Hypochaeris radicata	summer 2000	4.12	2	0.13		1.52	1	0.22		0.61	1	0.44		3.89	1	**0.05**	
	summer 2001	3.94	2	0.14		0.48	1	0.49		2.04	1	0.15		3.46	1	0.06	
	summer 2005	7.33	2	**0.03**		1.07	1	0.30		3.75	1	0.05		7.30	1	**0.01**	*
Leontodon species	summer 2000	0.49	2	0.78		0.04	1	0.83		0.47	1	0.49		0.21	1	0.64	
	summer 2001	8.43	2	**0.01**	*	0.72	1	0.40		3.94	1	**0.05**		7.97	1	**0.00**	*
	summer 2005	16.29	2	**0.00**	*	1.51	1	0.22		16.42	1	**<.0001**	*	8.99	1	**0.00**	*
Lotus corniculatus	summer 2000	0.63	2	0.73		0.23	1	0.63		0.13	1	0.72		0.59	1	0.44	
	summer 2001	0.34	2	0.84		0.03	1	0.87		0.30	1	0.58		0.19	1	0.67	
	summer 2005	6.58	2	**0.04**		0.05	1	0.83		5.65	1	**0.02**		5.45	1	**0.02**	
Myosotis ramosissima	summer 2000	2.00	2	0.37		0.00	1	1.00		1.00	1	0.32		1.00	1	0.32	
	summer 2001	12.52	2	**0.00**	*	0.06	1	0.81		8.85	1	**0.00**	*	8.18	1	**0.00**	*
	summer 2005	13.33	2	**0.00**	*	4.72	1	**0.03**		12.07	1	**0.00**	*	2.27	1	0.13	
Ononis repens	summer 2000	2.40	2	0.30		1.44	1	0.23		2.09	1	0.15		0.22	1	0.64	
	summer 2001	1.59	2	0.45		1.57	1	0.21		0.78	1	0.38		0.08	1	0.77	
	summer 2005	4.12	2	0.13		1.25	1	0.26		3.97	1	**0.05**		0.70	1	0.40	
Phleum arenarium	summer 2000	4.42	2	0.11		1.06	1	0.30		4.14	1	**0.04**		1.43	1	0.23	
	summer 2001	4.41	2	0.11		0.24	1	0.63		4.43	1	**0.04**		2.10	1	0.15	
	summer 2005	6.46	2	**0.04**		0.09	1	0.77		5.11	1	**0.02**		6.30	1	**0.01**	*
Poa pratensis	summer 2000	0.31	2	0.86		0.04	1	0.83		0.13	1	0.72		0.30	1	0.59	
	summer 2001	0.29	2	0.87		0.05	1	0.82		0.29	1	0.59		0.09	1	0.76	
	summer 2005	21.55	2	**<.0001**	*	0.08	1	0.78		16.93	1	**<.0001**	*	18.16	1	**<.0001**	*
Sedum acre	summer 2000	1.44	2	0.49		1.21	1	0.27		0.95	1	0.33		0.00	1	0.95	
	summer 2001	5.81	2	0.05		1.44	1	0.23		5.85	1	**0.02**	*	1.38	1	0.24	
	summer 2005	18.48	2	**<.0001**	*	0.02	1	0.89		16.90	1	**<.0001**	*	15.42	1	**<.0001**	*
Senecio jacobaea	summer 2000	3.57	2	0.17		0.02	1	0.89		2.87	1	0.09		2.43	1	0.12	
	summer 2001	3.33	2	0.19		3.08	1	0.08		0.02	1	0.88		1.90	1	0.17	
	summer 2005	32.95	2	**<.0001**	*	4.43	1	**0.04**		28.38	1	**<.0001**	*	17.18	1	**<.0001**	*
Tortula ruralis var. ruraliformis	summer 2000	2.24	2	0.33		0.09	1	0.76		2.27	1	0.13		0.98	1	0.32	
	summer 2001	0.40	2	0.82		0.56	1	0.45		0.09	1	0.76		0.00	1	0.99	
	summer 2005	12.02	2	**0.00**	*	1.09	1	0.30		12.03	1	**0.00**	*	7.53	1	**0.01**	*

Appendix 8 (part III)

Veronica arvensis	summer 2000	1.06	2	0.59	1.05	1	0.30	0.35	1	0.56	0.21	1	0.64	
	summer 2001	2.14	2	0.34	0.35	1	0.56	1.93	1	0.16	0.73	1	0.39	
	summer 2005	7.37	2	**0.03**	0.79	1	0.38	3.98	1	**0.05**	7.55	1	**0.01**	*

Appendix 8 (part IV): Results of Kruskal-Wallis-tests of the cover of some plant species and some other vegetation parameters in the exclosures in IJzermonding, with treatment as independent variable, analysed per season. Chi-square = chi-square-value obtained by the test. DF = degrees of freedom. P = significance value obtained by the test. * indicates that P is still significant after sequential Bonferroni correction. L+R+S = all grazers. R+S = rabbits and smaller herbivores, but no larger grazers. S = only herbivores that are smaller than rabbits.

Dune Fossile de Ghyvelde		**All treatments**				**L+R+S versus R+S**				**L+R+S versus S**				**R+S versus S**			
	Period	**Chi-square**	**DF**	**P**		**Chi-square**	**DF**	**P**		**Chi-square**	**DF**	**P**		**Chi-square**	**DF**	**P**	
mean vegetation height (cm)	summer 2000	32.95	2	**<.0001**	*	16.41	1	**<.0001**	*	27.24	1	**<.0001**	*	6.06	1	**0.01**	*
	summer 2001	39.75	2	**<.0001**	*	28.33	1	**<.0001**	*	30.41	1	**<.0001**	*	0.70	1	0.40	
	summer 2005	14.32	2	**0.00**	*	8.83	1	**0.00**	*	12.65	1	**0.00**	*	0.12	1	0.73	
% bare soil cover	summer 2000	3.20	2	0.20		2.42	1	0.12		2.26	1	0.13		0.22	1	0.64	
	summer 2001	3.42	2	0.18		2.17	1	0.14		3.18	1	0.07		0.00	1	0.95	
	summer 2005	12.87	2	**0.00**	*	5.00	1	**0.03**		3.57	1	0.06		10.77	1	**0.00**	*
% cover of woody plants	summer 2000	12.14	2	**0.00**	*	1.25	1	0.26		12.69	1	**0.00**	*	5.82	1	**0.02**	*
	summer 2001	8.26	2	**0.02**	*	0.01	1	0.92		6.43	1	**0.01**	*	6.71	1	**0.01**	*
	summer 2005	0.53	2	0.77		0.31	1	0.58		0.44	1	0.51		0.03	1	0.85	
% cover of non-woody plants	summer 2000	3.84	2	0.15		1.19	1	0.27		1.15	1	0.28		3.43	1	0.06	
	summer 2001	0.16	2	0.92		0.05	1	0.83		0.03	1	0.86		0.16	1	0.69	
	summer 2005	15.91	2	**0.00**	*	3.90	1	**0.05**		7.06	1	**0.01**	*	14.00	1	**0.00**	*
% graminoid species cover in the non-woody cover	summer 2000	0.03	2	0.98		0.04	1	0.83		0.01	1	0.92		0.00	1	0.99	
	summer 2001	1.29	2	0.52		0.73	1	0.39		1.20	1	0.27		0.01	1	0.92	
	summer 2005	18.03	2	**0.00**	*	0.33	1	0.56		18.34	1	**<.0001**	*	10.67	1	**0.00**	*
% annual plant species cover in the non-woody cover	summer 2000	5.79	2	0.06		0.20	1	0.66		3.87	1	**0.05**		4.60	1	**0.03**	
	summer 2001	0.36	2	0.84		0.16	1	0.69		0.33	1	0.57		0.05	1	0.83	
	summer 2005	2.97	2	0.23		0.82	1	0.36		3.28	1	0.070		0.46	1	0.50	
% cover of mosses	summer 2000	1.33	2	0.51		1.31	1	0.25		0.59	1	0.44		0.09	1	0.76	
	summer 2001	2.08	2	0.35		2.25	1	0.13		0.77	1	0.38		0.12	1	0.73	
	summer 2005	5.33	2	0.070		3.82	1	0.051		0.06	1	0.81		3.78	1	0.052	
% cover of litter	summer 2000	7.72	2	**0.02**		5.18	1	**0.02**		6.64	1	**0.01**	*	0.00	1	0.95	
	summer 2001	5.56	2	0.06		0.52	1	0.47		5.01	1	**0.03**		2.78	1	0.10	
	summer 2005	34.69	2	**<.0001**	*	3.00	1	0.08		29.52	1	**<.0001**	*	22.78	1	**<.0001**	*
number of species per plot	summer 2000	0.70	2	0.70		0.49	1	0.48		0.00	1	0.95		0.55	1	0.46	
	summer 2001	2.45	2	0.29		0.02	1	0.88		1.65	1	0.20		2.00	1	0.16	
	summer 2005	6.78	2	**0.03**		2.34	1	0.13		7.17	1	**0.01**	*	0.93	1	0.34	
Simpson's index of diversity	summer 2000	3.73	2	0.16		0.01	1	0.94		2.36	1	0.12		3.20	1	0.07	
	summer 2001	6.22	2	**0.04**		1.77	1	0.18		1.26	1	0.26		6.24	1	**0.01**	*
	summer 2005	2.00	2	0.37		1.43	1	0.23		0.08	1	0.78		1.37	1	0.24	

Appendix 9 (part I)

Agrostis capillaris	summer 2000	4.17	2	0.12		3.61	1	0.06		0.04	1	0.84		2.58	1	0.11	
	summer 2001	6.21	2	**0.04**		6.89	1	**0.01**	*	0.60	1	0.44		1.87	1	0.17	
	summer 2005	16.69	2	**0.00**	*	0.98	1	0.32		11.42	1	**0.00**	*	14.47	1	**0.00**	*
Aira praecox	summer 2000	4.41	2	0.11		1.76	1	0.18		0.54	1	0.46		4.22	1	**0.04**	
	summer 2001	3.19	2	0.20		0.48	1	0.49		1.42	1	0.23		3.00	1	0.08	
	summer 2005	1.60	2	0.45		1.00	1	0.32		0.60	1	0.44		0.00	1	1.00	
Carex arenaria	summer 2000	0.73	2	0.70		0.20	1	0.66		0.43	1	0.51		0.51	1	0.48	
	summer 2001	9.32	2	**0.01**	*	6.34	1	**0.01**	*	0.15	1	0.70		7.48	1	**0.01**	*
	summer 2005	1.63	2	0.44		0.26	1	0.61		1.76	1	0.18		0.57	1	0.45	
Cladonia furcata	summer 2000	1.05	2	0.59		0.74	1	0.39		0.00	1	0.97		0.80	1	0.37	
	summer 2001	0.13	2	0.93		0.19	1	0.66		0.02	1	0.88		0.00	1	0.95	
	summer 2005	3.32	2	0.19		1.02	1	0.31		3.27	1	0.07		0.78	1	0.38	
Dicranum scoparium	summer 2000	1.66	2	0.44		0.67	1	0.41		0.14	1	0.70		1.73	1	0.19	
	summer 2001	1.66	2	0.44		1.58	1	0.21		0.58	1	0.45		0.28	1	0.60	
	summer 2005	0.37	2	0.83		0.00	1	0.95		0.25	1	0.62		0.32	1	0.57	
Festuca species	summer 2000	6.73	2	**0.03**		0.21	1	0.64		5.91	1	**0.02**	*	3.89	1	**0.05**	
	summer 2001	3.78	2	0.15		0.32	1	0.57		3.60	1	0.06		1.70	1	0.19	
	summer 2005	1.21	2	0.54		0.03	1	0.86		1.08	1	0.30		0.80	1	0.37	
Galium verum	summer 2000	0.06	2	0.97		0.02	1	0.88		0.06	1	0.80		0.01	1	0.92	
	summer 2001	0.57	2	0.75		0.02	1	0.88		0.33	1	0.57		0.50	1	0.48	
	summer 2005	0.09	2	0.96		0.06	1	0.81		0.07	1	0.79		0.01	1	0.94	
Geranium molle	summer 2000	1.90	2	0.39		0.28	1	0.60		1.68	1	0.19		0.92	1	0.34	
	summer 2001	4.17	2	0.12		1.44	1	0.23		0.72	1	0.40		4.12	1	**0.04**	
	summer 2005	4.24	2	0.12		3.42	1	0.06		2.25	1	0.13		0.01	1	0.93	
Hypnum cupressiforme	summer 2000	0.21	2	0.90		0.05	1	0.83		0.20	1	0.65		0.06	1	0.80	
	summer 2001	1.15	2	0.56		0.04	1	0.84		1.16	1	0.28		0.62	1	0.43	
	summer 2005	2.40	2	0.30		2.53	1	0.11		0.15	1	0.70		0.67	1	0.41	
Luzula campestris	summer 2000	6.43	2	**0.04**	*	5.86	1	**0.02**	*	2.34	1	0.13		1.45	1	0.23	
	summer 2001	13.63	2	**0.00**	*	10.64	1	**0.00**	*	9.15	1	**0.00**	*	0.16	1	0.69	
	summer 2005	21.28	2	**<.0001**	*	13.45	1	**0.00**	*	15.26	1	**<.0001**	*	1.83	1	0.18	
Plagiomnium affine	summer 2000	1.93	2	0.38		0.01	1	0.91		1.63	1	0.20		1.17	1	0.28	
	summer 2001	0.27	2	0.88		0.00	1	0.95		0.24	1	0.62		0.15	1	0.70	
	summer 2005	6.76	2	**0.03**		1.14	1	0.29		3.74	1	0.05		5.86	1	**0.02**	

Appendix 9 (part II)

Pseudoscleropodium purum	summer 2000	0.41	2	0.82		0.26	1	0.61		0.34	1	0.56		0.00	1	0.95
	summer 2001	2.68	2	0.26		2.07	1	0.15		1.85	1	0.17		0.00	1	0.98
	summer 2005	9.53	2	**0.01**	*	4.96	1	**0.03**		8.34	1	**0.00**	*	0.78	1	0.38
Rumex acetosella	summer 2000	1.43	2	0.49		1.35	1	0.25		0.00	1	0.99		0.80	1	0.37
	summer 2001	0.09	2	0.96		0.06	1	0.80		0.00	1	0.95		0.06	1	0.80
	summer 2005	4.33	2	0.11		0.54	1	0.46		2.53	1	0.11		3.82	1	0.05
Senecio jacobaea	summer 2000	0.52	2	0.77		0.06	1	0.81		0.71	1	0.40		0.05	1	0.82
	summer 2001	0.59	2	0.75		0.01	1	0.93		0.72	1	0.40		0.19	1	0.66
	summer 2005	17.41	2	**0.00**	*	6.29	1	**0.01**	*	15.18	1	**<.0001**	*	5.22	1	**0.02**
Veronica arvensis	summer 2000	0.81	2	0.67		0.00	1	1.00		0.61	1	0.44		0.61	1	0.44
	summer 2001	5.44	2	0.07		4.21	1	**0.04**		1.00	1	0.32		1.93	1	0.16
	summer 2005	1.13	2	0.57		0.35	1	0.56		0.28	1	0.59		1.14	1	0.29

Appendix 9 (part III): Results of Kruskal-Wallis-tests of the cover of some plant species and some other vegetation parameters in the exclosures in Dune Fossile de Ghyvelde, with treatment as independent variable, analysed per season. Chi-square = chi-square-value obtained by the test. DF = degrees of freedom. P = significance value obtained by the test. * indicates that P is still significant after sequential Bonferroni correction. L+R+S = all grazers. R+S = rabbits and smaller herbivores, but no larger grazers. S = only herbivores that are smaller than rabbits.

Study area	Period	Exclosure	ordination technique	All treatments			L+R+S versus R+S			L+R+S versus S			R+S versus S		
				Chi-square	DF	P	Chi-square	DF	P	Chi-square	DF	P	Chi-square	DF	P
IJzermonding	Summer 2000	Excl 1	PCA	13.20	2	**0.00** *	8.31	1	**0.00** *	7.41	1	**0.01** *	5.03	1	**0.03**
		Excl 2	PCA	13.05	2	**0.00** *	3.69	1	0.05	8.31	1	**0.00** *	8.31	1	**0.00** *
		Excl 3	PCA	2.84	2	0.24	0.92	1	0.34	0.23	1	0.63	3.69	1	0.05
		Excl 4	DCA	12.77	2	**0.00** *	8.31	1	**0.00** *	5.03	1	**0.03**	6.56	1	**0.01** *
		Excl 5	PCA	11.56	2	**0.00** *	0.41	1	0.52	8.31	1	**0.00** *	8.31	1	**0.00** *
	Summer 2001	Excl 1	PCA	13.56	2	**0.00** *	8.31	1	**0.00** *	7.41	1	**0.01** *	5.77	1	**0.02**
		Excl 2	PCA	10.05	2	**0.01** *	1.26	1	0.26	6.56	1	**0.01** *	7.41	1	**0.01** *
		Excl 3	PCA	2.29	2	0.32	0.10	1	0.75	1.64	1	0.20	1.64	1	0.20
		Excl 4	DCA	10.98	2	**0.00** *	8.31	1	**0.00** *	7.41	1	**0.01** *	0.64	1	0.42
		Excl 5	PCA	12.77	2	**0.00** *	6.56	1	**0.01** *	8.31	1	**0.00** *	5.03	1	**0.03**
	Summer 2005	Excl 1	DCA	12.54	2	**0.00** *	2.56	1	0.11	8.31	1	**0.00** *	8.31	1	**0.00** *
		Excl 2	PCA	13.93	2	**0.00** *	6.56	1	**0.01** *	8.31	1	**0.00** *	7.41	1	**0.01** *
		Excl 3	PCA	10.05	2	**0.01** *	0.64	1	0.42	8.31	1	**0.00** *	5.77	1	**0.02** *
		Excl 4	PCA	2.29	2	0.32	0.23	1	0.63	2.08	1	0.15	1.26	1	0.26
		Excl 5	PCA	14.75	2	**0.00** *	8.31	1	**0.00** *	8.31	1	**0.00** *	7.41	1	**0.01** *
Ghyvelde	Summer 2000	Excl 1	PCA	7.52	2	**0.02** *	0.41	1	0.52	5.03	1	**0.03**	5.77	1	**0.02** *
		Excl 2	PCA	10.98	2	**0.00** *	8.31	1	**0.00** *	7.41	1	**0.01** *	0.41	1	0.52
		Excl 3	PCA	2.84	2	0.24	0.92	1	0.34	0.23	1	0.63	3.69	1	0.05
		Excl 4	PCA	14.36	2	**0.00** *	6.56	1	**0.01** *	8.31	1	**0.00** *	8.31	1	**0.00** *
		Excl 5	PCA	13.13	2	**0.00** *	6.56	1	**0.01** *	8.31	1	**0.00** *	5.77	1	**0.02**
	Summer 2001	Excl 1	DCA	6.14	2	**0.05** *	3.10	1	0.08	5.03	1	**0.03**	0.92	1	0.34
		Excl 2	PCA	11.82	2	**0.00** *	8.31	1	**0.00** *	6.56	1	**0.01** *	3.10	1	0.08
		Excl 3	PCA	8.64	2	**0.01** *	5.03	1	**0.03**	6.56	1	**0.01** *	1.26	1	0.26
		Excl 4	DCA	13.56	2	**0.00** *	7.41	1	**0.01** *	8.31	1	**0.00** *	5.77	1	**0.02**
		Excl 5	DCA	6.74	2	**0.03**	8.31	1	**0.00** *	0.92	1	0.34	0.92	1	0.34
	Summer 2005	Excl 1	PCA	12.78	2	**0.00** *	3.10	1	0.08	8.31	1	**0.00** *	8.31	1	**0.00** *
		Excl 2	PCA	5.19	2	0.07	2.08	1	0.15	4.33	1	**0.04** *	1.26	1	0.26
		Excl 3	PCA	5.77	1	**0.02** *	5.77	1	**0.02**	-	-	-	-	-	-
		Excl 4	DCA	10.05	2	**0.01** *	5.77	1	**0.02**	8.31	1	**0.00** *	0.64	1	0.42
		Excl 5	DCA	0.10	1	0.75	0.10	1	0.75	-	-	-	-	-	-

Appendix 10: Results of Kruskal-Wallis-tests on the scores of the pqs on the first ordination axis, with treatment as independent variable, per season and per exclosure. Chi-square = chi-square-value obtained by the test. DF = degrees of freedom. P = significance value obtained by the test. * indicates that P is still significant after sequential Bonferroni correction. L+R+S = all grazers. R+S = rabbits and smaller herbivores, but no larger grazers. S = only herbivores that are smaller than rabbits.

Species name	Dutch name	Family
Agrostis capillaris	Gewoon struisgras	Poaceae
Agrostis stolonifera	Fioringras	Poaceae
Ammophila arenaria	Helm	Poaceae
Anthriscus caucalis	Fijne kervel	Apiaceae
Arenaria serpyllifolia	Zandmuur	Caryophyllaceae
Arrhenatherum elatius	Glanshaver	Poaceae
Avenula pubescens	Zachte haver	Poaceae
Brachythecium albicans	Bleek dikkopmos	Brachytheciaceae
Brachythecium rutabulum	Gewoon dikkopmos	Brachytheciaceae
Bromus hordeaceus subsp. *thominei*	Duindravik	Poaceae
Bromus sterilis	IJle dravik	Poaceae
Bryonia dioica	Heggenrank	Cucurbitaceae
Bryum species	Knikmos	Bryaceae
Calamagrostis epigejos	Duinriet	Poaceae
Carex arenaria	Zandzegge	Cyperaceae
Cerastium species	Hoornbloem	Caryophyllaceae
Ceratodon purpureus	Purpersteeltje	Ditrichaceae
Cirsium species	Vederdistel	Asteraceae
Cladonia furcata		Cladoniaceae
Cladonia pyxidata		Cladoniaceae
Crepis capillaris	Klein streepzaad	Asteraceae
Cynoglossum officinale	Veldhondstong	Boraginaceae
Dactylis glomerata	Gewone kropaar	Poaceae
Elymus athericus	Strandkweek	Poaceae
Elymus repens	Kweek	Poaceae
Erigeron canadensis	Canadese fijnstraal	Asteraceae
Erodium cicutarium	Gewone reigersbek	Geraniaceae
Erophila verna	Vroegeling	Brassicaceae
Eurhynchium praelongum	Fijn laddermos	Brachytheciaceae
Kiemplant Fabaceae		Fabaceae
Festuca species	Zwenkgras	Poaceae
Galium aparine	Kleefkruid	Rubiaceae
Galium verum	Geel walstro	Rubiaceae
Geranium molle	Zachte ooievaarsbek	Geraniaceae
Hippophae rhamnoides	Duindoorn	Elaeagnaceae
Holcus lanatus	Gestreepte witbol	Poaceae
Homalothecium lutescens	Smaragdmos	Brachytheciaceae
Hypochaeris radicata	Gewoon biggenkruid	Asteraceae
Hypnum cupressiforme	Gewoon klauwtjesmos	Hypnaceae
Kiemplant dicotyl		
Kiemplant monocotyl		
Koeleria macrantha	Smal fakkelgras	Poaceae
Lamium purpureum	Paarse dovenetel	Lamiaceae
Leontodon species	Leeuwentand	Asteraceae
Lolium perenne	Engels raaigras	Poaceae
Lotus corniculatus	Gewone rolklaver	Fabaceae

Appendix 11 (part I)

Medicago minima	Kleine rupsklaver	Fabaceae
Myosotis arvensis	Akkervergeet-mij-nietje	Boraginaceae
Myosotis ramosissima	Ruw vergeet-mij-nietje	Boraginaceae
Ononis repens	Kruipend stalkruid	Fabaceae
Peltigera canina		Peltigeraceae
Phleum arenarium	Zanddoddengras	Poaceae
Phleum pratense	Gewoon timoteegras	Poaceae
Plantago coronopus	Hertshoornweegbree	Plantaginaceae
Plantago lanceolata	Smalle weegbree	Plantaginaceae
Poa pratensis	Veldbeemdgras	Poaceae
Rhynchostegium megapolitanum	Duinsnavelmos	Brachytheciaceae
Rubus caesius	Dauwbraam	Rosaceae
Rumex acetosella	Schapenzuring	Polygonaceae
Saxifraga tridactylites	Kandelaartje	Saxifragaceae
Sedum acre	Muurpeper	Crassulaceae
Senecio jacobaea	Jacobskruiskruid	Asteraceae
Sonchus species	Melkdistel	Asteraceae
Stellaria media	Vogelmuur	Caryophyllaceae
Taraxacum species	Paardenbloem	Asteraceae
Tortula ruralis var *ruraliformis*	Groot duinsterretje	Pottiaceae
Tragopogon pratensis	Gele morgenster	Asteraceae
Trifolium arvense	Hazenpootje	Fabaceae
Trifolium campestre	Liggende klaver	Fabaceae
Trifolium dubium	Kleine klaver	Fabaceae
Trifolium repens	Witte klaver	Fabaceae
Trifolium scabrum	Ruwe klaver	Fabaceae
Valerianella locusta	Gewone veldsla	Valerianaceae
Veronica arvensis	Veldereprijs	Scrophulariaceae
Vicia species	Wikke	Fabaceae
Vulpia species	Langbaardgras	Poaceae

Appendix 11 (part II): Species list of the plant species found in the pqs in IJzermonding (from spring 2000 until summer 2005).

Species name	Dutch name	Family
Acer platanoides	Noorse esdoorn	Sapindaceae
Agrostis capillaris	Gewoon struisgras	Poaceae
Aira praecox	Vroege haver	Poaceae
Anthoxanthum odoratum	Gewoon reukgras	Poaceae
Aphanes inexspectata	Kleine leeuwenklauw	Rosaceae
Arenaria serpyllifolia	Zandmuur	Caryophyllaceae
Avenula pubescens	Zachte haver	Poaceae
Brachythecium rutabulum	Gewoon dikkopmos	Brachytheciaceae
Kiemplant Brassicaceae		Brassicaceae
Bromus hordeaceus subsp. *thominei*	Duindravik	Poaceae
Bryum species	Knikmos	Bryaceae
Calamagrostis epigejos	Duinriet	Poaceae
Calliergonella cuspidata	Gewoon puntmos	Amblystegiaceae
Cardamine hirsuta	Kleine veldkers	Brassicaceae
Carex arenaria	Zandzegge	Cyperaceae
Kiemplant Caryophyllaceae		Caryophyllaceae
Cerastium species	Hoornbloem	Caryophyllaceae
Ceratodon purpureus	Purpersteeltje	Ditrichaceae
Cladina arbuscula		Cladoniaceae
Cladonia furcata		Cladoniaceae
Cladonia pyxidata		Cladoniaceae
Claytonia perfoliata	Witte winterpostelein	Portulacaceae
Climacium dendroides	Boompjesmos	Climaciaceae
Crataegus monogyna	Eénstijlige meidoorn	Rosaceae
Crepis capillaris	Klein streepzaad	Asteraceae
Cynoglossum officinale	Veldhondstong	Boraginaceae
Dicranum scoparium	Gewoon gaffeltandmos	Dicranaceae
Erodium cicutarium	Gewone reigersbek	Geraniaceae
Erophila verna	Vroegeling	Brassicaceae
Eurhynchium praelongum	Fijn laddermos	Brachytheciaceae
Kiemplant Fabaceae		Fabaceae
Festuca species	Zwenkgras	Poaceae
Galium aparine	Kleefkruid	Rubiaceae
Galium verum	Geel walstro	Rubiaceae
Geranium molle	Zachte ooievaarsbek	Geraniaceae
Hieracium umbellatum	Schermhavikskruid	Asteraceae
Holcus lanatus	Gestreepte witbol	Poaceae
Hypochaeris radicata	Gewoon biggenkruid	Asteraceae
Hypnum cupressiforme	Gewoon klauwtjesmos	Hypnaceae
Kiemplant dicotyl		
Kiemplant monocotyl		
Leontodon species	Leeuwentand	Asteraceae
Ligustrum vulgare	Wilde liguster	Oleaceae
Luzula campestris	Gewone veldbies	Juncaceae
Muscari comosum	Kuifhyacint	Hyacinthaceae
Myosotis ramosissima	Ruw vergeet-mij-nietje	Boraginaceae
Peltigera species		Peltigeraceae
Phleum arenarium	Zanddoddengras	Poaceae

Appendix 12 (part I)

Plagiomnium affine	Rondbladig boogsterrenmos	Mniaceae
Pleurochaete squarrosa	Hakig kronkelbladmos	Pottiaceae
Poa pratensis	Veldbeemdgras	Poaceae
Polytrichum juniperinum	Zandhaarmos	Polytrichaceae
Populus tremula	Ratelpopulier	Salicaceae
Pseudoscleropodium purum	Groot laddermos	Brachytheciaceae
Racomitrium canescens	Grijze bisschopmuts	Grimmiaceae
Ranunculus bulbosus	Knolboterbloem	Ranunculaceae
Rosa canina	Hondsroos	Rosaceae
Rubus species	Braam	Rosaceae
Rumex acetosella	Schapenzuring	Polygonaceae
Sambucus nigra	Gewone vlier	Adoxaceae
Saxifraga tridactylites	Kandelaartje	Saxifragaceae
Sedum acre	Muurpeper	Crassulaceae
Senecio jacobaea	Jacobskruiskruid	Asteraceae
Senecio sylvaticus	Boskruiskruid	Asteraceae
Sonchus species	Melkdistel	Asteraceae
Stellaria media	Vogelmuur	Caryophyllaceae
Taraxacum species	Paardenbloem	Asteraceae
Teesdalia nudicaulis	Klein tasjeskruid	Brassicaceae
Tortula ruralis var *ruraliformis*	Groot duinsterretje	Pottiaceae
Urtica dioica	Grote brandnetel	Urticaceae
Veronica arvensis	Veldereprijs	Plantaginaceae
Veronica officinalis	Mannetjesereprijs	Plantaginaceae
Vicia species	Wikke	Fabaceae
Viola curtisii	Duinviooltje	Violaceae

Appendix 12 (part II): Species list of the plant species found in the pqs in Dune Fossile de Ghyvelde (from spring 2000 until summer 2005).

IJzermonding	
	Ammophila arenaria was present in the three treatments in spring and summer 2000, but disappeared from L+R+S and R+S in 2001 and had completely gone in 2005.
	Anthriscus caucalis, which was not present in each treatment in spring 2000 (missing in L+R+S) and summer 2001 (absent in L+R+S and R+S), and even completely lacking in summer 2000, was present in the three treatments in summer 2005.
	Arrhenatherum elatius and *Bromus sterilis*, completely absent in 2000, appeared in S in 2001 and were present in R+S and S in 2005.
	Erodium cicutarium is present in all treatments until 2001, but disappeared from S in 2005.
	During the four field periods, *Peltigera canina* was never found in S and in summer 2000 and summer 2005, it was also not found in R+S.
	Plantago lanceolata was found in R+S only in spring 2000 and summer 2001; however, it was missing in S only in summer 2000 and was present in all the treatments in summer 2005.
	Trifolium campestre, totally absent in summer 2000, is present in all treatments in spring 2000 and summer 2001, but disappeared from S in summer 2005.
	Vulpia sp. was found only in R+S in spring 2000, but was present in the three treatments in summer 2000 and 2001. Nevertheless, in summer 2005, it had disappeared from S.
Ghyvelde	
	In 2000 and 2001, *Aira praecox* is present in all treatments, but in 2005, it is only observed in L+R+S. *Erodium cicutarium* was not found in R+S in spring 2000 and was absent from S in summer 2000. In summer 2001, it was not seen at all, and in 2005, we observed it again in R+S.
	Myosotis ramosissima was present in all treatments in 2000, but was missing in S in 2001 and was observed in L+R+S only in 2005.
	Until 2001, *Peltigera* sp. was observed in the three treatments, but it completely disappeared from the pqs in 2005.
	Seedlings of *Populus tremula* were completely absent in spring 2000, but appeared in S in summer 2000. In summer 2005, they had also expanded to R+S.
	Tortula ruralis var. *ruraliformis* was found in all treatments in 2000, disappeared from L+R+S in 2001 and had completely gone in 2005.
	Urtica dioica, found only in S in spring 2000 and only in L+R+S in summer 2000, was observed in L+R+S and S in 2001 and in all treatments in 2005.
	Viola curtisii, observed in all treatments in 2000, was missing in S in 2001. In 2005, it could only be seen in R+S.

Appendix 13: Qualitative differences in species composition between the treatments in IJzermonding and Ghyvelde.

Grasslands in Puyenbroeck, 2006 *(Photographs by N. Somers)*

CHAPTER 3

Does vegetation height predict spatial foraging activity of the rabbit (*Oryctolagus cuniculus* L.)?

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Abstract

The wild rabbit (*Oryctolagus cuniculus* L.) is predicted to forage according to an unimodal functional response curve, which means that they would prefer a short to intermediate vegetation height for foraging rather than a high vegetation where food availability is higher. The higher food quality of a short-grazed vegetation is supposed to be responsible for this. A possible consequence is the occurrence of facilitation: by grazing, large herbivores can make the vegetation more suitable for smaller grazers. By performing two different studies in two study areas, we tested whether rabbits indeed prefer to graze in shorter vegetation, by comparing rabbit activity in short (grazed or mown) and ungrazed vegetation. Mown vegetation in the most productive grassland site proved to be preferred by the animals, as indicated by an experimental set-up. There were no indications that this was due to a difference in forage quality. In a more complex, natural situation, rabbits were not facilitated by large grazers: they did not prefer to graze in the shorter vegetation. We suggest that experimental studies might reveal phenomena that are masked under more complex field conditions, for instance due to variation in productivity of the grassland or the occurrence of self-facilitation.

Introduction

Generally, food intake by mammalian herbivores is predicted to increase asymptotically with food density (Type II functional response, HOLLING, 1959) (OKSANEN *et al.*, 1981; LUNDBERG, 1988; LUNDBERG & ÅSTRÖM, 1990; GROSS *et al.*, 1993). Food requirements, however, can be expected to differ among taxa. While larger herbivores may tolerate forage of low nutritional quality if available in sufficiently large quantities, the high metabolic rate and small digestive system of medium-sized grazers (DEMMENT & VAN SOEST, 1985) entail a need for higher-quality forage, albeit in smaller quantities (PRINS & OLFF, 1998; OLFF *et al.*, 2002). As fibre concentration of above-ground vegetation increases and nitrogen concentration decreases during the process of ageing (BOS, 2002), a vegetation consisting of fully-grown, mature plants is on average of lower nutritional quality than short swards consisting of regrown plants ('grazing lawns' *sensu* MCNAUGHTON, 1984). Hence, food intake rate of medium-sized grazers can be expected to be lower under high availability of low-quality food, and higher under low to intermediate availability of high-quality food (Type IV functional response; DEKKER & VAN LANGEVELDE, 2007), as reflected by unimodal, dome-shaped response curves (BOS *et al.*, 2002b; DURANT *et al.*, 2003; VAN LANGEVELDE *et al.*, 2008).

Presumed differences in functional response between smaller and large herbivores have given rise to the concept of 'feeding facilitation' among herbivore assemblages, i.e. larger herbivores creating 'grazing lawns' for smaller species (ARSENAULT & OWEN-SMITH, 2002). If coexistence of herbivore communities would partly (or entirely) depend on facilitation (PRINS & OLFF, 1998; FARNSWORTH *et al.*, 2002), removal or extinction of one or more bulk feeders might directly affect persistence of smaller-sized species (HUISMAN & OLFF, 1998) and, in the longer term, have significant evolutionary-ecological implications (MCNAUGHTON, 1984; CARDINALE *et al.*, 2002). Evidence in favour of feeding facilitation has been provided by studies on natural herbivore assemblages (MCNAUGHTON, 1976; KRUEGER, 1986; STAHL *et al.*, 2006) as well as on assemblages consisting of wild and domestic herbivores (e.g. GORDON, 1988; BOS *et al.*, 2002c; KUIJPER, 2004; AUSTRHEIM *et al.*, 2007; MYSTERUD & AUSTRHEIM, 2008). Other studies, however, failed to support such relationships (SINCLAIR & NORTON-GRIFFITHS, 1982) or revealed trends opposite to those predicted (VAN DER WAL *et al.*, 1998). Apart from methodological grounds, heterogeneity in support of facilitation in medium-sized herbivores may stem from ecological differences among studies. First, the balance of facilitation and competition may depend on plant productivity (KUIJPER *et al.*, 2004; DEKKER & VAN LANGEVELDE, 2007), i.e. with decreasing plant productivity, interspecific competition for food can be expected to increase. Second, facilitation may show strong seasonality, e.g. only occur during the plant growing seasons (FRYXELL, 1991; ARSENAULT & OWEN-SMITH, 2002) or when food resources become limited (RUEDA *et al.*, 2008). Third, feeding facilitation can be expected to be function of the difference in body mass among coexisting herbivores (PRINS & OLFF, 1998); i.e. when differences are either very small or very large, smaller species may not (or to a lesser extent) benefit from the presence of larger ones. Body mass can here be considered as a global measure determining energy and protein requirements of the herbivore and its capacity to ingest and digest the vegetation. Fourth, the density of the large and medium-sized herbivores influences the outcome of the interaction (BAKKER *et al.*, 2009). Facilitation is expected to be stronger at moderate large herbivore grazing intensity and at a low medium-sized herbivore density. Finally, if high-density grazing by medium-sized herbivores results in grazing lawns, as has been observed in geese (VAN DER GRAAF *et al.*, 2002) and rabbits (BAKKER *et al.*, 2005), facilitation by larger herbivores may be replaced by self-facilitation (ARSENAULT & OWEN-SMITH, 2002).

While rabbits are widely assumed to be facilitated by larger grazers (e.g. WILLIAMS *et al.*, 1974; WALLAGE-DREES, 1982; OOSTERVELD, 1983; DREES, 1989; DREES, 1998; BOS *et al.*, 2002a), supporting evidence is mainly anecdotic or indirect. Only limited systematic research is available about the putative preference of rabbits for previously grazed (or clipped) vegetation and the hence deductible facilitative effect of large grazers on rabbits: purely experimental studies suggest the preference of the rabbit for shorter swards (IASON *et al.*, 2002; BAKKER *et al.*, 2009); more descriptive field studies indicate a preference for swards of medium plant standing crop (VAN DE KOPPEL *et al.*, 1996) or find no consistent effect of large herbivores on rabbits (OLFF & BOERSMA, 1998). We conducted two field experiments to test if, and to what extent, wild rabbits (*Oryctolagus cuniculus* L.) are facilitated by domestic herbivores deployed in nature management. One experiment was a short-term experiment under strictly controlled conditions in a homogeneous grazing habitat, while the second experiment was performed during a long-term period, in a complex natural environment with a spatially heterogeneous grazing habitat. We hypothesized that rabbits will prefer grazing in vegetation that has been kept at low to intermediate height (by means of grazing or mowing) above grazing in higher vegetation.

Materials and methods

Study area

Experiments were carried out in three study sites. Grazing-simulating mowing experiments were conducted in the Flemish Provincial Domain 'Puyenbroeck' (Wachtebeke, Belgium, 51°9'11" N, 3°52'43" E, managed by the Provincial Government East-Flanders) ca. 75 km W from Nieuwpoort. At this site, wild rabbits are the dominant natural grazers (absence of large grazers). Exclosure experiments were conducted in coastal dune grasslands at the Flemish Nature Reserve 'IJzermonding' (Nieuwpoort, Belgium; 51°9'4" N, 2°43'57" E; managed by ANB, Flemish Government) and the French Nature Reserve 'Dune Fossile de Ghyvelde' (Ghyvelde, France, 51°2'48" N, 2°33'02" E; managed by Conservatoire du Littoral), ca. 25 km apart. At both sites, wild rabbits comprise the dominant natural grazers while large domestic herbivores are used for grazing management (IJzermonding: Mergelland sheep, *Ovis aries* L.; Ghyvelde: Haflinger pony, *Equus caballus* L.). More information about these dune areas can be found in Chapter 1.

Mowing experiment

We selected two flat, monotonous lawns (henceforth referred to as 'grassland 1' and 'grassland 2') in the study area Puyenbroeck, both bordered by a Poplar plantation and mown at regular intervals. In each grassland, a single 72 x 30 m study plot was delineated, immediately bordering the woodland (grassland 1) or ca. 2 m away from it (grassland 2). Both plots were divided into eight strips of equal width (numbered 1-8 in Fig. 1), and twelve 75 x 75 cm pqs were distributed evenly across each strip (totalling 96 pqs per grassland). In a first experimental period (spring 2006), all grassland strips were mown at equal height ('equal' treatment). In the second period (spring-summer 2006), even strips were mown at equal height, while odd strips were left untouched ('even' treatment). In the third period (summer-autumn 2006), even strips were left untouched, and odd strips were mown at equal height ('odd' treatment).

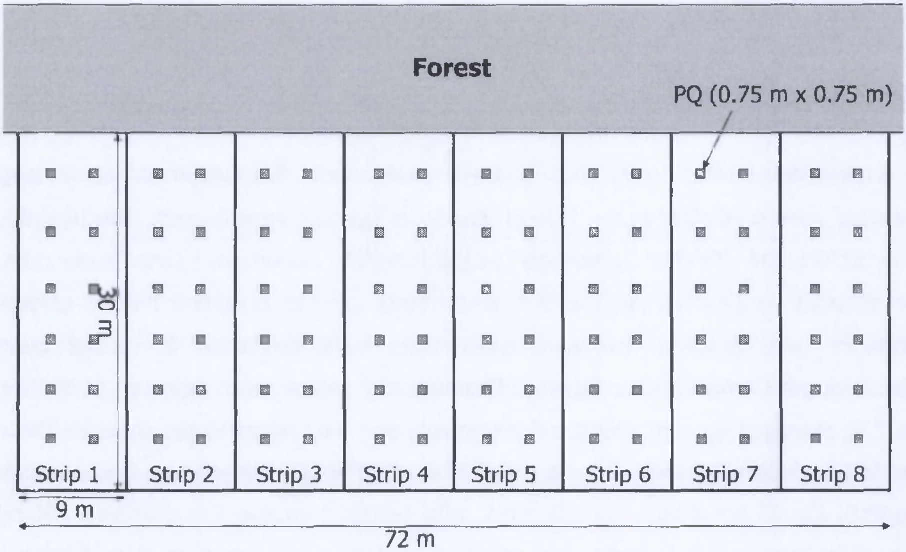

Figure 1: Schematic presentation of one grassland of the mowing experiment. There are eight strips of 9 m by 30 m, all bordering the forest. Within each strip, 12 pqs of 75 cm by 75 cm are marked off.

Before the onset of the experiment, all rabbit pellets were removed in each pq. At the end of the three experimental periods ('equal' 25-26 April 2006; 'even' 10-12 July 2006; 'odd' 26-27 September 2006), the total numbers of pellets in each pq were counted and subsequently removed. To estimate the rate of pellet decay, a total of 50 freshly collected pellets were exposed in two small rabbit-proof exclosures in each grassland at the beginning of each experimental period and were counted at the end of this experimental period. On 8 November 2006, x,y-coordinates of all rabbit burrows located in and around each grassland were recorded using GPS (Garmin GPS map 76 – see Fig.2) The mean distance between each pq and the ten most close by rabbit holes was calculated.

On every counting date, vegetation height was measured at the centre of each pq, by lowering down a disc (diameter 15 cm) with a central slot around a vertical ruler, measuring the height at which the disc touches the vegetation first (combination of "drop disc method" and "sward stick method" described in STEWART *et al.* 2001). The mowing treatments proved to be adequate to establish significant differences in vegetation height between odd and even strips during the 'even' and 'odd' treatment, while vegetation height was similar in all strips during the 'equal' treatment (see Appendix 1).

In addition, four plant samples per grassland were collected by clipping aboveground vegetation. We collected on average 12 g dry weight per sample, consisting of a mix of the present plant species. The samples were distributed equally over short and long strips. They were dried at 60°C (WTB Binder with controller RD 2 EED/FED; Binder, Tuttlingen, Germany) until no further mass loss was detected, after which the dried plants were grinded. The percentage of crude protein (CP) and of cell wall constituents (cellulose, hemicellulose and lignin, which were derived from NDF (neutral detergent fibre), ADF (acid detergent fibre) and ADL (acid detergent lignin)) were obtained by Near Infrared Spectroscopy (NIRS – for more information see GIVENS *et al.* (1997)). A FOSS Feed and Forage Analyzer was used, combined with Winisi software (FOSS, Brussels, Belgium). The calibration for the NIRS was carried out by performing wet analyses for approximately 10% of the samples, following the method of Kjeldahl for CP and GOERING & VAN SOEST (1970) and VAN SOEST *et al.* (1991) for cell wall constituents. Forage quality for rabbits was approximated by DP (digestible protein percentage) and DE (digestible energy). Amounts of digestible protein were estimated by multiplying CP with the mean digestibility coefficient of CP for grasses (value of 0.70; MAFF, 1986). Digestible energy was estimated by multiplying Gross Energy (based on MAFF 1986) with a coefficient of digestibility, quantified as 0.867 – 0.0012 ADF (g/kg DM) (DE BLAS *et al.*, 1992).

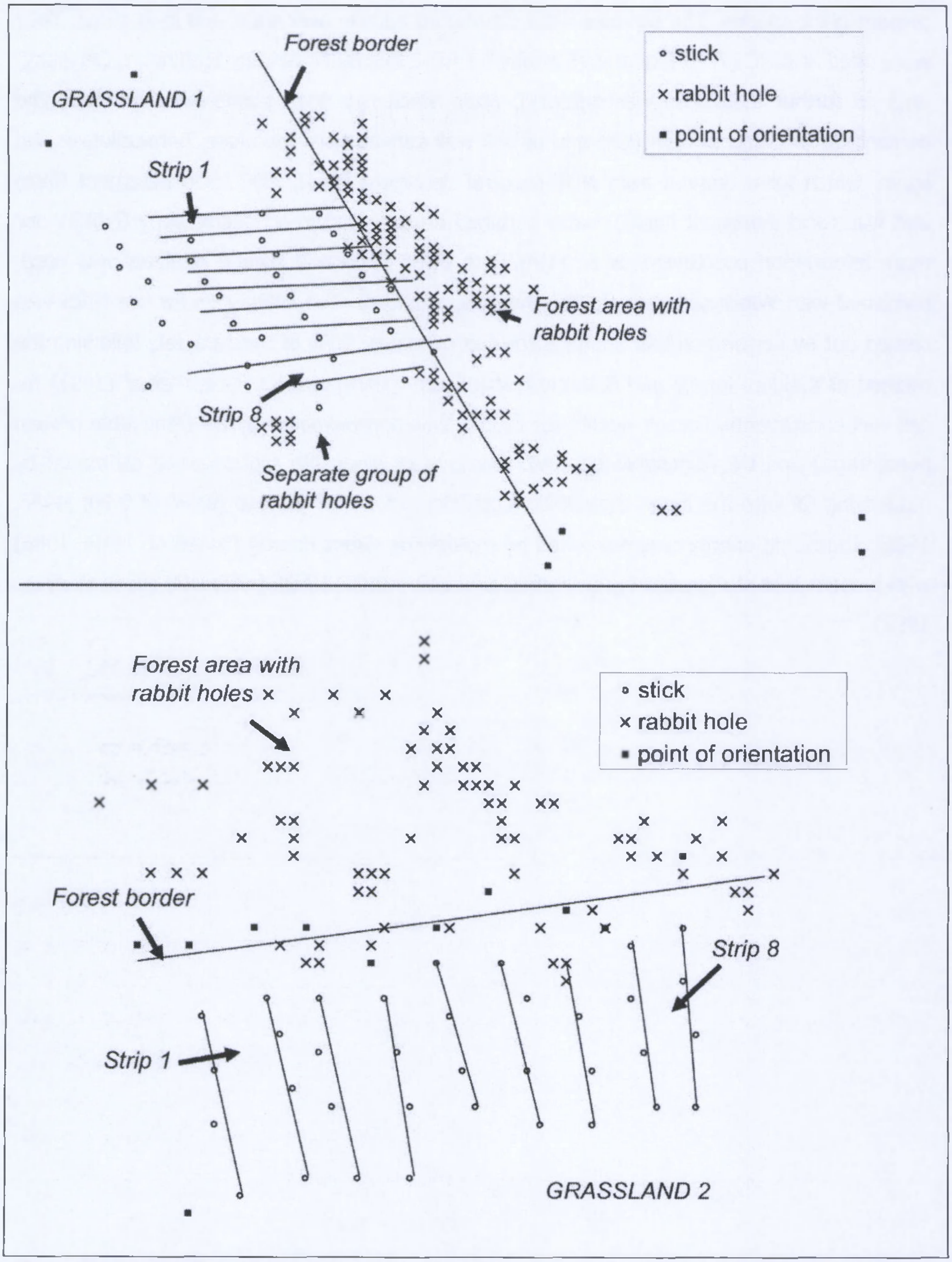

Figure 2: Presentation of the two grasslands of the mowing experiment, based on coordinates obtained by GPS. The rabbit holes, the forest border and the sticks (marking off the strips in each grassland) are indicated. In grassland 1, a separate group of rabbit holes can be seen near strip 8. All other rabbit holes are situated in or near the forest.

Exclosure experiment

Between August 1999 - April 2000, a total of five exclosures (each consisting of three treatments of 350 x 350 cm) were set up along shrub-grassland edges in coastal dune grasslands at IJzermonding and Ghyvelde (Fig. 3). Within each exclosure treatment, six 75 x 75 cm permanent quadrants (pqs) were delineated and assigned to one of the three treatments: (i) larger herbivores, rabbits and smaller herbivores allowed (L+R+S); (ii) larger herbivores excluded (R+S); (iii) larger herbivores and rabbits excluded (S). L+R+S treatments were not fenced, R+S treatments were fenced with non rabbit-proof wire, S treatments were fenced with rabbit-proof wire.

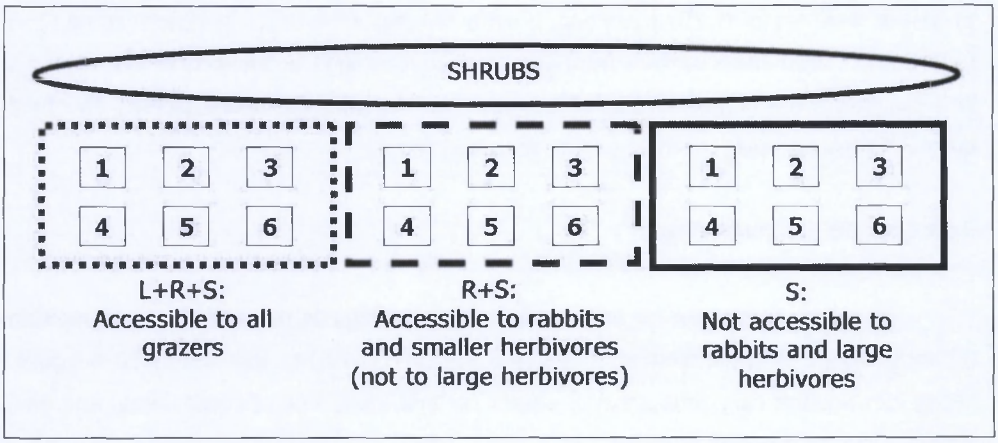

Figure 3: Schematic presentation of one exclosure-group. Each group is positioned near shrubs and consists of one L+R+S-treatment (accessible to all herbivores), one R+S-treatment (accessible to rabbits and smaller herbivores, not to larger herbivores) and one S-treatment (accessible to small herbivores but not to rabbits and larger herbivores). Six pqs of 75 cm by 75 cm are marked off within each treatment.

Between April-September 2000 and August 2001- September 2005, monthly or bi-monthly counts of rabbit pellets were performed in L+R+S and R+S pqs. To estimate the rate of pellet decay, 100 freshly collected pellets were placed in the S treatment within each exclosure and the remaining pellets were counted during the following visit.

During April 2000 (spring season), June-September 2000, July-September 2001, and August-September 2005 (summer season), vegetation height in each pq was measured (Appendix 2) at 25 randomly selected points by lowering down a disc (diameter 15 cm) with a central slot around a vertical ruler, measuring the height at which the disc touches the vegetation first (see higher). We did not measure vegetation height during the other seasons, but it was visually observed that the patterns in those other seasons were similar to those measured in the earlier mentioned seasons. As the grazing treatment did not result in significant differences in vegetation height in IJzermonding (except for summer 2005), the results of this study area (Appendix 3) are not discussed further. In Ghyvelde, the grazing treatment did significantly influence the vegetation height, except for spring 2000. Therefore, spring 2000 was omitted from the analyses of the data of Ghyvelde. Additional vegetation measurements are described in Chapter 2.

In August-September 2005 (summer season), the aboveground vegetation of a random selection of pqs was totally clipped. Generally, two pqs per exclosure and per treatment were sampled. (By exception, 3 extra samples were taken in IJzermonding (2 in L+R+S and 1 in S) and 5 samples from Ghyvelde are missing (1 accidentally in L+R+S and 4 in S because some ponies succeeded to graze the vegetation of these plots).) The food quality of these samples was analysed as described above.

Preference for vegetation height

To assess preference for low versus high vegetation in the exclosure and mowing experiments, we used the method of TAYLOR & WILLIAMS (1956) to estimate rabbit densities, taking into account daily production of pellets per individual, rate of pellet decay, and time interval between consecutive counts:

$$\text{number of rabbits/ha} = \frac{m2.k1 - m1.k2}{g.(k1 - k2)} \cdot \frac{\ln(k1 / k2)}{(t2 - t1)}$$

in which

m1, m2: mean number of pellets per plot during the first (1) and second (2) count, at the level of the study site (exclosure experiment) or individual pq (mowing experiment); as pellets were removed from each plot after each count, m1=0; t2-t1: time interval between two consecutive counts; k1, k2: rate of pellet decay based on samples of 50 (mowing experiments) or 100 (exclosure experiments) pellets exposed away from rabbits; g: number of pellets produced per rabbit and per day, estimated at 333 which was the average of 360 pellets/ind/day reported by LOCKLEY (1962; 1976) and 305 pellets/ind/day based on nine days' counts of pellets of six domestic rabbits (Chapter 5). Rabbit densities were calculated per date, grassland and plot for mowing experiments and per date, study area and treatment (L+R+S, R+S) for exclosure experiments.

Statistical analysis

Effects of mowing on rabbit activity in Puyenbroeck was evaluated with generalized mixed models, one for the control period (when even and odd strips both were mown) and one for the two treatment periods together. Fixed factors included TREATMENT (even or odd strip (control) or long or short strip (treatment)), DISTANCE (the mean distance from the pq to the ten most close rabbit holes) and SEASON (only for the 'treatment'-model, in order to correct for the seasonal differences between the two treatment periods). The GRASSLAND (1-2), STRIP (1-16) and PQ (1-192) were included as random factors. Pqs in which the number of rabbit pellets equalled zero during both of the two treatment periods have been omitted from the analysis. Treatment effects on DP and DE were evaluated by Kruskal-Wallis-tests.

After applying square root transformation of rabbit activity in the dune areas, we used paired t-tests to evaluate differences between exclosure treatments. Treatment effects on DP and DE were evaluated by a general linear model, with TREATMENT as the only factor.

The statistical tests were performed with SAS 9.1 (SAS Institute Inc., Cary, North Carolina).

Results

The mowing experiment

The estimated presence of rabbits was similar for even and odd strips in the control period (similar vegetation height – Table 1 and Table 2). The vegetation height was also similar for the odd and even strips (Appendix 1), although significantly higher in grassland 2 than in grassland 1. As the vegetation was quite low in the control period, 'long' strips during the following experimental periods would originate from fresh, regrowing vegetation. The distance between the pq and the rabbit burrows negatively influenced the rabbit activity. Therefore, this measure was also included in the mixed model for the treatment periods.

Significant differences in rabbit presence between the short and long strips were observed during the mowing treatments (Table 2): the number of rabbits was higher in the strips mown short than in the unmown strips. There was also a significant difference between the presence of rabbits between season 1 and 2: the rabbit presence was much higher in season 1. No effect of distance could be observed here.

The Kruskal-Wallis-tests comparing DP and DE for the even and odd strips indicate that there were no significant differences between the strips.

	Grassland	Description of treatment	Strip	Average ± standard error
Number of rabbits	1	Similar vegetation height	Even = similar	100 ± 67
			Odd = similar	100 ± 62
		'Even' treatment	Even = short	228 ± 65
			Odd = long	102 ± 59
		'Odd' treatment	Odd = short	61 ± 31
			Even = long	0 ± 0
	2	Similar vegetation height	Even = similar	91 ± 34
			Odd = similar	200 ± 54
		'Even' treatment	Even = short	263 ± 93
			Odd = long	99 ± 20
		'Odd' treatment	Odd = short	29 ± 9
			Even = long	16 ± 8
DP (%)	1	Similar vegetation height	Even = similar	11.23 ± 0.96
			Odd = similar	10.17 ± 1.33
		'Even' treatment	Even = short	9.51 ± 1.13
			Odd = long	7.47 ± 0.16
		'Odd' treatment	Odd = short	14.40 ± 0.30
			Even = long	13.16 ± 0.91
	2	Similar vegetation height	Even = similar	14.03 ± 1.56
			Odd = similar	14.22 ± 1.28
		'Even' treatment	Even = short	8.30 ± 0.93
			Odd = long	6.70 ± 0.17
		'Odd' treatment	Odd = short	11.94 ± 0.33
			Even = long	11.43 ± 1.11
DE (MJ / kg DM)	1	Similar vegetation height	Even = similar	11.67 ± 0.00
			Odd = similar	11.30 ± 0.43
		'Even' treatment	Even = short	10.97 ± 0.08
			Odd = long	9.49 ± 0.05
		'Odd' treatment	Odd = short	11.02 ± 0.20
			Even = long	11.05 ± 0.00
	2	Similar vegetation height	Even = similar	12.26 ± 0.06
			Odd = similar	12.15 ± 0.17
		'Even' treatment	Even = short	11.10 ± 0.18
			Odd = long	11.84 ± 0.00
		'Odd' treatment	Odd = short	10.44 ± 0.20
			Even = long	11.22 ± 0.24

Table 1: Averages and standard error of the number of rabbits, DP (percentage of digestible protein) and DE (MJ per kg dry matter) of the mowing experiment. The results are presented per grassland, per treatment period and subdivided by even and odd strips (which also represents the short and long vegetation height in the two last treatment periods).

		Effect					
Effect on the number of rabbits			Num DF	Den DF	F-value	Prob	Estimate
	Control	Even or odd strip	1	76	0.89	0.349	Even: -83.89; Odd: 0.00
		Distance	1	76	4.78	**0.032**	-8.42
			Covariance parameter estimates				
		Grassland	0.00				
		Strip	18457.00				
		PQ	0.00				
		Residual	52071.00				
			Num DF	Den DF	F-value	Prob	Estimate
	Treatment	Short or long strip	1	167	5.43	**0.021**	Long: -87.72; Short: 0.00
		Season	1	167	16.38	**<.0001**	Season 1: 152.40; Season 2: 0.00
		Distance	1	167	0.63	0.429	-2.10
			Covariance parameter estimates				
		Grassland	0.00				
		Strip	9370.43				
		PQ	0.00				
		Residual	0.00				
Effect on DP			Grassland	Chi-square	DF	Prob	
	Control	Even or odd strip	1	0.60	1	0.439	
			2	0.00	1	1.000	
	'Even' treatment	Short or long strip	1	2.40	1	0.121	
			2	2.40	1	0.121	
	'Odd' treatment	Short or long strip	1	2.40	1	0.121	
			2	0.00	1	1.000	
Effect on DE			Grassland	Chi-square	DF	Prob	
	Control	Even or odd strip	1	0.00	1	1.000	
			2	0.00	1	1.000	
	'Even' treatment	Short or long strip	1	2.40	1	0.121	
			2	2.40	1	0.121	
	'Odd' treatment	Short or long strip	1	0.00	1	1.000	
			2	2.40	1	0.121	

Table 2: Results of the mixed models applied to the number of rabbits and the Kruskal-Wallis-tests applied to DP (digestible protein) and DE (digestible energy) of the mowing experiment. The results are presented for the control period and the treatment periods separately. Num DF = numerator degrees of freedom. Den DF = denominator degrees of freedom. DF = degrees of freedom. F-value = test statistic obtained by the mixed model. Chi-square = test statistic obtained by the Kruskal-Wallis-test. Prob = the significance level obtained by the test.

The exclosure-experiment

The estimated rabbit presence did not differ significantly when comparing L+R+S and R+S, except for winter 2003, where the rabbit number was significantly higher in L+R+S (Table 3). Vegetation height was significantly higher in R+S in summer 2000, summer 2001 and summer 2005 (Appendix 2). There were no significant differences in DP and DE between the treatments.

GHYVELDE		Average ± standard error		Paired t-test or GLM		
		L+R+S	R+S	DF	t-value or F-value	Prob
Rabbits per year	2000	22 ± 1	25 ± 6	1	-0.42	0.749
	2001	5 ± 2	6 ± 2	4	-0.93	0.406
	2002	21 ± 4	23 ± 5	11	-0.95	0.365
	2003	35 ± 5	31 ± 5	9	1.12	0.292
	2004	59 ± 10	62 ± 12	9	-0.75	0.470
	2005	22 ± 4	27 ± 5	6	-2.40	0.053
Rabbits per season	Spring	57 ± 10	61 ± 11	8	-0.94	0.373
	Summer	33 ± 6	37 ± 7	13	-1.74	0.106
	Autumn	21 ± 5	19 ± 4	11	0.54	0.599
	Winter	17 ± 4	16 ± 3	10	0.28	0.785
Rabbits per season and year	Summer 2000	22 ± 1	25 ± 6	1	-0.42	0.749
	Summer 2001	6 ± 2	8 ± 3	1	-13.09	0.049
	Autumn 2001	4 ± 3	5 ± 2	2	-0.30	0.792
	Winter 2002	3 ± 1	5 ± 2	2	-1.27	0.331
	Spring 2002	37 ± 4	45 ± 7	2	-2.82	0.106
	Summer 2002	27 ± 4	27 ± 7	2	0.11	0.925
	Autumn 2002	15 ± 6	13 ± 3	2	0.34	0.767
	Winter 2003	24 ± 2	16 ± 2	2	11.70	**0.007**
	Spring 2003	56 ± 22	47 ± 1	1	0.34	0.793
	Summer 2003	38 ± 12	46 ± 21	1	-0.83	0.558
	Autumn 2003	29 ± 1	26 ± 1	2	3.46	0.075
	Winter 2004	36 ± 16	33 ± 11	1	0.37	0.775
	Spring 2004	86 ± 19	94 ± 22	2	-1.39	0.299
	Summer 2004	78 ± 5	90 ± 3	1	-1.50	0.374
	Autumn 2004	36 ± 14	32 ± 8	2	0.35	0.761
	Winter 2005	13 ± 3	14 ± 3	2	-0.55	0.638
	Spring 2005	27 ± n.a.	38 ± n.a.	n.a.	n.a.	n.a.
	Summer 2005	30 ± 1	36 ± 3	2	-3.21	0.085
Rabbits - all data		31 ± 4	32 ± 4	45	-1.10	0.277
DP (%)	Summer 2005	7.28 ± 0.35	6.99 ± 0.28	1/17	0.45	0.513
DE (MJ / kg DM)	Summer 2005	9.01 ± 0.44	8.24 ± 0.23	1/17	2.52	0.131

Table 3: Results of the rabbit counts and DP and DE in Ghyvelde. Averages and standard errors are presented per treatment (L+R+S = accessible to all herbivores; R+S: accessible to rabbits and smaller herbivores, but not to large grazers). Rabbit counts are presented by year, by season, by season and year or all together. The results of the paired t-tests (for the rabbit counts) and the GLM (for DP and DE) are presented. DF = degrees of freedom. T-value or F-value = test statistic obtained by the test. Prob = the significance level obtained by the test. n.a. = not applicable (only one data point available).

Discussion

The results of the clipping experiment in Puyenbroeck indicate that the rabbits preferred a lower vegetation, while this pattern was not observed in the dune area of Ghyvelde. A drastic, short-term experimental mowing regime confirmed our hypothesis that rabbits do prefer shorter swards (leading to facilitation), but a long-term grazing trial under more complex natural circumstances did not confirm the hypothesis (and did neither indicate the opposite possibility of competition). Two remarkable considerations concerning Puyenbroeck are 1. that after reversing the place of the treatment (the reversal of the even and the odd strips), the results are still significant and 2. that during the control period (similar vegetation height in all strips), a period in which vegetation height was not significantly different between the strips, there was no such preference for the odd or even strips. This makes the finding that facilitation of rabbits in Puyenbroeck is probable even stronger. Considering the significant differences of the vegetation height during the two experimental periods, in both grasslands, we can conclude that vegetation height determined the activity pattern of the rabbits in Puyenbroeck. This is consistent with the findings of BAKKER *et al.*, (2009) and IASON *et al.* (2002).

However, our results do not suggest that this preference is caused by a difference in vegetation quality, when comparing the lower and higher vegetation. Despite the fact that we earlier proved that rabbits are able to distinguish between food samples of different quality (SOMERS *et al.*, 2008; Chapter 4), this quality does not seem to interfere with their preference for short swards. Table 1 shows that overall the average DP of short vegetation is slightly higher than that of the taller vegetation, suggesting a higher quality of the shorter vegetation. These differences proved to be not significant though. As the sample size of the quality measurements is rather low, we could wonder whether the differences are really not significant or whether the sample size was simply too small to detect significant differences. Nevertheless, even five years of different grazing regimes in Ghyvelde also did not significantly alter the food quality between the treatments, so we should consider some alternative hypotheses to explain the preference of the rabbits for short vegetation. IASON *et al.* (2002) propose that the preference of their study rabbits for short vegetation originated from antipredator considerations of the animals (better visibility of predators in an open vegetation). Also, short swards could improve communication with other rabbit individuals in case of predator detection (warning by upturned white tail). Foraging decisions are assumed to be titrations of costs and benefits within and across patches; herbivores would balance conflicting demands for food acquisition and safety (KOTLER & BLAUSTEIN, 1995). This means that it is possible that the unimodal functional response curve of rabbits is not caused by food quality considerations, but is simply the price to pay when they want to graze in a safe way: a large return of high biomass is not possible because it is too dangerous. However, the experiments of BAKKER *et al.* (2005) and DEKKER *et al.* (2007) show that rabbits are sensitive to perceived predation risk, but this does not influence the average spatial distribution of their grazing pressure. Rather, the rabbits shifted the time of foraging or did increase total foraging time (spending more time on vigilance). Another suggestion is that forage efficiency (handling time) in a dense vegetation is higher than in a low, open vegetation (VAN DE KOPPEL *et al.*, 1996). It is clear that further research on these hypotheses is necessary.

In Ghyvelde, facilitation does not seem to be present yet, but maybe there is a chance that it would start to occur in future years: the balance between competition and facilitation might have shifted towards a neutral point due to the growing difference between the vegetation height during the experiment, and maybe, it will further shift towards facilitation in the future? According to KUIJPER *et al.* (2008), species competing each other for the same resources on short time-scale might well be facilitating each other when looking at larger time-scales (e.g. while taking plant species replacement into account). Also, when facilitation occurs, its effect becomes stronger over the years (BAKKER *et al.*, 2009). However, at present, we have no indications that facilitation will occur in Ghyvelde while facilitation in the mowing experiment at Puyenbroeck is already occurring after a few months.

So we consider some possible factors masking or preventing facilitation in the dune area. A first possible explanation is that vegetation height in Ghyvelde in L+R+S is mostly not that much lower than unmown strips in Puyenbroeck. Maybe, the vegetation in Ghyvelde should simply be shorter to allow facilitation. However, mean vegetation height in Ghyvelde is probably quite high as a consequence of the height of the shrub layer. The grass layer itself was probably not that much higher than the mown strips in Puyenbroeck.

An important possible explanation comes from the different plant productivity levels of the two study areas. The study sites in Ghyvelde have a relatively low productivity. Possibly, each herbivore is just taking what it can get, as the availability of food is quite low. In the highly productive grasslands of Puyenbroeck, regrowth is occurring much quicker, and after mowing, the stimulation of the formation of a grazing lawn is starting directly, leading to the occurrence of rabbit facilitation.

Seasonality did not seem to have any effect on the grazing behaviour of the rabbits. The rabbits in IJzermonding and Ghyvelde did not change their vegetation preferences during the different seasons, neither during the growing season, neither in the season when resources are limiting, which is the winter season for the rabbits in our study areas (WALLAGE-DREES, 1988). The experiment in Puyenbroeck was stopped before the start of the winter, but it would be an interesting suggestion for further research to extend this experiment during a longer period, in order to investigate whether seasonality is influencing the behaviour of rabbits towards short and high vegetation.

An alternative explanation involves the type of herbivore involved in the different areas. When there is no optimum difference in body mass to allow facilitation (PRINS & OLFF, 1998), facilitation cannot be expected. This is shown by the decrease of rodents in the presence of cattle (KEESING, 1998; BAKKER *et al.*, 2009; STEEN *et al.*, 2005) and the decrease of insects in the presence of lagomorphs (GRAYSON & HASSALL, 1985; HUNTZINGER *et al.*, 2008), although sometimes the abundance of insects is increased by vertebrate grazing (RAMBO & FAETH, 1999). The herbivores involved in Ghyvelde (ponies) are not the same as the 'large herbivore' in Puyenbroeck (an artificial one: the mowing machine). However, since this artificial 'grazer' was 'grazing' large quantities of forage, we could consider it a model for a large, generalist herbivore (a bulk feeder) present in large densities. As this bulk feeder was facilitating the rabbits of Puyenbroeck, we have no reason to assume that the body mass of the ponies of Ghyvelde would be too large to allow for facilitation. We can conclude that body mass differences between the herbivores of our two areas are not sufficient to explain the different results of these areas.

We could not compare the densities of large herbivores in both study areas, as the "large herbivore" was artificial in Puyenbroeck. We can however compare rabbit density between the areas. As facilitation would indeed be more important in areas with low rabbit density (BAKKER *et al.*, 2009), we would expect that facilitation would occur in Ghyvelde rather than in Puyenbroeck. This was however not the case, so we conclude that rabbit density in Ghyvelde is not too high to obtain facilitation.

Finally, self-facilitation (ARSENAULT & OWEN-SMITH, 2002) could be a valuable alternative explanation for the lack of visible feeding facilitation in Ghyvelde. It might be possible that the grazing pressure inside the exclosures of Ghyvelde is so high that they prefer to maintain a grazing lawn inside this exclosure, thereby avoiding possible competitive interactions with herbivores outside the exclosure. Nevertheless, as the vegetation height in Ghyvelde is increasing over the years, we can assume that the rabbits are not able to maintain the grazing lawns themselves and in the future, facilitation will be more necessary than ever before.

This research aimed to investigate whether rabbits indeed do prefer short vegetation above longer vegetation, as predicted by an unimodal functional response. Clearly, the conditions under which facilitation is tested are determining for the research outcome. Straight experimental conditions are able to reveal clear patterns, which is probably due to the simplicity of the environment. In complex natural situations, many more factors could influence the behaviour of the animals, thereby possibly masking trends that could effectively be present. Also, we cannot exclude that some biological factors (like large herbivore species, grassland productivity, season, herbivore density) are the reason for the observed differences between Puyenbroeck and Ghyvelde, which stresses our opinion that facilitation should be tested under various conditions, and that one study indicating no facilitation in a herbivore assemblage should not be considered decisive. Further, our results did not indicate a higher food quality to be responsible for the rabbits' preference for short vegetation in a high-productive grassland. Although some plausible alternative explanations have been discussed, none of them seem to be entirely satisfying on their own. Also on this topic, further research is clearly necessary.

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Appendices

	Grassland	Description of treatment	Strip	Average ± standard error		
Vegetation height	1	Similar vegetation height	Even = similar	8.19	±	0.46
			Odd = similar	8.52	±	1.10
		'Even' treatment	Even = short	6.69	±	0.29
			Odd = long	44.00	±	3.96
		'Odd' treatment	Odd = short	9.23	±	0.45
			Even = long	15.40	±	1.90
	2	Similar vegetation height	Even = similar	4.73	±	0.43
			Odd = similar	4.92	±	0.36
		'Even' treatment	Even = short	10.83	±	0.42
			Odd = long	19.15	±	2.05
		'Odd' treatment	Odd = short	8.92	±	0.27
			Even = long	14.96	±	0.93
Results Mixed Model	Similar vegetation height		Num DF	Den DF	F-value	Prob
		Even or odd strip	1	6	0.17	0.699
		Grassland	1	6	25.95	**0.002**
		Interaction (omitted)	1	6	0.01	0.912
			Covariance parameter estimates			
		Position burrows		0.01		
		Residual		0.02		
	'Even' treatment		Num DF	Den DF	F-value	Prob
		Short or long strip	1	6	272.94	**<.0001**
		Grassland	1	6	5.76	0.053
		Interaction (included)	1	6	80.35	**0.000**
			Covariance parameter estimates			
		Position burrows		0.00		
		Residual		0.02		
	'Odd' treatment		Num DF	Den DF	F-value	Prob
		Short or long strip	1	6	72.57	**0.000**
		Grassland	1	6	2.14	0.194
		Interaction (omitted)	1	6	0.02	0.883
			Covariance parameter estimates			
		Position burrows		0.03		
		Residual		0.01		

Appendix 1: Averages and standard error of the vegetation height of the mowing experiment. Results of the mixed models applied to the vegetation height, after ln transformation, are also presented. The results presented for the fixed effects of the mixed models are the results after omitting the interaction, when this interaction was not significant. The interaction term of the 'even' treatment was significant. Least squares means were calculated for all the interaction combinations: even strips x grassland 1: estimate = 1.90; odd strips x grassland 1: estimate = 3.77; even strips x grassland 3:

estimate = 2.38; odd strips x grassland 3: estimate = 2.94. As the interaction was significant, we conclude that the effect of the mowing was different for the two grasslands, but the least squares estimates show that nevertheless, the vegetation was highest for the odd (not mown) strips. The results are presented per grassland, per treatment and subdivided by even and odd strips (which also represents the short and long vegetation height in the two last treatment periods). Num DF = numerator degrees of freedom. Den DF = denominator degrees of freedom. DF = degrees of freedom. F-value = test statistic obtained by the mixed model. Prob = the significance level obtained by the test.

Vegetation height (cm)		Average ± standard error		Kruskal-Wallis-test		
		L+R+S	R+S	DF	Chi-square	Prob
Ghyvelde	Spring 2000	7.89 ± 3.60	4.74 ± 0.16	1	2.55	0.110
	Summer 2000	17.82 ± 2.02	29.58 ± 2.18	1	16.41	**<.0001**
	Summer 2001	16.48 ± 1.94	28.11 ± 1.88	1	28.33	**<.0001**
	Summer 2005	24.56 ± 4.92	32.07 ± 3.02	1	8.83	**0.003**
IJzer-monding	Spring 2000	4.90 ± 0.47	4.78 ± 0.42	1	0.01	0.935
	Summer 2000	17.71 ± 1.81	19.03 ± 1.95	1	0.52	0.469
	Summer 2001	21.53 ± 2.11	24.88 ± 2.18	1	1.30	0.255
	Summer 2005	49.71 ± 4.29	67.52 ± 4.94	1	6.21	**0.013**

Appendix 2: Results of the vegetation height measurements in Ghyvelde and IJzermonding. Averages and standard errors are presented per treatment (L+R+S = accessible to all herbivores; R+S: accessible to rabbits and smaller herbivores, but not to large grazers). Vegetation height is presented by the four measurement periods. The results of the Kruskal-Wallis-tests (comparing L+R+S with R+S) are presented. DF = degrees of freedom. Chi-square = test statistic obtained by the Kruskal-Wallis-test. Prob = the significance level obtained by the test.

IJZERMONDING		Average ± standard error		Paired t-test or GLM		
		L+R+S	R+S	DF	t-value or F-value	Prob
Rabbits per year	2000	40 ± 8	62 ± 11	5	-5.15	**0.004**
	2001	67 ± 10	80 ± 16	4	-1.53	0.200
	2002	26 ± 6	47 ± 9	11	-6.11	**<.0001**
	2003	12 ± 2	25 ± 3	9	-7.33	**<.0001**
	2004	16 ± 4	21 ± 3	8	-2.20	0.059
	2005	11 ± 2	18 ± 4	5	-2.45	0.058
Rabbits per season	Spring	38 ± 7	54 ± 11	10	-3.32	**0.008**
	Summer	28 ± 7	42 ± 10	12	-5.52	**0.000**
	Autumn	23 ± 7	34 ± 6	12	-4.27	**0.001**
	Winter	13 ± 3	26 ± 5	10	-5.43	**0.000**
Rabbits per season and year	Spring 2000	51 ± 8	73 ± 19	2	-2.56	0.125
	Summer 2000	39 ± 11	62 ± 3	1	-2.69	0.226
	Autumn 2000	12 ± n.a.	28 ± n.a.	n.a.	n.a.	n.a.
	Summer 2001	76 ± 13	107 ± 27	1	-2.77	0.220
	Autumn 2001	62 ± 15	62 ± 13	2	-0.24	0.833
	Winter 2002	29 ± 5	41 ± 11	2	-1.51	0.270
	Spring 2002	56 ± 11	89 ± 17	2	-7.72	**0.016**
	Summer 2002	14 ± 2	29 ± 10	2	-2.23	0.155
	Autumn 2002	6 ± 2	27 ± 3	2	-17.02	**0.003**
	Winter 2003	6 ± 1	21 ± 2	2	-7.43	**0.018**
	Spring 2003	10 ± 5	18 ± 2	1	-1.76	0.329
	Summer 2003	15 ± 4	25 ± 1	1	-2.36	0.255
	Autumn 2003	16 ± 4	34 ± 10	2	-5.54	**0.031**
	Winter 2004	7 ± 1	18 ± 2	1	-2.52	0.241
	Spring 2004	29 ± 3	26 ± 6	1	1.33	0.412
	Summer 2004	19 ± 13	24 ± 10	1	-1.31	0.415
	Autumn 2004	11 ± 2	17 ± 5	2	-1.53	0.266
	Winter 2005	10 ± 3	21 ± 9	2	-2.27	0.151
	Spring 2005	17 ± n.a.	17 ± n.a.	n.a.	n.a.	n.a.
	Summer 2005	10 ± 2	14 ± 0	1	-1.87	0.312
Rabbits - all data		25 ± 3	39 ± 4	47	-9.17	**<.0001**
DP (%)	Summer 2005	6.18 ± 0.10	6.41 ± 0.32	1/20	0.58	0.454
DE (MJ / kg DM)	Summer 2005	8.53 ± 0.24	8.29 ± 0.32	1/20	0.37	0.551

Appendix 3: Results of the rabbit counts and DP and DE in IJzermonding. Averages and standard errors are presented per treatment (L+R+S = accessible to all herbivores; R+S: accessible to rabbits and smaller herbivores, but not to large grazers). Rabbit counts are presented by year, by season, by season and year or all together. The results of the paired t-tests (for the rabbit counts) and the GLM (for DP and DE) are presented. DF = degrees of freedom. T-value or F-value = test statistic obtained by the test. Prob = the significance level obtained by the test. n.a. = not applicable (only one data point available).

Feeding trial, 2005 *(Photographs by N. Somers)*

CHAPTER 4

Food quality affects diet preference of rabbits: experimental evidence

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Abstract

When foraging, small mammalian herbivores do not show a preference for the forage with the highest biomass, which can be explained by several hypotheses (e.g. antipredator considerations, more difficult handling of tall swards and/or the higher nutritional quality of shorter grasses). We tested the ability of rabbits to discriminate between plants of different nutritional value and whether they prefer the most nutritious. A feeding trial in which rabbits (*Oryctolagus cuniculus* L.) were offered two different types of grasses (fertilised and unfertilised) was executed under experimental conditions. The rabbits preferred the grasses with the highest protein percentage, when controlling for sward height/plant biomass. This observation is equivalent to results obtained in geese and provides experimental evidence about the capability of rabbits to select for plants with the highest nutritional quality.

Introduction

Predicting the impact of herbivores on their environment requires insight into the criteria by which grazers select food patches. The mechanism of functional response (reviewed in CRAWLEY, 1983) predicts herbivores to prefer patches with the highest biomass and plant size (LUNDBERG, 1988; GROSS *et al.*, 1993), in order to obtain as much forage as possible in a given time period. However, some studies evidenced that medium-sized herbivores, such as rabbits (*Oryctolagus cuniculus* L.) and geese, prefer rather swards of intermediate height (OLFF *et al.*, 1997; WILMSHURST *et al.*, 2000; HASSALL *et al.*, 2001; IASON *et al.*, 2002; BAKKER *et al.*, 2005). Several explanations for this phenomenon have been mentioned. IASON *et al.* (2002) and VAN DE KOPPEL *et al.* (1996) suggested that rabbits and hares, *Lepus europaeus* (PALLAS), prefer vegetation with medium standing crop swards because predators are most likely to occur in the cover offering higher vegetation. Moreover, a tall sward is more difficult to handle for medium-sized animals. A dislike for tall swards by brent geese, *Branta bernicla bernicla* (L.), and barnacle geese, *Branta leucopsis* (BECHSTEIN), was explained by the larger costs of handling, more difficult locomotion, and decreased predator detection in the taller vegetation (VAN DER GRAAF *et al.*, 2002).

However, preference for shorter swards may also be related to their higher food quality: grazing creates fastly growing and nutritionally-rich vegetation (so called 'grazing lawns', MCNAUGHTON, 1984), due to plant compensatory mechanisms operating after defoliation (MATTSON, 1980; MCNAUGHTON, 1983). Large herbivores need a larger plant biomass, but can tolerate low plant quality, while smaller herbivores can persist on small quantities of food on the condition that the plants are of high nutritional quality (OLFF *et al.*, 2002). Medium-sized hindgut fermenters (e.g. the rabbit) depend on highly digestible forage because they have high metabolic requirements and their digestive system is very small (DEMMENT & VAN SOEST, 1985). The creation of grazing lawns by large herbivores may hence result in 'feeding facilitation' (ARSENAULT & OWEN-SMITH, 2002) benefiting smaller grazers. Hunger may strengthen this preference for nutritionally-rich forage (CRAWLEY, 1983).

The wild rabbit is considered to have a preference for shorter swards. For example, MORENO & VILLAFUERTE (1995) noticed that rabbit grazing pressure was higher in fresh, re-growing vegetation (after burning). Being a central-place forager (SCHOENER, 1979), foraging of this herbivore is concentrated in the neighbourhood of the burrow (DEKKER, 2007), less than 20 m from cover (MORENO & VILLAFUERTE, 1995). With increasing distance from the burrow, grazing pressure of rabbits gradually decreases, causing a gradient pattern. Consequently, vegetation height increases and nitrogen concentration of forage decreases, due to repeated grazing of the rabbits, stimulating fresh regrowth (BAKKER *et al.*, 2005). BAKKER *et al.* (2005) conclude that the grazing pattern has to be explained by food quality.

Univocal discrimination of factors determining feeding preferences is only possible in strictly controlled choice experiments (so-called 'cafeteria-trials'; CRAWLEY, 1983). Experimentally enhancing food quality in field experiments by fertilisation increases both biomass (BALL *et al.*, 2000) and plant height. Moreover, feeding preferences are very difficult to measure in the field (CRAWLEY, 1983). For these reasons, we conducted a laboratory experiment to test the ability of rabbits to discriminate between grasses of low and high nutritional quality, while controlling for sward height or vegetation biomass. We predicted that rabbits would prefer grasses of the highest forage quality either when offered grass swards of comparable height or cut grasses of the same biomass.

Materials and methods

Study species

Twelve domestic rabbits (six males and six females, all between 1 and 3 years old) of the breed 'Steenkonijn' were used. The Steenkonijn is the oldest Belgian rabbit breed, and is most closely related to the wild rabbit (WERNER, 1980). Therefore, the behaviour of these animals is supposed to be comparable to the behaviour of their wild ancestor. The rabbits were housed in wire mesh pens (65 cm x 110 cm, height: 60 cm) such that each individual could see a single other individual. This allowed social contact between the animals (DUNCAN *et al.*, 2006). A vaccination against myxomatosis and viral haemorrhagic syndrome was administered. All the individuals received water *ad libitum*, and were fed with a mixture of commercial rabbit pellets and grains (Bonito 96, Aveve, Belgium). From the first day of the feeding trials, the pellet feeding was discontinued, so that the rabbits depended for their feeding on the experimental plants, supplemented with straw that was provided in the pens.

We used *Festuca rubra* as forage in all trials. *Festuca rubra* plants were grown from seeds (Herbiseed, Twyford, England), sown on a mixture of 50% dune sand and 50% potting soil, in seed trays of 40 cm by 45 cm during July – October 2004. Immediately after sowing, halve of the seed trays (selected at random) received 30 g of organic fertiliser (8% nitrogen, 6% phosphorus, 7% potassium, 3% magnesium and 38% organic matter), further referred to as 'fertilised plants' (F+). All trays received an inorganic fertiliser twice (once one month after sowing and once in March 2005). The 'fertilised plants' received 7.5 g of inorganic fertiliser (20% nitrogen, 5% phosphorus and 8% potassium) at a time; the 'unfertilised plants' (F-) received 2.5 g at a time.

The trays were put inside the greenhouse immediately after sowing, and were watered every two days. From December 2004 until March 2005, the trays were put outside for better aeration. Fungicide (sulphur) was added twice to cope with a mildew infection, and an infection of aphids was treated by using a mix of piperonylbutoxide and pyrethrine.

Experimental design

The feeding trial took place in an experimental pen (Figure 1) of 104 cm depth, 91 cm width and 73 cm height, connected to a smaller pen (36 cm by 26 cm by 30 cm) from which the rabbit was not able to see the surroundings. Two grass swards (trimmed just before the start of the trial to an height of 13 cm (further called short swards ('S')) or of 33 cm (tall swards ('T'))) or two dishes with clipped grass (100 g per dish) were put in the larger pen, on the opposite side of the entrance from the small pen. A partition of 40 cm high (in the middle between the two swards or dishes) divided the large pen into two halves.

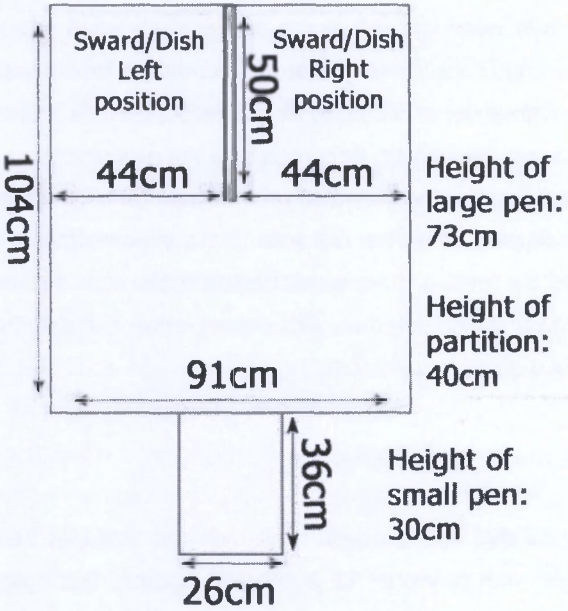

Figure 1: Design of the experimental pen.

In the sward trial (22-29 April 2005), there were four groups of three rabbits and two treatments (two combinations of swards): F+T versus F-T and F+S versus F-S. Each two groups of rabbits received the treatments in a different order. This total design was replicated once, but with a reverse of the left-hand and the right-hand sward. During the clipping trial (2-5 May 2005), the rabbits received dishes with clipped fertilised grass on one side of the pen, and unfertilised grass on the other side (F+ versus F-). In the replicate of the clipping trial, the position of F+ and F- was reversed. Since the design was randomized and fully balanced, possible effects of the order of treatments were minimized.

Before the start of the feeding trial, four learning days were organised: the rabbits were placed in the pen to habituate to the pen, the grass and the observer. Research carried out with ruminants shows that food preferences develop because of the experience of postingestive effects (satiety or malaise) and their interaction with the senses of mainly taste and smell (PROVENZA, 1995). Herbivores learn about grass quality through foraging consequences, which they link with preingestive cues necessary to recognize the value of the forage (GINANE *et al.*, 2005). Although this was only evidenced for ruminants, it is reasonable to assume that ruminants and non-ruminants do not differ in the non-cognitive aspects of how feedback is processed (PROVENZA, 1995). Non-ruminants have indeed been found to be able to discriminate between foods, even when the differences are relatively small (POST, 1993). This means that the rabbits were only able to select the most nutritious food if they had the opportunity to experience the differences in postingestive effects between F+ and F- grasses. This condition was fulfilled through the learning days preceding the experiment.

At the start of each experimental session, the individual was weighed (to estimate its body condition) and placed in the small pen. When the entrance to the experimental pen was opened and the rabbit approached the feeding trays, we started an observation session of 20 minutes, from a central point which did not interfere with the experiment. A detailed description of the foraging behaviour of the rabbit was noted.

Forage quality analysis

After each session, plant material that had not been consumed was removed from the pen and dried at 60°C (WTB Binder with controller RD 2 EED/FED (Binder, Tuttlingen, Germany)), until no more mass loss was detected, after which the dried plants were grinded. The percentage of crude protein (CP) and of cell wall constituents (cellulose, hemicellulose and lignin, which were derived from NDF (neutral detergent fibre), ADF (acid detergent fibre) and ADL (acid detergent lignin)) were obtained by Near Infrared Spectroscopy (NIRS –

for more information see GIVENS *et al.* (1997)). A FOSS Feed and Forage Analyzer was used, combined with Winisi software (FOSS, Brussels, Belgium). The calibration for the NIRS was carried out by performing wet analyses for approximately 10% of the samples, following the method of Kjeldahl for CP and following the protocols of GOERING & VAN SOEST (1970) and VAN SOEST *et al.* (1991) for cell wall constituents.

The amount of digestible protein (DP) was calculated by multiplying CP with the mean digestibility coefficient of CP in grasses (value of 0.70; MAFF, 1986). DE (Digestible Energy) was calculated based on ADF, by multiplying GE (Gross Energy, value based on MAFF (1986)) by GE_D (coefficient of digestibility of Gross Energy, in which GE_D is defined as follows: $GE_D = 0.867 - 0.0012$ ADF (g/kg DM) (DE BLAS *et al.*, 1992).

Statistical analysis

The feeding preference of rabbits for different types of grasses was tested by Mixed Linear Models, with INDIVIDUAL as random effect and "relative foraging time" as dependent variable. This last variable was calculated by timing individual feeding bouts per sward or dish, starting from the moment when the experimental individual had tasted from either both swards or both dishes until the end of the session, divided by the total time left until the end of the session. As vegetation height or biomass were controlled for, differences in handling time were not expected, so that the relative foraging time can be considered a good measure for intake rate. Furthermore, the level of satiety after eating (PROVENZA, 1995; GINANE *et al.*, 2005) will have been higher for the more nutritious grasses. Since mammals learn to link the taste of the forage to the satiety level, directed foraging behaviour can only start from the moment they have tasted both grasses. Therefore, the relative foraging time, as defined above, is the most appropriate measure describing their preference.

Four independent categorical variables were included in the model: FERTILISATION (0/1), POSITION OF THE SWARD OR DISH (left or right), DAY and SEX. We started with a full model including all two-factor and higher-order interactions and applied a backward stepwise selection procedure.

The effect of FERTILISATION on forage quality was analysed by a General Linear Model. We tested the effect of fertilisation as a categorical variable separately on the percentages of CP, cellulose, hemicellulose, lignin, DP and DE. Mixed regression analysis modelling repeated measurements at individual level was used to examine shifts in weight during the feeding trials.

All statistical analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, North Carolina).

Results

Table 1 summarizes the effect of fertilisation, day, sex and the position of the sward or dish (and all possible interaction terms) on the relative foraging time. Fertilised swards and dishes were preferred over unfertilised ones, as can be seen in Figure 2: the mean of the relative foraging time was lower for F-plants (sward trial: mean = 20.02% ± 3.31 SE, N = 48; clipping trial: mean = 18.25 ± 4.74%, N = 24) than for F+plants (sward trial: mean = 31.26 ± 3.31%, N = 48; clipping trial: mean = 37.13 ± 4.74%, N = 24).

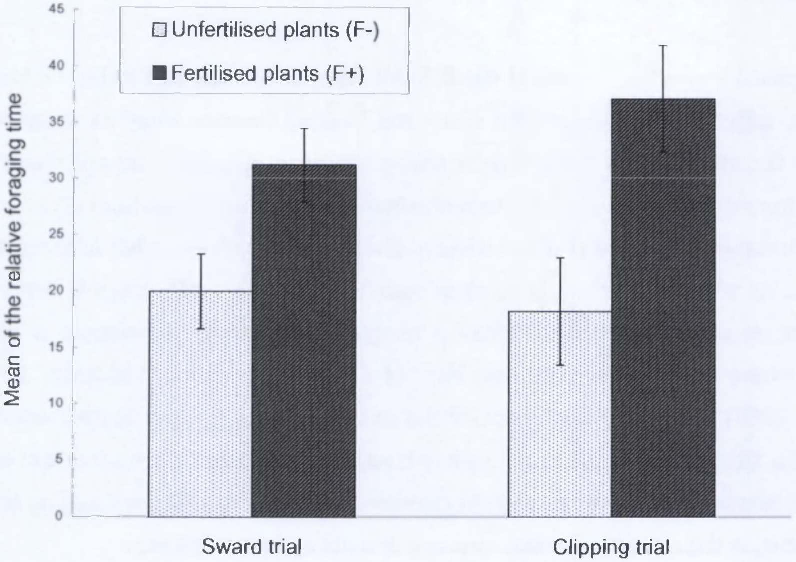

Figure 2: Mean and standard error of the relative foraging time of rabbits, when offered the choice between fertilised and unfertilised grasses, controlling for sward height (sward trial) or plant biomass (clipping trial). The relative foraging time was calculated by timing individual feeding bouts per sward or dish, starting from the moment when the experimental individual had tasted from either both swards or both dishes until the end of the session, divided by the total time left until the end of the session.

Sward trial	**Fixed effects**	*num d.f.*	*den d.f.*	*F*	*P*
	Fertilisation (F)	1	94	5.74	**0.02**
	Day (D)	7	87	1.21	0.3
	Position grass (P)	1	86	0.84	0.36
	Sex (S)	1	85	0.35	0.56
	F*P	1	84	0.85	0.36
	P*D	7	77	1.05	0.4
	D*S	7	70	0.9	0.51
	F*S	1	69	0.3	0.59
	P*S	1	68	0.02	0.89
	F*D	3	65	0.19	0.9
	F*P*D	3	62	1.72	0.17
	F*D*S	7	55	1.08	0.39
	P*D*S	3	52	0.29	0.83
	F*P*S	1	51	0.03	0.87
	F*P*D*S	3	48	0.23	0.87
	Random effect	*estimate*	*residual*		
	Individual	0	525.03		
Clipping trial	**Fixed effects**	*num d.f.*	*den d.f.*	*F*	*P*
	Fertilisation (F)	1	46	7.86	**0.01**
	Sex (S)	1	45	3.45	0.07
	Day (D)	1	44	0.08	0.78
	Position grass (P)	1	43	0	0.97
	F*S	1	42	2.97	0.09
	D*S	1	41	0.3	0.59
	P*D	1	40	0.26	0.61
	P*S	1	39	0.22	0.64
	F*D	1	38	0.2	0.66
	F*P	1	37	0.04	0.85
	F*D*S	1	36	0.98	0.33
	F*P*D	1	35	0.21	0.65
	P*D*S	1	34	0.1	0.76
	F*P*S	1	33	0.04	0.85
	F*P*D*S	1	32	0.13	0.72
	Random effect	*estimate*	*residual*		
	Individual	0	633.67		

Table 1: Results of the Mixed Linear Model testing for the effect of the four main factors (fertilisation, position, day and sex) and interactions on the relative foraging time, during a backward stepwise selection. The relative foraging time was calculated by timing individual feeding bouts per sward or dish, starting from the moment when the experimental individual had tasted from either both swards or both dishes until the end of the session, divided by the total time left until the end of the session. The p-values are these from the last step before the respective variable was removed. Num d.f. = numerator degrees of freedom, den d.f. = denominator degrees of freedom.

There were some significant differences between fertilised and unfertilised plants (Table 2). CP and cellulose percentages were significantly higher in the F+plants, as well in the sward trial as in the clipping trial. In the clipping trial, also the hemicellulose percentage was higher in the F+plants, while the lignin percentage was lower in these plants. The value of digestible protein percentage was significantly higher in the F+plants, compared to the F-plants. This applied to the sward trial as well as to the clipping trial. Also, the DE percentage differed significantly between F+ and F-plants, with a higher DE percentage in F-plants, in the sward trial, but not in the clipping trial.

The effect of day on the relative foraging time was not significant, although the rabbits lost weight during the trials. These losses were statistically significant in both trials (sward trial: $F_{1,11} = 519.51$, estimate for time effect = -22.74, $P < 0.001$; clipping trial: $F_{1, 11.3} = 7.11$, estimate for time effect = -18.50, $P = 0.02$).

	num d.f.	*den d.f.*	*F*	*P*	*mean F-plants ± SE*	*mean F+plants± SE*
Sward trial						
CP (%)	1	94	60.95	**< 0.001**	10.77 ± 0.28	13.91 ± 0.28
Cellulose (%)	1	94	19.99	**< 0.001**	21.86 ± 0.25	23.43 ± 0.25
Hemicellulose (%)	1	94	0.21	0.65	22.62 ± 0.25	22.79 ± 0.25
Lignin (%)	1	94	0.36	0.55	3.17 ± 0.07	3.23 ± 0.07
DP (%)	1	94	60.95	**< 0.001**	7.54 ± 0.20	9.73 ± 0.20
DE (MJ / kg DM)	1	94	24.46	**< 0.001**	10.54 ± 0.05	10.17 ± 0.05
Clipping trial						
CP (%)	1	46	57.65	**< 0.001**	10.17 ± 0.30	13.44 ± 0.30
Cellulose (%)	1	46	10.32	**< 0.001**	21.55 ± 0.22	22.56 ± 0.22
Hemicellulose (%)	1	46	17.22	**< 0.001**	21.54 ± 0.19	22.65 ± 0.19
Lignin (%)	1	46	19.5	**< 0.001**	2.77 ± 0.07	2.32 ± 0.07
DP (%)	1	46	57.65	**< 0.001**	7.12 ± 0.21	9.41 ± 0.21
DE (MJ / kg DM)	1	46	2.69	0.11	10.70 ± 0.05	10.57 ± 0.05

Table 2: Results of the General Linear Model testing for the effect of fertilisation on forage quality measures of standing crop (sward trial) and clipped grass material (clipping trial). F-plants = unfertilised plants, F+plants = fertilised plants. CP = % Crude Protein , DP = % Digestible Protein, DE = Digestible Energy (MJ per kg dry matter). Num d.f. = numerator degrees of freedom, den d.f. = denominator degrees of freedom.

Discussion

The results of the experiment showed that only fertilisation had a significant influence on the preference of the animals: the relative foraging time was longer for the fertilised forage, both in the sward and in the clipping trial. The forage quality analysis revealed a higher percentage of both crude and digestible protein in F+plants, compared to F-plants.

This was to be expected, since nitrogen is a principal component of the used fertilisers and its concentration is strongly related to protein concentration. We also observed a higher percentage of cellulose and hemicellulose, although the latter only in the F+plants from the clipping trial, in which lignin decreased. As forage quality is believed to be enhanced by the protein level (LANGVATN & HANLEY, 1993) and to be diminished by the fibre concentration (OLFF *et al.*, 1997), the question raises whether fertilisation effectively resulted in a higher food quality. Moreover, in the sward trial, the F+plants even had a lower digestible energy content. However, it is reasonable to assume that the increase of digestible protein is the most important factor determining forage quality. Since some amino acids cannot be synthesized by the animal's body itself, animals need amino acids, immediately available from the forage to maintain body conditions constant (FRAGA, 1998). The close agreement between the sum of individual amino acids levels in the body of the rabbit and the CP concentration (FRAGA, 1998), indicates that CP concentration provides a good estimate of forage quality. We can hence conclude that the rabbits selected the forage with the highest nutritional quality.

This preference for high quality forage has been suggested for rabbits (KUIJPER *et al.*, 2004; RÖDEL, 2005) and also for other relatively small mammal herbivores, e.g. small ruminants (WILMSHURST *et al.*, 2000) and mountain hares (*Lepus timidus* L.) (LINDLÖF *et al.*, 1974). However, studies eliminating the correlation between forage quality and sward height/biomass are scarce. Therefore, it is difficult to know whether the animals are really able to select for the higher nitrogen concentration, or whether this selection is just coincidental related to the selection of swards with medium standing crop. Some studies concerning geese (HASSALL *et al.*, 2001; BOS *et al.*, 2002; HASSALL & LANE, 2005) showed the capability of these birds to discriminate between high and low quality forage, by eliminating the relationship between forage quality and sward height. BAKKER *et al.* (2005) executed a field experiment which eliminated the relationship between distance from the rabbit burrow and forage quality, and showed that forage of a higher quality is preferred, even when farther from the burrow (and thus with a higher sward height). However, the highest (and farthest) swards in this trial were on average approximately as high as the short swards in our study. Therefore, it is possible that sward height in their trial did not show enough variation to really affect the rabbit behaviour. However, their results are confirmed by the results of the present study, controlling for plant height and biomass, which clearly indicate that plant quality, particularly nitrogen and related protein concentration, is a crucial factor for selecting foraging patches in rabbits.

The other factors included in the model (position of the sward or dish, experimental day and sex) did not significantly influence the food preference of the rabbits. The day of feeding was of no importance, although CRAWLEY (1983) mentions that a hungry animal will be more selective. Since the animals lost weight during the experiments, it could be expected that the animals would become more selective towards the end of the experiment, but this was not confirmed. Similar results were obtained by DUNCAN *et al.* (2006) who found no evidence that nutritional plane had an overall effect on the proportion of several plant species eaten during preference tests carried out with herbivores. The authors relate this to the more extreme forage deficits in the wild. Similarly, the scarcity of food during winter time in the temperate regions will cause stronger feelings of hunger than the rabbits in our experiment experienced.

Although we evidenced that food quality is important for determining preferences, we were not able to exclude the importance of antipredation considerations and other sward height related issues in diet selection (see also Chapter 3): other factors, besides nutrient concentration, may also have played a role during the decision process of the rabbits. Other research indeed showed that rabbits also choose the swards with the lowest biomass, when there are no nutritional differences between the swards of different heights (IASON *et al.*, 2002). However, the field experiment of BAKKER *et al.* (2005) demonstrates that the presence of predators causes a shift in the moment of feeding, but does not affect patch preferences. The presented results clearly showed that nutritional content plays an important role in the observed preference of medium-sized herbivores for swards of intermediate size, but further research is needed to unravel the relative importance of other potentially contributing factors.

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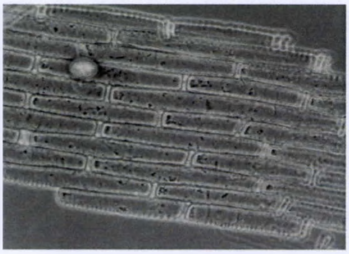
Growing plants, 2003
(Photograph by N. Somers)

Feeding trial, 2003
(Photograph by N. Somers)



Epidermal fragment of
Festuca rubra
(Photograph by K. De Maeyer)

CHAPTER 5

The determination of the diet of rabbits (*Oryctolagus cuniculus* L.) using microhistological faecal analysis: an experimental evaluation

Nele Somers, Beatrijs Bossuyt, Luc Lens & Maurice Hoffmann

Abstract

Microhistological faecal analysis to assess the diet of herbivores is a widely used method: the diet of the herbivore is qualified and quantified by identifying plant epidermal fragments which can be found in the pellets of this animal. The method has several advantages, but some limitations have been mentioned in the past. A clear consensus on the accuracy of this technique is lacking. So we performed a feeding trial with rabbits (*Oryctolagus cuniculus* L.) to compare the known diet with the results obtained by faecal analysis. The main goal of the study was to find out whether the diet composition can be reliably derived from faecal analysis (despite problems of differential digestion). Therefore, we also determined the number of identified fragments which is necessary to obtain a good result and investigated whether the best quantification of the diet is originating from fragment counts or from fragment area measurements. It is concluded that only the composition of grass diets can be estimated from pellet analyses, due to a higher digestion intensity of forbs. We recommend the identification of 150 epidermal fragments and quantification by area measurements. Further research is needed to develop new, more reliable methods to determine the diet composition of wild grazers more accurately.

Introduction

Microhistological faecal analysis has been widely used for determining the diet composition of wild herbivores (e.g. WILLIAMS *et al.*, 1974; PUTMAN, 1984; DE JONG *et al.*, 1995; TREYDTE *et al.*, 2006; YOSHIHARA *et al.*, 2008), including lagomorphs (e.g. BHADRESA, 1977; HOMOLKA, 1982; HOMOLKA, 1987; HOMOLKA, 1988; DUFFY *et al.*, 1996; PUIG *et al.*, 2007). The method is based on the fact that epidermal plant material is preserved well during digestion, so that epidermal plant fragments are preserved in the stomach and the faeces. The characteristics of these fragments are different for different plant species, and so they are useful to microscopically identify the plant species that have been eaten by the animal.

Determining diet composition by analysing faeces has several advantages over other methods (HOLECHEK *et al.*, 1982). Utilization studies (estimation of the level of utilization of plants by direct observation of living plant material) suffer from many confounding factors (e.g. trampling, weathering) and accuracy is lacking. Direct observation of the animals' grazing behaviour is not accurate for wild animals that are difficult to approach. Fistula sampling is also not possible for free-living wild herbivores. Stomach analyses need sacrificing the animals. Hence, as all these methods have practical and/or accuracy limitations, when wanting to investigate the diet of a wild rabbit (*Oryctolagus cuniculus* L.) population during the course of a year (see Chapter 6), microhistological faecal analysis is the most plausible option. A continuous collection of pellets can be taken from the same rabbit population without seriously disturbing them (DUSI 1949). Moreover, not only qualitative data are derived from these analyses, but most researchers also try to quantify the proportion of each plant species in the herbivore diet, mostly by counting the fragments (e.g. DUFFY *et al.*, 1996), sometimes by measuring the area of the fragments (e.g. BHADRESA, 1977).

However, there are a lot of studies testing the validity of the results of faecal analysis (e.g. STEWART, 1967; SPARKS & MALECHEK, 1968; FREE *et al.*, 1970; HAVSTAD & DONART, 1978; VAVRA *et al.*, 1978; PULLIAM & NELSON, 1979; SMITH & SHANDRUK, 1979; VAVRA & HOLECHEK, 1980; HOLECHEK & VAVRA, 1981; LESLIE *et al.*, 1983; MCINNIS *et al.*, 1983; SAMUEL & HOWARD, 1983; WALLAGE-DREES, 1988; ALIPAYO *et al.*, 1992; MAIA *et al.*, 2003) and several aspects of faecal analysis have been questioned by them.

When evaluating faecal analysis, the problem of the disappearing forbs (SAMUEL & HOWARD, 1983) is very often encountered. It seems that forbs are often underestimated in faecal analysis, while grasses are overestimated (e.g. VAVRA *et al.*, 1978;

SMITH & SHANDRUK, 1979; LESLIE *et al.*, 1983; MCINNIS *et al.*, 1983; WALLAGE-DREES, 1988). Possible causes are enumerated by SAMUEL & HOWARD (1983): maybe the epidermis of forbs is not being separated readily from lower layer cells; forb fragments might not survive the grinding which is involved in sample preparation or slide preparation; the proportion of forbs might also be altering during the digestion process. This last option (differential digestion between grasses and forbs) is mostly accepted as causing the disappearance of forbs, and even called an obvious shortcoming of the method (VAVRA *et al.*, 1978), but see also SANDERS *et al.* (1980), HOMOLKA (1986) and ALIPAYO *et al.* (1992). According to PULLIAM & NELSON (1979), there is also a significant higher digestibility in annual grasses than in most perennial grasses. Thus, a major questing regarding faecal analysis is whether it should rather be used as a qualitative than as a quantitative technique (HOLECHEK & VALDEZ, 1985).

A second problem associated with faecal analysis is the lack of a consensus about how many fragments have to be identified before reaching a stable and accurate estimate of the diet. In his pioneering research, DUSI (1949) took a small portion of a pellet, examined the whole slide and identified all fragments encountered. Later authors used numerous methods: making five slides per sample and looking at 20 locations per slide where each species was identified (SPARKS & MALECHEK, 1968; FREE *et al.*, 1970), 500 fragments per sample (ALIPAYO *et al.*, 1992), making 3 slides and using 60 fragments in total (VAVRA & HOLECHEK, 1980), identifying all fragments in 40 microscopic fields per slide (VAVRA *et al.*, 1978), 100 fragments (10 slides) per pellet using four pellet groups (MAIA *et al.*, 2003), analysing 200 fragments per sample (WALLAGE-DREES, 1988).

Third, there is some doubt whether just counting the fragments and calculating their frequency is a good method. VAVRA & HOLECHEK (1980) state that it becomes obvious that there is a large variation in structural breakdown of plant species during digestion. During digestion, different plant species break up into fragments that significantly differ in size (PUTMAN, 1984), so that counts would not be a valid means of estimating the proportions of epidermis ingested (STEWART, 1967). Estimating the proportion of plant species in the diet by measuring the area of the plant fragments could be a solution, but is used by only few authors (e.g. DE JONG *et al.*, 1995).

Taking into account these uncertainties about the method of microhistological faecal analysis, we decided to carry out a feeding trial to evaluate and optimize the technique, before using it in our studies of the diet of wild rabbits (see Chapter 6).This was necessary as we did not encounter any research in which several plant species (as well forbs as grasses; as well annuals as perennials) were fed in a known amount, followed by faecal analysis in which several aspects of the analysis were tested simultaneously: proportions of the plants eaten were compared to the proportion of plants in the faeces; area and frequency measures were compared and it was tested how many fragments have to be identified before achieving a reliable result. An additional reason for doing this trial is the possibility that effects on digested material may also differ among animal species (SMITH & SHANDRUK 1979), so animal species specific research might be needed.

During the feeding trial, six rabbits were fed three known diets. Pellets were collected and analyzed by using microhistological analysis (including measuring the area of the fragments). The goal of the research was answering the question whether faecal analysis can be used as a reliable quantitative method to determine the diet of the rabbit: is there a good accordance between the diets fed and the diet estimated resulting from the faecal analysis (taking into account forbs and grasses)? We tested how many fragments have to be identified and taken into account before reaching a good result and whether measuring the area of the fragments offers better results than simply counting the fragments.

Materials and methods

Study species

During the feeding trial, six domestic rabbits (three males and three females, all one year old) of the breed 'Steenkonijn' were used. The Steenkonijn is the oldest Belgian rabbit breed, and is closely related to the wild rabbit (WERNER, 1980). (Wild rabbits in captivity proved to behave very anxious and stressed. Therefore, we decided to use domestic rabbits during the feeding trial.) The rabbits were housed in wire mesh pens (65cm x 110cm, height: 60cm), of which the floor was covered with sand (no straw, in order to prevent the rabbit to eat plant material that was not involved in the experiment). The animals received a vaccination against myxomatosis and RHD and a basic diet - a mixture of commercial rabbit pellets (Bonito 96, Aveve, Belgium) was provided. Water was always available *ad libitum.*

Five plant species were grown in greenhouse conditions in order to feed them to the rabbits during the course of a feeding trial: *Arrhenatherum elatius* (perennial grass), *Festuca rubra* (perennial grass), *Poa annua* (annual grass, although sometimes biennial), *Galium verum* (perennial forb) and *Stellaria media* (annual forb). All plants were grown from seeds (provided by Ecoflora, Halle, Belgium and Herbiseed, Twyford, England), sown on a mixture of 50% calcareous dune sand and 50% potting soil, in seed trays of 40cm by 45cm in May – June 2003.

Feeding trial and collection of the pellets

In order to compare the known diet of the rabbits with the composition of their faeces, we conducted a feeding trial in which a known quantity of several plant species was fed to the animals. The rabbit pellets were collected and prepared for faecal analysis.

Before the start of the experiment, the different plant species were fed to the rabbits to allow them to get used to the plants. The feeding trial started on the 27th of September 2003 and ended on the 16th of October 2003.

Plant material was administered to the rabbits in one of three combinations: "grass vs. forb", "perennial vs. annual" and "perennial grasses vs. annual grasses" (see Table 1). The six rabbits were subdivided into three groups (each consisting of one male and one female). These rabbit groups received the three plant combinations each in a different order during three feeding periods, each consisting of five feeding days (a latin square design, NETER *et al.* (1996)). On the third feeding day of each period, all rabbit pellets were removed from the pen. On the fourth and fifth feeding day, as well as on the day after the fifth feeding day, all rabbit pellets in the individual pens were collected and frozen. Since the mean retention time of the rabbit varies between 9 and 30 hours, with an average of 19 hours (see review of CARABAÑO & PIQUER (1998), we waited until the fourth feeding day before collecting the first pellets for investigation. This gave us the certainty that no other plant species, fed before the start of the feeding period, would be present in the faeces. After the last pellet collection and on the day thereafter, a mixture of 20g of all plant species was fed to the animals. After these two habituation days, a new feeding period was started. This structure was continued until the end of the third feeding period and the last pellet collection after the third feeding period. So in the end, nine pellet samples had been taken from each of the six rabbits, from which three samples originated from the same plant combination. This finally resulted in 54 samples. The mean number of pellets per rabbit and per day was 305 ± 12.

As mentioned before, also the animal species involved may influence the result of faecal analysis. Leporids (the wild rabbit included) are known to reingest soft faeces (caecotrophy). The soft faeces are ingested after excretion directly from the anus (HIRAKAWA, 2001), so we only collected hard faeces. The two types of pellets are formed by a separation mechanism in the proximal colon, so the difference between the soft and hard faeces is not due to the food having passed once or twice (HIRAKAWA, 2001), as is sometimes misinterpreted. The soft faeces mainly contain greater proportions of protein, minerals and vitamins, while the hard faeces are enriched in fibrous components (CARABAÑO & PIQUER, 1998; HIRAKAWA, 2001). Therefore, we can suppose that the fibrous, indigestible epidermal plant fragments on which the method of faecal analysis is based are especially present in the hard faeces, and so we expect that caecotrophy is not likely to influence the results of faecal analysis.

	Plant combinations		
	Grass vs. forb	Perennial vs. annual	Perennial grasses vs. annual grasses
Amount of plants fed	*25g Festuca rubra* *20g Galium verum* *35g Stellaria media*	*20g Arrhenatherum elatius* *30g Poa annua* *30g Stellaria media*	*30g Arrhenatherum elatius* *30g Festuca rubra* *30g Poa annua*
Amount of plants eaten effectively	*23g Festuca rubra* *12g Galium verum* *34g Stellaria media*	*19g Arrhenatherum elatius* *29g Poa annua* *30g Stellaria media*	*29g Arrhenatherum elatius* *29g Festuca rubra* *29g Poa annua*

Table 1: Overview of the identity and amount of the plant species in the three plant combinations fed to and effectively eaten by six experimental rabbits.

The plant material was not always eaten completely by the rabbits. We collected residual plant material before feeding fresh plants to the animals, and measured the dry weight of the uneaten plants. To correct for the desiccation of the plant material, 10g of each plant species was placed each day in the neighbourhood of the pens, and weighed on the next day. By subtracting the spoiled plant weight (corrected for desiccation) from the fed plant weight, we calculated the real amount of plant material per plant species eaten by each rabbit. Per plant combination, these values were averaged over all the rabbits. From now on, we consider these values (see Table 1) as the plant amount eaten by the rabbits.

Preparation of the faeces

From the frozen pellets, 30 pellets per sample were selected at random to be prepared for microscopical analysis. The preparation method was based on the method followed by DE JONG *et al.* (1995). The selected pellets were put in an autoclave (Certo Clav Multicontrol) with some distilled water, and sterilized for five minutes at a temperature of 125°C. After cooling down, the water was poured off, using a plankton screen (openings of 100µm), and thereafter the pellet sample was crushed. We weighed 5g of that sample and put it in a blender (Waring Blender 1l, 120-240 volt) for being mixed during one minute in order to create a homogeneous pellet sample (PULLIAM & NELSON, 1979; MAIA *et al.*, 2003). The water was poured off using the plankton screen again and the sample was put in a small pot, filled with ethanol 70% and closed by a lid. The samples were conserved this way.

Microscopical analysis of the faeces

For microscopical analysis, a drop of the sample was put on a microscopic slide and covered by a cover slip. Per sample, 30 slides were made. Each slide was examined using a microscope (Olympus BX41) at magnification 10x10. Ten epidermal plant fragments where identified per slide, in at least two transects. (Particles smaller than 10µm x 10µm were discarded.) In total, 300 plant fragments were identified per pellet sample. Identification of the fragments was done by comparing the fragments with reference photographs of epidermal fragments of the plant species administered. The reference collection was created by K. De Maeyer. Plant material was collected in the field; epidermal fragments were taken from the plants, cleared by household bleach and put on microscopic slides. Photographs of the fragments were taken, and the characteristics of the fragments were noted. As distinguishing between the fragments of *Galium verum* and *Stellaria media* was not always possible, they were both noted as "dicot"; the same problem was encountered with *Festuca rubra* and *Arrhenatherum elatius*, therefore both noted as "perennial grass", at least in the trials where these species were fed simultaneously.

The area of each plant fragment was measured and noted by using a grid of 100 small squares, each square representing an area of 100µm x 100µm at the magnification used.

Statistical analysis

Due to technical problems, five of the 54 samples dried out completely. The analyses of these samples were left out of all statistical analyses. From these five samples, two originated from the grass-forb combination, two from the grass combination and one from the perennial-annual combination. So in total, 49 samples were taken into account for the statistical analysis. To avoid nested data, we randomised the data by using Excel: the order in which the fragments were encountered was not respected anymore and doubles were possible.

For each plant combination, we formulated a plant species ratio (calculated either with the amount of plant species effectively eaten or with the area or frequency of the epidermal fragments of the plant species in the faeces). For the plant combination "grass vs. forb", we calculated the ratio of *Festuca rubra* (a grass) over the sum of the two dicot species *Galium verum* and *Stellaria media*. For the plant combination "perennial vs. annual", we calculated the ratio of *Arrhenatherum elatius* (perennial) over the sum of the two annual species *Poa annua* and *Stellaria media*. For the plant combination "perennial grasses vs. annual grasses", we calculated the ratio of the sum of *Festuca rubra* and *Arrhenatherum elatius* over the amount of *Poa annua*.

Chi-square analyses were performed for testing the differences (or resemblances) between the plant amounts eaten and plant quantity obtained by the faecal analysis (after examining 25, 50, ..., 300 fragments). Using mixed linear models, we tested (separately for each plant combination) whether the area of epidermal fragments is statistically different between PLANT SPECIES. This information should be very useful in the discussion whether using frequency or area of fragments. The random factor used in the model was the SESSION NUMBER, which is a combination of the day of pellet collection and the rabbit from which the pellets were collected. For the combination "perennial grasses vs. annual grasses", we calculated the difference between the ratio eaten and the ratio obtained by measuring fragment area on one hand, and the difference between the ratio eaten and the ratio obtained by counting fragments. This was done for 100, 150, 200, 250 and 300 fragments. A Wilcoxon Signed Rank Test was performed on these calculated values to test whether measuring area or counting fragments gives a different view of the true diet. The statistical tests were performed using SAS 9.1 (SAS Institute Inc., Cary, North Carolina).

Results

When comparing the three plant combinations, "perennial grasses vs. annual grasses" was the plant combination showing the most stable evolution (Figure 1). The two other combinations were not very stable when considering less than 100 fragments, indicating that results can still change a lot when examining extra fragments. However, when examining more than 150 fragments, the results were better, although never as stable as for the plant combination "perennial grasses vs. annual grasses".

The proportion of the different plant species in the plant mixes eaten by the rabbits differed significantly from the proportion of these plants in the pellets, at least for the combinations "grass vs. forb" and "perennial vs. annual" (Table 2). For the plant combination "perennial grasses vs. annual grasses", no significant differences were found, indicating that the amount of grass species eaten by a rabbit was well reflected by the proportion of these species in the rabbit pellets, whether observed as number of fragments or as fragment area.

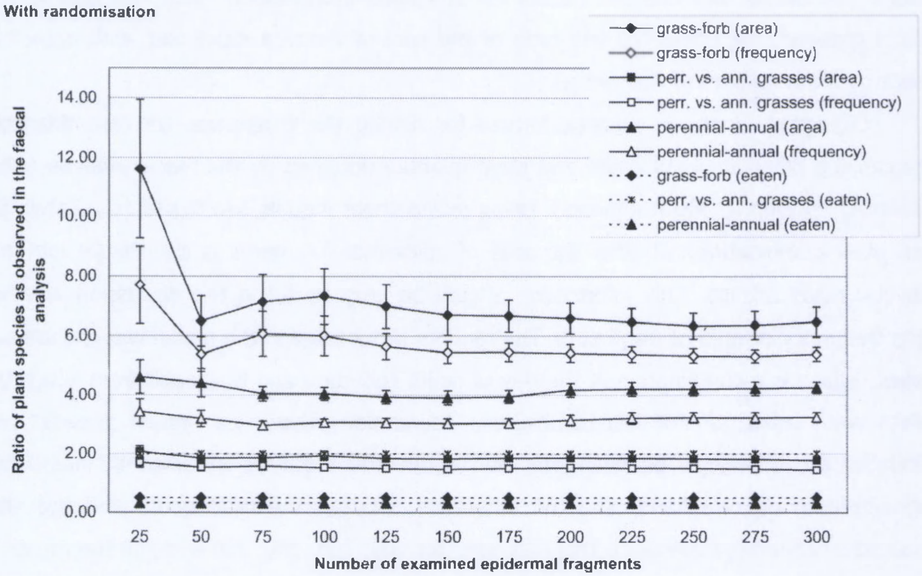


Figure 1: The evolution of the ratio of the plant species after examining 25, 50, ..., 300 epidermal fragments. For the plant combination "grass vs. forb", we calculated the ratio of *Festuca rubra* (a grass) over the sum of the two dicot species *Galium verum* and *Stellaria media*. For the plant combination "perennial vs. annual", we calculated the ratio of *Arrhenatherum elatius* (perennial) over the sum of the two annual species *Poa annua* and *Stellaria media*. For the plant combination "perennial grasses vs. annual grasses", we calculated the ratio of the sum of *Festuca rubra* and *Arrhenatherum elatius* over the amount of *Poa annua*. Standard errors are shown.

There were significant differences between the areas of epidermal fragments among different plant species, for the three plant combinations. In the combination "grass vs. forb" ($F_{1,\ 4783}$ = 13.79, p = 0.0002), dicots had the smallest area (lsmean = 4.46 ± 0.24) compared to *Festuca rubra* (lsmean = 5.27 ± 0.15). In the combination "perennial vs. annual" ($F_{2,\ 5081}$ = 58.91, $p < 0.0001$), *Arrhenatherum elatius* had the largest area (lsmean = 5.84 ± 0.13), compared to the two annuals *Poa annua* (lsmean = 4.48 ± 0.09) and *Stellaria media* (lsmean = 4.30 ± 0.15). In the combination "perennial grasses vs. annual grasses" ($F_{1,\ 4783}$ = 69.37, $p < 0.0001$), the annual *Poa annua* had the smallest area (lsmean = 4.40 ± 0.17) compared to the perennial grasses (lsmean = 5.43 ± 0.16).

After stabilisation of the calculated ratio (from 100-150 fragments onwards, see above), the ratio calculated by the area of the fragments was always larger than the one calculated by the frequency of the species (Figure 1). The differences between the area results and the frequency results were smallest for the combination "perennial grasses vs.

annual grasses". The ratio of the epidermal fragments in the plant combination "perennial grasses vs. annual grasses" was closer to the true amount of eaten plant species by the rabbits when considering the area of the fragments instead of the frequency of the fragments (Figure 2). The difference between the two methods was shown by the Wilcoxon Signed Rank Test, in which p-values were always significant ($p < 0.01$). This observation was true in any of the number of analyzed fragments, i.e. 100, 150, 200, 250 as well as 300 fragments. A similar comparison was not made for the two other plant combinations, since the relationship between the amount of plants eaten did not match the amount of plant species in the pellets (see above), nor in the case of considering the area of the fragments, neither when considering their frequency.

Grass vs. forb	Area of the fragments			Frequency of fragments		
Number of examined fragments	**DF**	**Chi-square**	**Prob**	**DF**	**Chi-square**	**Prob**
25	1	67.73	<.0001	1	18.67	<.0001
50	1	73.75	<.0001	1	27.30	<.0001
75	1	94.49	<.0001	1	37.97	<.0001
100	1	106.67	<.0001	1	44.70	<.0001
125	1	113.58	<.0001	1	50.38	<.0001
150	1	114.99	<.0001	1	55.29	<.0001
175	1	122.50	<.0001	1	59.60	<.0001
200	1	125.06	<.0001	1	63.42	<.0001
225	1	126.71	<.0001	1	66.83	<.0001
250	1	127.65	<.0001	1	69.91	<.0001
275	1	129.81	<.0001	1	72.70	<.0001
300	1	133.13	<.0001	1	75.23	<.0001

Perennial vs. annual	Area of the fragments			Frequency of fragments		
Number of examined fragments	**DF**	**Chi-square**	**Prob**	**DF**	**Chi-square**	**Prob**
25	2	13.36	0.0013	2	5.05	0.080
50	2	16.66	0.0002	2	6.89	0.032
75	2	22.24	<.0001	2	11.32	0.004
100	2	22.84	<.0001	2	11.91	0.003
125	2	24.53	<.0001	2	13.51	0.001
150	2	21.99	<.0001	2	13.12	0.001
175	2	23.08	<.0001	2	14.10	0.001
200	2	25.88	<.0001	2	15.18	0.001
225	2	27.88	<.0001	2	16.75	0.000
250	2	28.39	<.0001	2	17.40	0.000
275	2	28.55	<.0001	2	18.13	0.000
300	2	28.59	<.0001	2	18.06	0.000

Perennial grasses vs. annual grasses	Area of the fragments			Frequency of fragments		
Number of examined fragments	**DF**	**Chi-square**	**Prob**	**DF**	**Chi-square**	**Prob**
25	1	0.02	0.897	1	0.37	0.545
50	1	0.31	0.579	1	0.83	0.362
75	1	0.29	0.592	1	0.75	0.387
100	1	0.09	0.767	1	1.14	0.286
125	1	0.22	0.638	1	1.19	0.276
150	1	0.23	0.628	1	1.45	0.228
175	1	0.19	0.665	1	1.26	0.262
200	1	0.17	0.679	1	1.28	0.257
225	1	0.16	0.690	1	1.31	0.253
250	1	0.18	0.669	1	1.32	0.250
275	1	0.22	0.640	1	1.48	0.223
300	1	0.31	0.575	1	1.77	0.184

Table 2: Results of the chi-square-tests that compares the amount of the different plant species eaten by the rabbits with the area or frequency of these plant species in the faeces. The analysis was done separately after analysing 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275 and 300 fragments and separately for each of the three plant combinations. When the result is significant, the differences between the plan amount eaten and the results of the faecal analysis are significantly different. DF = degrees of freedom. Chi-square = chi-square test statistic obtained by the test. Prob = the significance level obtained by the test.

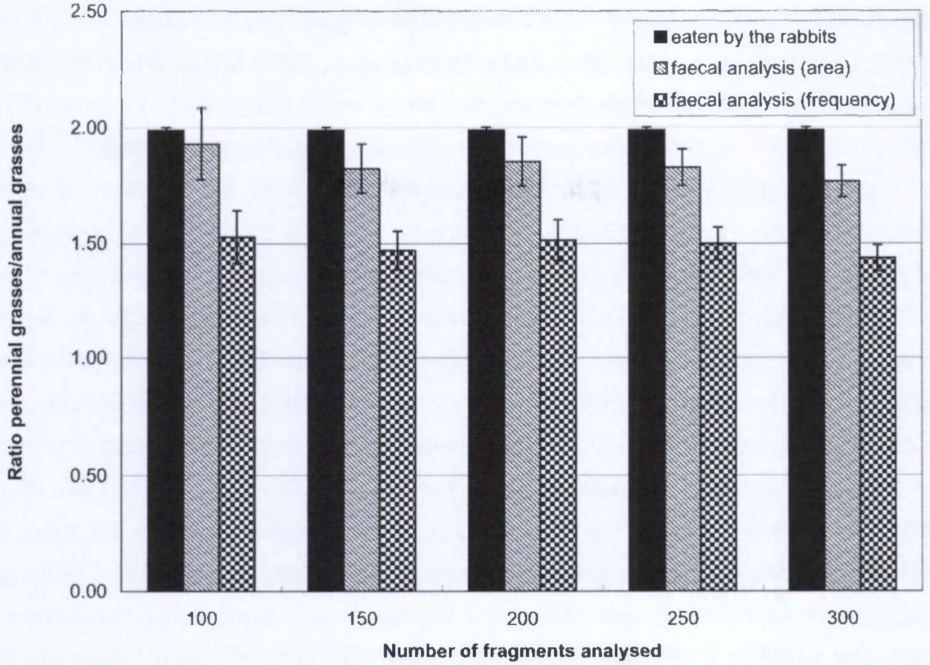


Figure 2: The ratio of the sum of *Festuca rubra* and *Arrhenatherum elatius* over the amount of *Poa annua* (in the plant combination "perennial grasses vs. annual grasses") is shown. This ratio is compared for the plant amounts effectively eaten by six rabbits and the ratios calculated from the area and the frequency of epidermal fragments in the pellets of these rabbits. The comparison is made after examining 100, 150, 200, 250 and 300 epidermal fragments. Standard errors are shown.

Discussion

The results of our feeding trial show that the known diet of the rabbits was only reflected qualitatively and quantitatively in the epidermis fragments in the pellets when concerning diets that consist only of grasses. Identifying 150 epidermal fragments should be recommended, and the use of area measurements has to be preferred over simply counting the fragments.

When evaluating the comparison between the ratio of plant species eaten and the ratio obtained by faecal analysis, we can conclude that the results of the faecal analysis are unreliable when concerning diets including forbs (the grass-forb-diet and the perennial-annual-diet): comparing the amount eaten and analysed in the faecal pellets results in very significant differences for these two diets. The third diet, comprising only grass species, seems to be reflected reliably in the faeces. Thus, we should conclude that the disproportional disappearance of forbs as compared to grass species (VAVRA *et al.*, 1978; SMITH & SHANDRUK, 1979; LESLIE *et al.*, 1983; MCINNIS *et al.*, 1983; SAMUEL & HOWARD, 1983; WALLAGE-DREES, 1988), is severely distorting the proportions of the plant species found in the faecal pellets. Differential digestion makes the microhistological results unreliable as far as forb consumption is concerned. As faecal material taken from the intestines of some ruminants contains more species than faeces taken from the ground, SMITH & SHANDRUK (1979) suppose that also weather conditions could alter the composition of the faeces, and moreover, the degree of over- and underestimation could be significantly affected by the season (LESLIE *et al.*, 1983). As the pellets analysed in our study were taken freshly from captive rabbits (not exposed to fluctuating weather conditions), we should assume that pellets collected in the field will expose even more distorted results than presented in Table 2. Some authors tried to establish correction factors (DEARDEN *et al.*, 1975; LESLIE *et al.*, 1983; BARTOLOMÉ *et al.*, 1985), but these correction factors should not only be calculated for each plant species, but also for specific study areas and seasons (LESLIE *et al.*, 1983), and probably even for each herbivore species and the age of the animals concerned (STEWART, 1967). This would be a very time-consuming process, and for some rare herbivore species, this would probably be impossible. As SMITH & SHANDRUK (1979) conclude, a close agreement between the diet and the faeces can only be reached when the herbivore has mainly eaten grasses; the identification of forbs in faeces on rangelands dominated by forbs poses an important problem (FREE *et al.*, 1970).

From each pellet sample, 300 fragments have been identified. This quantity proved to be far more numerous than really needed to obtain a stable result. When less than 100 fragments are identified, the ratio of plant species observed in the faecal pellets is not yet stabilized, especially when considering the data from the grass-forb-diet and the perennial-annual-diet. From the measurement of 100 fragments onwards, results become more stable, especially when looking at the grasses-diet. We may conclude that we should identify at least 100 fragments for obtaining a more or less stable result. However, when including a safety margin, we recommend identifying 150 plant fragments in the analysis.

Comparing the area of the fragments of different plant species confirms the results of VAVRA & HOLECHEK (1980) and PUTMAN (1984): forbs and annual grasses break down in fragments that are significantly smaller than the fragments of perennial grasses. Our results indicate that the plant ratio is usually higher for the area measurements. As mentioned above, this plant ratio obtained by faecal analysis is only reliable for the grasses diet, but also in this diet, we can see a clear distinction between the frequency and area measurements. The tests indicated that frequency as well as area measurements give a good estimate of the real diet, but that the area measurement gives an even more reliable estimate. As a consequence, we support the view of STEWART (1967) that area measurements are an improvement when comparing to fragment counts. STEWART (1967) also tested a third quantification method, the point quadrate method, but concluded that it would be a too time-consuming process to enable sufficient analyses to reduce the very high standard error he obtained by this method.

We can conclude that the most important limitation of microhistological faecal analysis is the problem of differential digestion of forbs. As it is hardly possible to overcome this shortcoming of the method, there is a need for better methods. MARTINS *et al.* (2002) and FERREIRA *et al.* (2007) describe a method for estimating diet composition by using n-alkanes. Particular patterns of concentrations of n-alkanes in cuticular wax are specific to individual plant species or species groups and can be compared to n-alkane concentrations in faeces (by gas chromatography). However, this method is limited to the number of n-alkane markers available (probably limited to 9) and, as a consequence, this method is only useful when dealing with simple diets. For the study of complex diets, pooling plant species into groups is necessary. Therefore, further research to increase the number of markers is needed. CAUT *et al.* (2008) investigated the possibility to compare stable isotope ratios of nitrogen and carbon in resources and consumer tissues. Using hairs of the consumer could be used as a non-lethal method to assess the diet. However, this method is still under development.

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Dune Fossile de Ghyvelde, 2005 *(Photographs by N. Somers)*

CHAPTER 6

The diet of the wild rabbit (*Oryctolagus cuniculus* L.): selection of plant species in an acid dune grassland

Nele Somers, Beatrijs Bossuyt, Luc Lens & Maurice Hoffmann

Abstract

Rabbits (*Oryctolagus cuniculus* L.) are expected to forage on high quality food. Experimental research showed that they select for high quality forage within the same plant species. However, the spatial scale on which diet preferences are studied could influence the results, so it is not clear whether the same diet selection mechanisms are operating at the plant species level: little is known about plant species selection in the field, the seasonal pattern of this selection, and the mechanisms of this possible selection. Therefore, we studied the diet composition of wild rabbits in an acid dune grassland, and compared it to the availability of food throughout the different seasons of the year. Rabbits proved to select indeed their food items: they do not just eat according to forage availability, and seasonality does influence the results. Plant quality did not prove to be the main trigger to select for certain species in field conditions. Although it has been proven that rabbits are able to select for the better quality of forage at the monospecific plant patch level, this study indicates that other triggers are at least equally important in determining the diet selection at the plant species level.

Introduction

Patterns and processes of diet selection of mammalian grazing herbivores have been extensively studied in ecological studies (e.g. Belovsky, 1984; Wilmshurst *et al.*, 2000). The twin goals of their diet selection are maximizing nutrient intake and minimizing toxin intake (Duncan & Gordon, 1999). Provenza (1995) proposes that these goals can be obtained by a postingestive feed-back system, by which herbivores relate the satiety or malaise after eating particular food items with the taste and smell of these items. Although Provenza (1995) states that ruminants and non-ruminants do not differ in the non-cognitive aspects of how feedback is processed, research about diet selection of medium-sized non-ruminant herbivores, such as the rabbit (*Oryctolagus cuniculus* L.), is very limited. Based on the smaller volume of the digestive tract of medium-sized hindgut-fermenters, such as the rabbit, and the need for quickly digestible food to fulfil their high metabolic needs (Demment & Van Soest, 1985), smaller herbivores can be expected to need plants of higher quality than larger herbivores (Olff *et al.*, 2002).

The outcome of food selection studies strongly depend on the scale of the study: a natural hierarchy of selection processes can be identified (Johnson, 1980). For example, at first order, there is the geographical range in which the animal occurs; second order selection determines the home range of a group of rabbits; third order selection is about which habitat components of the home range are being used for foraging and then there is fourth order selection of particular food items within one habitat patch (Johnson, 1980). The prediction about food selection based on food quality by medium-sized herbivores, such as rabbits, was already shown by experimental research (Bakker *et al.*, 2005; Somers *et al.*, 2008), proving the important role of protein concentration for rabbits when selecting food patches, which can be considered as third order selection. However, little is known about selection of food plants by rabbits (fourth order). Many researchers described the diet composition of the wild rabbit (Williams *et al.*, 1974; Bhadresa, 1977; Chapuis, 1979; Homolka, 1987; Duffy *et al.*, 1996; Wolfe *et al.*, 1996) and other lagomorphs (Homolka, 1982; Puig *et al.*, 2007; Pauperio & Alves, 2008; Seccombe-Hett & Turkington, 2008), but only a few also tried to determine diet preferences (e.g. Bhadresa, 1977; Chapuis, 1979). To our knowledge, studies combining data about diet preferences with plant quality data (to test the role of plant quality when selecting food plants) are not available. Also other factors (such as toxicity of the plants) could influence diet preferences as well. Since none of the plant species considered in this comparative study of a selection of abundantly present plant species are known as species rich in toxic secondary compounds, we could entirely focus on plant quality. Due to the seasonal aspect of plant characteristics, among which forage quality traits, diet selection could be influenced by seasonality (CHAPUIS & FORGEARD, 1982). Therefore, diet selection has to be studied in all seasons, in order to take into account these possible seasonal differences.

In this study we try to answer the following questions: do rabbits select for specific plant species or do they just eat according to the plant availability? If they do select, is plant quality one of the criteria they use? To answer these questions we selected an acid coastal dune grassland situation as a model in a coastal dune area richly populated by rabbits. Diet availability and diet composition were studied and compared during all four seasons. We hypothesized that rabbits have preferences at the plant species level, and that these preferences would vary among different seasons. We additionally hypothesized that forage quality would be an important determinant of forage selection by rabbits.

Materials and methods

Study area and research plots

The research was carried out in the French Nature Reserve 'Dune Fossile de Ghyvelde' (Ghyvelde, France, 51°2'48" N, 2°33'02" E; managed by Conservatoire du Littoral). At this site, wild rabbits comprise the dominant natural grazers, while large herbivores are used for grazing management (Haflinger horse, *Equus caballus* L.; LAMOOT & HOFFMANN, 2004). More information about this dune area can be found in Chapter 1.

Four similar grassland study plots (replicates) of 30 m x 30 m have been selected in the study area. In each study plot, 4 transects of 30 m were established, 10 m apart from each other (see Figure 1). Five permanent quadrates (pqs) of 0.75 m x 0.75 m were distributed evenly along each transect, so that there were 20 pqs in each of the four study plots, which resulted in a total of 80 pqs.

Diet availability

For every season, diet availability was estimated in all four study plots: 30th of November 2005 – 21st of December 2005 (further called 'Autumn 2005), 6th of February 2006 – 3rd of March 2006 ('Winter 2005-2006), 2nd of May 2006 – 29th of May 2006 ('Spring 2006') and 31st of July 2006 – 10th of August 2006 ('Summer 2006'). Although small, the size of the study plot represents a rabbit home range size similar to the one described by DEKKER *et al.* (2006), so we consider plant availability in the study plots as a good estimate of what was available for the rabbits living in or near the study plots (within their home range). Since the distance between the individual plots always largely exceeded 100 m, and since normal home ranges of rabbit groups measured in Europe are mostly smaller than 1 ha (DEKKER *et al.*, 2006), we can assume that all four plots were foraged by different rabbit groups.

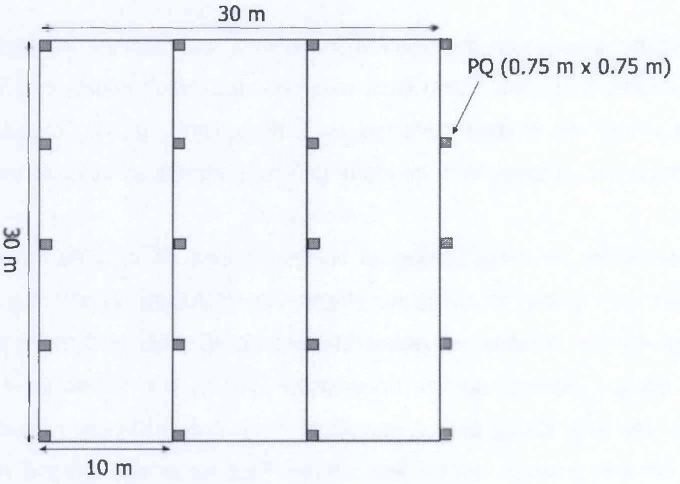


Figure 1: Schematic presentation of one study plot. In each plot, there are four transects of 30 m; the distance between two transects is 10 m. Along each transect, five pqs of 75 cm by 75 cm are marked off, distributed evenly over the transect.

In each pq, all plant species (except for mosses and lichens) were identified (following VAN DER MEIJDEN (1996) and LAMBINON *et al.* (1998)) and their estimated cover was noted using the Londo scale (LONDO, 1975). Estimating plant cover is a method regularly used for estimating diet availability (e.g. DE JONG *et al.*, 1995). This method is less destructive than clipping the plant species for determining their biomass (BHADRESA, 1977) and was therefore more usable for our study, comparing diet availability in different seasons at the same place. PAYNE (1974) confirms that using cover values alone as an index of herbage production differences is a very useful and less time-consuming method.

Using Pcord4, a TWINSPAN-table of the relevés of spring 2006 was made, in order to distinguish between different vegetation types. On the 7th of August 2006, all study plots were visited, and a vegetation type was assigned to every point in a grid with distance 1 m between the points of the grid.

Combining the data of the vegetation relevés and the spatial distribution of the vegetation types, we calculated the average presence (expressed as percentage of cover) of individual plant species per plot and per season. This measure was interpreted as the availability of these species as a food item.

Diet composition

In each season, synchronously with the vegetation relevés, we collected 30 fresh rabbit pellets within (or maximal 5 m away from) each research plot. Fresh pellets can be distinguished from pellets which are several days old by their smooth, glossy, mucous coating (DUSI, 1949). Pellets were collected from as many different latrines as possible and frozen after collection.

The pellets were prepared for microhistological analysis based on DE JONG *et al.* (1995). The selected pellets were put in an autoclave (Certo Clav Multicontrol) with some distilled water, and sterilized for five minutes at a temperature of 125°C. After cooling down, the water was poured off, using a plankton screen (openings of 100µm), and thereafter the pellet sample was crushed. We weighed 5g of that sample and put it in a blender (Waring Blender 1l, 120-240 volt) for being mixed during one minute. The water was poured off using the plankton screen again and the sample was put in a small pot, filled with ethanol 70% and closed by a lid. The samples were conserved this way.

For microscopical analysis, a drop of the sample was put on a microscopic slide and covered by a cover slip. Per sample, 30 slides were made. Each slide was examined using a microscope (Olympus BX41) at magnification 10 x 10. Ten epidermal plant fragments where identified per slide, in at least two transects. (Particles smaller than 10µm x 10µm were discarded.) Only monocotyledonous species were identified and noted (see Chapter 5). In total, 150 plant fragments were identified per pellet sample (see Chapter 5). Identification of the fragments was done by comparing the fragments with reference photographs (DE MAEYER, unpublished) of epidermal fragments of the plant species growing in the study area, near or in five exclosures (see Chapter 2). Plant material was collected in the field; epidermal fragments were taken from the plants, cleared by household bleach and put on microscopic slides. Photographs of the fragments were taken, and the characteristics of the fragments were noted.

The area of each plant fragment was measured and noted by using a grid of 100 small squares, each square representing an area of 100 µm x 100 µm at the magnification used (see Chapter 5).

Food quality

To assess the food quality of the food plants, 63 food samples were taken from Ghyvelde in the summer of 2007. The following grass species have been sampled: *Agrostis capillaris* (6 samples), *Aira praecox* (1 sample), *Anthoxanthum odoratum* (22 samples), *Carex arenaria* (13 samples), *Festuca rubra* (9 samples) and *Luzula campestris* (12 samples). The average sample dry weight was 13 g. Plant species were collected by clipping aboveground vegetation and were separately dried at 60°C (WTB Binder with controller RD 2 EED/FED; Binder, Tuttlingen, Germany) until no further mass loss was detected, after which the dried plants were grinded separately. The percentage of crude protein (CP) and of cell wall constituents (cellulose, hemicellulose and lignin, which were derived from NDF (neutral detergent fibre), ADF (acid detergent fibre) and ADL (acid detergent lignin)) were obtained by Near Infrared Spectroscopy (NIRS – for more information see GIVENS *et al.* (1997)). A FOSS Feed and Forage Analyzer was used, combined with Winisi software (FOSS, Brussels, Belgium). The calibration for the NIRS was carried out by performing wet analyses for approximately 10% of the samples, following the method of Kjeldahl for CP and GOERING & VAN SOEST (1970) and VAN SOEST *et al.* (1991) for cell wall constituents. Seasonal changes in plant quality were studied using data of plant specimens sampled near Dune Fossile de Ghyvelde, approximately 5 km from the study area (EBRAHIMI, 2007) following a procedure similar to the one described above. Forage quality for rabbits was approximated by the amount of digestible protein (DP) and digestible energy (DE). Amounts of digestible protein were estimated by multiplying CP with the mean digestibility coefficient of CP for grasses (value of 0.70; MAFF, 1986). Digestible Energy was estimated by multiplying Gross Energy (based on MAFF, 1986) with a coefficient of digestibility, quantified as 0.867 – 0.0012 ADF (g/kg DM) (DE BLAS *et al.*, 1992).

Statistical analysis

After discarding plant species covering less than 3% in each plot in every season, the following six species were used in statistical analysis: *Agrostis capillaris*, *Aira praecox*, *Anthoxanthum odoratum*, *Carex arenaria*, *Festuca rubra* and *Luzula campestris*. There were no species occurring rarely in the vegetation, but abundantly in the pellets. Paired t-tests were used to analyse differences between the availability of a plant species in each 0.09 ha plot and in pellets collected within this plot. Whether the different plant species had a different food quality (DP and DE) and whether DP and DE were dependent on the season was tested by general linear models, followed by Tukey Post-hoc-tests. The statistical tests were performed using SAS 9.1 (SAS Institute Inc., Cary, North Carolina).

Results

When comparing plant availability with plant consumption, *Anthoxanthum* was selected during autumn and winter, while *Agrostis* was preferred during spring, summer and winter (Table 1 and Figure 2). *Luzula campestris*, in contrast, was avoided during spring, autumn and winter. Diet selection by rabbits during summer 2006 deviated from that inferred in all other seasons, with selective preference of *Festuca rubra* and selective avoidance of *Carex arenaria*.

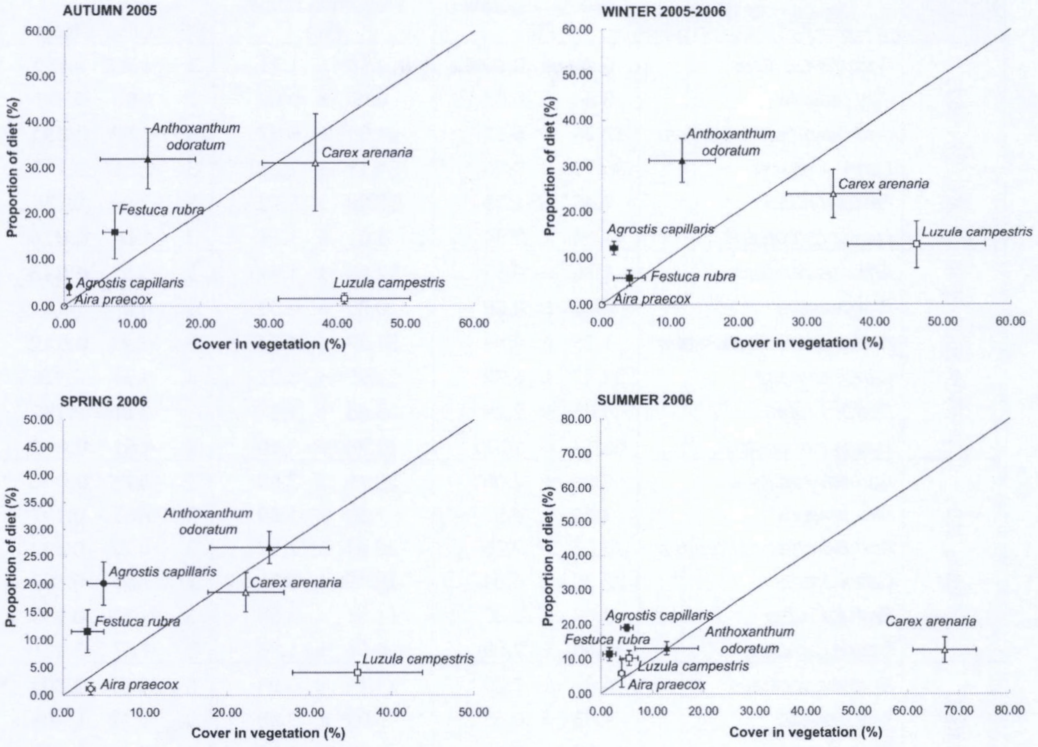


Figure 2: The relationship between the cover in the vegetation and the presence in the diet of six graminoid species, presented separately for four seasons. Error bars present the standard errors. The line y=x is drawn to determine preference for individual plant species. Plants that are above this line are positively selected by the rabbits; species under this line can be considered as avoided. (Method adapted from KUIJPER *et al.*, 2008)

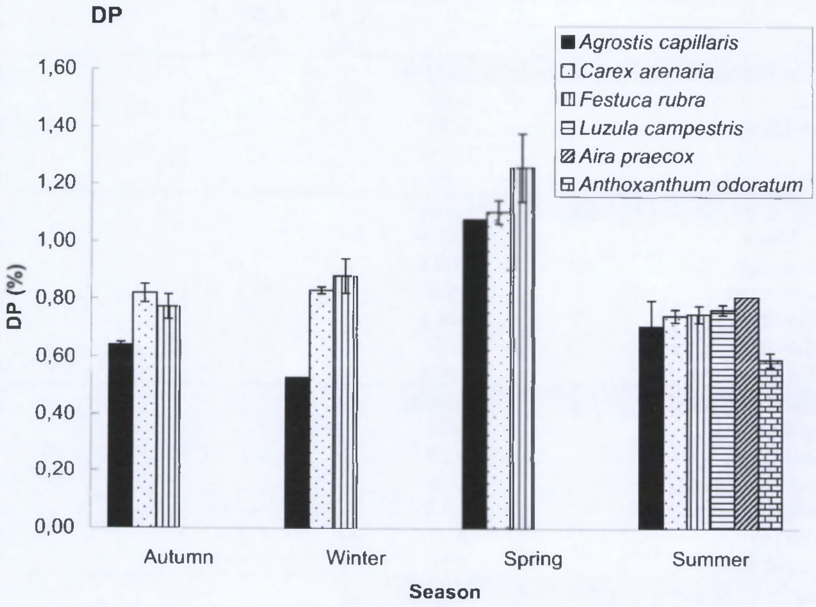
SEASON	**PLANT SPECIES**	Average ± standard error		Paired t-test			
		Cover in vegetation (%)	Proportion of diet (%)	DF	t-value	Prob	
AUTUMN 2005	*Agrostis capillaris*	0.76 ± 0.20	4.18 ± 1.71	3	-1.89	0.156	
	Aira praecox	0.04 ± 0.04	0.00 ± 0.00	3	1.00	0.391	
	Anthoxanthum odoratum	12.28 ± 6.95	31.94 ± 6.57	3	-3.87	**0.031**	
	Carex arenaria	36.77 ± 7.76	31.11 ± 10.61	3	0.34	0.757	
	Festuca rubra	7.48 ± 1.75	15.88 ± 5.78	3	-2.03	0.135	
	Luzula campestris	41.04 ± 9.64	1.61 ± 1.15	3	4.10	**0.026**	
WINTER 2005-2006	*Agrostis capillaris*	1.76 ± 0.55	12.06 ± 1.44	3	-5.37	**0.013**	
	Aira praecox	0.00 ± 0.00	0.00 ± 0.00	3	n.a.	n.a.	
	Anthoxanthum odoratum	11.76 ± 4.88	31.27 ± 4.82	3	-5.49	**0.012**	
	Carex arenaria	33.97 ± 6.92	23.95 ± 5.32	3	0.94	0.416	
	Festuca rubra	4.01 ± 2.29	5.66 ± 1.62	3	-2.06	0.131	
	Luzula campestris	46.17 ± 10.11	12.78 ± 5.10	3	4.61	**0.019**	
SPRING 2006	*Agrostis capillaris*	5.00 ± 2.00	20.11 ± 3.89	3	-6.84	**0.006**	*
	Aira praecox	3.44 ± 0.53	1.18 ± 1.09	3	1.65	0.197	
	Anthoxanthum odoratum	25.16 ± 7.25	26.64 ± 2.97	3	-0.22	0.842	
	Carex arenaria	22.30 ± 4.64	18.49 ± 3.51	3	0.59	0.599	
	Festuca rubra	3.04 ± 2.00	11.51 ± 3.86	3	-1.96	0.145	
	Luzula campestris	35.88 ± 7.89	4.06 ± 1.85	3	4.67	**0.019**	
SUMMER 2006	*Agrostis capillaris*	5.05 ± 1.20	19.14 ± 0.98	3	16.69	**0.001**	*
	Aira praecox	4.03 ± 0.65	5.94 ± 3.98	3	-0.42	0.701	
	Anthoxanthum odoratum	12.80 ± 6.20	13.19 ± 1.95	3	-0.08	0.938	
	Carex arenaria	67.29 ± 6.21	12.58 ± 3.69	3	6.35	**0.008**	*
	Festuca rubra	1.67 ± 1.24	11.52 ± 1.95	3	-8.56	**0.003**	*
	Luzula campestris	5.48 ± 1.74	10.35 ± 2.11	3	-5.09	**0.015**	*

Table 1: Averages and standard errors of the presence (percentage) of the grass species in vegetation cover and in rabbit pellets. The results of the paired t-tests are also presented. DF = degrees of freedom. T-value = test statistic obtained by the test. Prob = the significance level obtained by the test. n.a. = not applicable. * indicates that Prob is still significant after sequential Bonferroni correction.

DP of the six plant species was slightly significantly different (Table 2), but the Tukey Post Hoc did not reveal significant differences between the species. Also, the season proved to have an important influence, with a significantly higher DP in springtime (see also Figure 3). DE was not significantly different between plant species and seasons.

Effect on DP (GLM)		**DF**	**F-value**	**Prob**			
Season		3	31.95	**0.0004**			
Plant species		5	4.64	**0.044**			
Lsmean Season		**Lsmean**					
Autumn 2005		6.98					
Winter 2005-2006		6.90					
Spring 2006		11.66					
Summer 2006		6.86					
Tukey Post-hoc-tests Season		**Prob**					
Autumn vs. Winter		0.999					
Autumn vs. Spring		**0.001**					
Autumn vs. Summer		0.996					
Winter vs. Spring		**0.001**					
Winter vs. Summer		1.000					
Spring vs. Summer		**0.001**					
Lsmean Plant species		**Lsmean**					
Agrostis capillaris (1)		6.92					
Aira praecox (2)		9.24					
Anthoxanthum odoratum (3)		6.41					
Carex arenaria (4)		8.15					
Festuca rubra (5)		8.77					
Luzula campestris (6)		9.11					
Tukey Post-hoc-tests Plant species		**Prob**					
		(1)	(2)	(3)	(4)	(5)	(6)
	(1)	-	0.229	0.990	0.292	0.077	0.271
	(2)	0.229	-	0.194	0.811	0.992	1.000
	(3)	0.990	0.194	-	0.459	0.219	0.225
	(4)	0.292	0.811	0.459	-	0.821	0.875
	(5)	0.077	0.992	0.219	0.821	-	0.998
	(6)	0.271	1.000	0.225	0.875	0.998	-
Effect on DE (GLM)		**DF**	**F-value**	**Prob**			
Season		3	4.48	0.056			
Plant species		5	2.93	0.112			

Table 2: Results of the general linear model applied to DP and DE of the grass species. DF = degrees of freedom. F-value = test statistic obtained by the model. Prob = the significance level obtained by the test. Least square means (Lsmean) and Tukey Post-Hoc-test-results for the significant variables are also shown.



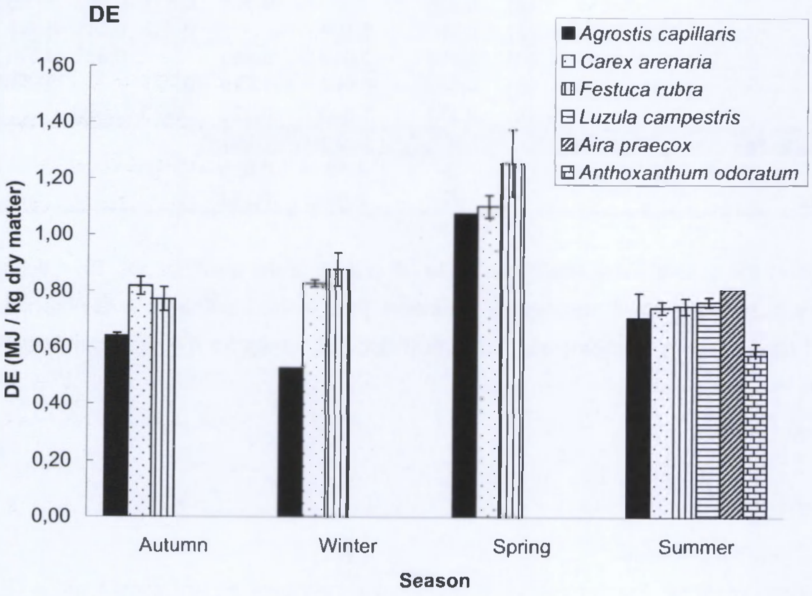


Figure 3: Average DP and DE for some grass species, for each season separately. Standard errors are shown. There were no measurements for *Luzula campestris, Aira praecox* and *Anthoxanthum odoratum* in autumn, winter and spring.

Discussion

Rabbits do not just eat what is available in their environment. Apart from preference for specific foraging patches (third order selection, see above - BAKKER *et al.*, 2005; SOMERS *et al.*, 2008), plant-specific traits also appear to affect diet selection (this study). The extent of preference or avoidance of individual plant species thereby differed among seasons, with the weakest effects recorded during autumn and winter.

As the animals do not just eat what they find, there should be a selection mechanism. Our hypothesis holds plant quality responsible for this selection and avoidance. The data on plant quality that were collected do not confirm this hypothesis though: the different plant species did not really prove to have a significantly different plant quality. However, plant traits other than nutritional quality may trigger selection or avoidance as well. For instance, the hairy habitus of *Luzula campestris* may deter rabbits. We do however not expect that this would indeed be the case, because *Luzula campestris* proved to be a preferred species in one season. Might rabbit diet preferences then be influenced by factors that do not come from the plant species? This does not seem to be the case: factors as antipredator considerations (KOTLER & BLAUSTEIN, 1995; IASON *et al.*, 2002), the distance between the food plants and the central-place (HAARBERG & ROSELL, 2006), the social rank of the animal (LOCKLEY, 1976; KRÜGER & FLAUGER, 2008) do seem to be especially important when testing diet preferences at the patch level (when deciding in which patch going to forage) and not on the species level. However, this consideration would not be true when different plant species are growing in different patches. This did not seem to be the case in our study area either, but studying spatial patterns of species in relation to feeding preferences of rabbits would be interesting. We recommend that the spatial distribution of plants should be explicitly incorporated in future studies on this topic. The distance between plant species could then also be included in analyses: when the previous plant you have eaten was very bad, the nearby plant will taste much better (negative contrasts, BERGVALL *et al.*, 2007). We should however mention that the social status should not have influenced our results, since pellets were sufficiently widely sampled to allow assuming that pellets of different rabbits were collected.

Whatever might be the reason for plant species preferences, we should realise that different study scales do influence the outcome of a study, as mentioned before. Studies revealing the preference of rabbits for high quality food came from patch-based studies (e.g. BAKKER *et al.*, 2005; SOMERS *et al.*, 2008) or group-based studies (MARTINS *et al.*, 2002) These last authors show a preference for high quality food items at group level: the group "grasses and forbs" was preferred above other food items, because of its high quality. There may even be finer levels on which food selection takes place: rabbits are able to select for plant parts, because of their small statue (Drees, 1992).

In conclusion, this study shows that the rabbits in our acid dune grassland system are selective grazers: they do not just eat what is available, but they do select for specific plant species, depending on the season. Plant quality did not prove to be the reason for the diet selection, contrary to our hypothesis. We recommend that patchiness of plant species should be incorporated in future studies concerning this topic. Moreover, it should be realized that the scale on which a study is carried out could strongly influence the results.

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Wild rabbit, Puyenbroeck, 2006 *(Photographs by M. Pevenage)*

CHAPTER 7

General discussion

The main hypothesis of the research is that introduced large herbivores facilitate rabbits (medium-sized herbivores) by modification of the vegetation. This modification involves creating short swards, creating denser (more productive) swards, creating swards that have a high food quality for rabbits and influencing vegetation composition. We expect that these modifications are not completely independent from each other: shorter swards are expected to have a higher food quality and to be more productive. We started from field observations under complex conditions to discover patterns and ended up with semi-controlled field- and controlled experimental research to reveal some causal mechanisms. By combining these results, we will now discuss the partial hypotheses which were presented in Chapter 1 and finally, we will discuss the implications of our research results for the main hypothesis.

Hypothesis 1: Large herbivores modify vegetation structure by grazing, thereby creating short and dense swards.

This research shows that large grazers modify vegetation structure, thereby creating short swards, but these were not denser than the high, ungrazed swards.

The combination of large grazers introduced in the study areas Dune Fossile de Ghyvelde and IJzermonding with rabbits keeps the vegetation structure in a more open state: vegetation height is lower and litter cover is significantly lower than in vegetation where the large grazers and rabbits were excluded (Chapter 2). Although we cannot ascribe these effects to the large grazers alone, the fact that grazing treatments with exclusion of only large grazers were intermediate between vegetation that was ungrazed and vegetation that was also grazed by large herbivores, suggests that the influence of large grazers was larger than the influence of the rabbits. We conclude that large grazers are at least able to create the typical low-canopy structure of grazing lawns.

The grazing did not result in denser swards though. In August-September 2005 (summer season), the aboveground vegetation of a random selection of pqs in the exclosures of Ghyvelde and IJzermonding was totally clipped and weighed (after drying) (Chapter 3). In Ghyvelde and IJzermonding, biomass concentration (i.e. the biomass per vertical layer of the vegetation; Table 1) was not significantly differing between the grazing treatments. A higher biomass concentration (dry weight divided by vegetation height - McNaughton, 1984) was predicted to be a second aspect of grazing lawns (van der Graaf *et al.*, 2005): ungrazed plants grow taller, more robust and occupy a larger, but less densely packed space than plants in more heavily grazed grasslands (McNaughton, 1984). We cannot confirm this part of the hypothesis and thereby affirm the conclusions of BELSKY (1986), who doubts the existence of overcompensation in grazed plants. However, we should realize that overcompensation of plant tissue might be possible only at low levels of herbivory (MCNAUGHTON, 1983) and that our study areas have a relatively low productivity. More productive areas might yield other results. Also, belowground biomass was not taken into account when testing for overcompensation (BELSKY, 1986), but it is reasonable to assume that belowground biomass is less useful for predicting sward use by rabbits. Results are also slightly contradictory to the findings of TAHMASEBI KOHYANI *et al.* (subm.), who found that grazing-tolerant grass species (*Agrostis stolonifera, Poa pratensis*) grew a larger number of tillers in grazed dune grassland than the same species in non-grazed dune grassland. On the other hand, they also found that two other - grazing intolerant - grass species (*Holcus lanatus, Festuca arundinacea*) showed a tiller number decrease phenomenon under grazing. All four species showed biomass decrease under grazing, which corroborates our findings in the exclosure experiment.

Hypothesis 2: Rabbits preferentially forage in short swards

This hypothesis was experimentally affirmed by this thesis (Chapter 3), proving that rabbits that could choose between unmown and shortly-mown vegetation strips (study area: Puyenbroeck) significantly preferred shortly-mown vegetation. We therefore conclude that rabbits do not forage following the classical Type II response (HOLLING, 1959), opposite to expectations of OKSANEN *et al.* (1981), LUNDBERG (1988), LUNDBERG & ÅSTRÖM (1990) and GROSS *et al.* (1993). A unimodal, dome-shaped Type IV functional response (BOS *et al.*, 2002; DURANT *et al.*, 2003; DEKKER & VAN LANGEVELDE, 2007; VAN LANGEVELDE *et al.*, 2008) is more likely the case for the rabbit.

However, the exclosure data from a less productive study area (Dune Fossile de Ghyvelde, Chapter 3), in which rabbit activity in vegetation grazed by horses and vegetation not grazed by horses was compared, did not yield similar results. Rabbits did not show foraging preferences for the treatment with presence of large herbivores, but neither for the treatment without horses. Nevertheless, vegetation height was significantly different between these treatments. It should be mentioned though that the "low" vegetation in Puyenbroeck was lower than the mean vegetation height in "low" swards in Ghyvelde, which were almost as high as the "long" swards in Puyenbroeck. So, in Ghyvelde, we have to conclude that vegetation height did not influence foraging behaviour of the rabbits, or that

GHYVELDE **Non-woody plants only**	**L+R+S**	**R+S**	**S**	**num**	**den**	**F**	**P**	**P Post Hoc Test**		
	Average ± S.E.	**Average ± S.E.**	**Average ± S.E.**	**d.f.**	**d.f.**			**L+R+S vs R+S**	**R+S vs S**	**L+R+S vs. S**
CP (%)	10.40 ± 0.50	9.98 ± 0.40	8.31 ± 0.35	2	22	5.18	**0.014**	0.755	**0.047**	**0.013**
Cellulose (%)	23.24 ± 2.28	27.67 ± 0.93	29.87 ± 1.20	2	22	3.93	**0.035**	0.129	0.647	**0.038**
Hemicellulose (%)	22.14 ± 0.83	23.71 ± 1.08	27.62 ± 0.62	2	22	7.30	**0.004**	0.442	**0.030**	**0.003**
Lignin (%)	8.66 ± 0.47	7.67 ± 0.26	7.27 ± 0.26	2	22	3.70	**0.041**	0.123	0.737	**0.049**
DP (%)	7.28 ± 0.35	6.99 ± 0.28	5.82 ± 0.24	2	22	5.18	**0.014**	0.755	**0.047**	**0.013**
DE (MJ / kg DM)	9.01 ± 0.44	8.24 ± 0.23	7.84 ± 0.23	2	22	2.93	0.074	0.216	0.704	0.077
Dry weight (g)	71.17 ± 6.55	85.31 ± 17.13	144.99 ± 12.22	2	22	6.81	**0.005**	0.717	**0.020**	**0.005**
Biomass concentration (g/cm)	5.73 ± 1.14	3.56 ± 1.09	5.36 ± 0.41	2	22	1.34	0.281	0.289	0.498	0.972
IJZERMONDING	**L+R+S**	**R+S**	**S**	**num**	**den**	**F**	**P**	**P Post Hoc Test**		
Non-woody plants only	**Average ± S.E.**	**Average ± S.E.**	**Average ± S.E.**	**d.f.**	**d.f.**			**L+R+S vs R+S**	**R+S vs S**	**L+R+S vs. S**
CP (%)	8.83 ± 0.14	9.16 ± 0.45	9.26 ± 0.17	2	29	0.72	0.497	0.665	0.966	0.504
Cellulose (%)	26.46 ± 1.02	27.42 ± 1.52	28.47 ± 0.99	2	29	0.73	0.489	0.834	0.817	0.456
Hemicellulose (%)	23.93 ± 0.54	24.36 ± 1.18	25.03 ± 0.78	2	29	0.44	0.651	0.931	0.849	0.625
Lignin (%)	7.56 ± 0.19	7.69 ± 0.28	8.86 ± 0.49	2	29	4.66	**0.018**	0.959	0.053	**0.022**
DP (%)	6.18 ± 0.10	6.41 ± 0.32	6.48 ± 0.12	2	29	0.72	0.497	0.665	0.966	0.504
DE (MJ / kg DM)	8.53 ± 0.24	8.29 ± 0.32	7.79 ± 0.18	2	29	2.20	0.129	0.778	0.387	0.113
Dry weight (g)	79.69 ± 6.74	107.50 ± 18.15	155.70 ± 13.40	2	29	8.91	**0.001**	0.289	**0.042**	**0.001**
Biomass concentration (g/cm)	1.89 ± 0.19	1.92 ± 0.31	1.92 ± 0.30	2	30	0.01	0.995	0.997	1.000	0.995

Table 1: Results of the plant analyses of Dune Fossile de Ghyvelde and IJzermonding. In August-September 2005 (summer season), the aboveground vegetation of a random selection of pqs in the exclosures of Ghyvelde and IJzermonding was totally clipped, weighed (after drying) and food quality of these samples was analysed. Biomass is expressed in average total dry weight (g) in 75 x 75 cm plots; biomass concentration is an indication of the vertical distribution (expressed in cm) of biomass in the vegetation. Averages and standard errors are presented per treatment (L+R+S = accessible to all herbivores; R+S: accessible to rabbits and smaller herbivores, but not to large grazers; S: accessible to small herbivores but not to rabbits and larger herbivores). The results of the statistical tests (general linear models testing the effect of TREATMENT, followed by Tukey Post Hoc Tests) are presented. CP = % Crude Protein. DP = % Digestible Protein. DE = Digestible Energy (MJ per kg dry matter). num d.f. = numerator degrees of freedom. den d.f. = denominator degrees of freedom. F = F-value, test statistic obtained by the GLM. P = the significance level obtained by the test.

vegetation was simply not short enough for obtaining facilitation, or that it interferes with other, unknown limiting factors.

Hypothesis 3: The short swards created by large grazers are of a higher nutritional quality (for rabbits) than ungrazed swards (grazing lawns)

Our results only partially indicate that large grazers create high quality swards. DP and DE were used as measures for food quality. DP was influenced in Ghyvelde: a lower DP concentration was observed when as well rabbits as large herbivores were excluded. There was however no influence when excluding only large herbivores (Table 1). DE was never positively influenced by grazing of large herbivores (Table 1) or by mowing (Chapter 3). Therefore, we cannot entirely confirm this hypothesis, which was predicted by results of e.g. COPPOCK *et al.* (1983) and RUESS (1984).

However, it is important to note that protein concentration in Ghyvelde is higher in vegetation grazed by all grazers or by rabbits, while fibre concentration is usually higher in exclosures without large grazers and rabbits. Also, we cannot exclude that other results might have been obtained when focusing on individual plant species, plant parts (FOX *et al.*, 1998) or belowground biomass (BELSKY, 1986). We should realize that many environmental factors may influence and obscure the effect of grazing on plant productivity and quality, e.g. soil nutrient availability, shading, grazer density, grazer species, grazing regime, intensity and frequency of defoliation (MILCHUNAS *et al.*, 1995). More research, focussing on different levels (swards, plant species, plant part) in different (high and low productive) habitats is needed to gain more insight into this hypothesis.

Hypothesis 4: When foraging, food quality is an important factor determining the diet choices of the rabbit.

We can partially affirm this hypothesis: third order food selection (selection between plant patches - JOHNSON, 1980) by rabbits was experimentally proven to be significantly influenced by food quality (Chapter 4). The small statue of the rabbit forced it to feed on high quality forage (DEMMENT & VAN SOEST, 1985; WILMSHURST *et al.*, 2000; OLFF *et al.*, 2002). In our trial, we were really able to show a causal relationship between food quality and diet selection, because we controlled for sward height or vegetation biomass, thereby avoiding the problem of interactions between food quality and food availability (e.g. BALL *et al.*, 2000). We were not able to test the effect of food availability itself, so a trade-off between food quality and food availability might still be possible.

The hypothesis is not confirmed though, when considering fourth order food selection (selection of particular food items within one habitat patch - JOHNSON, 1980). Rabbits do select between different plant species and they do not just eat according to forage availability, but this selectivity could not be related to plant quality (Chapter 6). In conclusion, whether rabbits are selective for high quality forage depends on the spatial scale of the study. Other factors might be involved too, e.g. general quality of the phytomass (nutrient poor versus nutrient rich environments), and the accompanying grazer species.

Hypothesis 5: Rabbits prefer the short swards created by large herbivores because of the higher nutritional value of these swards

This hypothesis cannot be affirmed by our results. In Puyenbroeck, wild rabbits preferred short swards for grazing, although no significant forage quality differences between short and high swards have been observed (Chapter 3). Therefore, we can conclude that vegetation height really was the factor that encouraged the rabbits to graze in the mown strips. When short-grazed vegetation is of higher quality than ungrazed vegetation, this might be an additional trigger for the rabbits to prefer these short swards. But the results of Puyenbroeck suggest that there should be additional other reasons that cause the preference of the rabbit for short swards.

An alternative explanation could be that the large grazers make more grass accessible to the rabbits, by reducing grass height and removing stems (VAN DE KOPPEL *et al.*, 1996; ARSENAULT & OWEN-SMITH, 2002). For example, grass stems could be considered as foraging deterrents (DRESCHER *et al.*, 2006). This alternative explanation has not deserved much attention by researchers studying wild rabbits. We suggest that this path should at least be explored. Other authors address the preference of rabbits for short swards to predation-related issues (IASON *et al.*, 2002; BAKKER *et al.*, 2009): less time is needed for vigilance in short swards (so that more time can be spent to foraging). We might hypothesize that central place foragers as rabbits need low vegetation for facilitating their flee towards the safe burrow, when predators are observed (BAKKER *et al.*, 2009). However, this hypothesis has not really been tested (BAKKER *et al.*, 2009) and the experiments of BAKKER *et al.* (2005) and DEKKER *et al.* (2007) show that rabbits, although sensitive to perceived predation risk, do not alter the average spatial distribution of their grazing pressure as a consequence of an increased perceived predation risk. Rather, the rabbits shifted the time of foraging or did increase total foraging time (spending more time on vigilance).

In conclusion, we found that rabbits are able to discriminate between forage of different food quality, that they do select for the most nutritious forage when this is the only differentiating factor and also that they possibly prefer a lower vegetation height for foraging. However, the reason why rabbits prefer short swards is not necessarily related to the expected high quality of these swards: selection for short swards may also occur when no differences in food quality are observed.

Hypothesis 6: Also rabbits might be able to create short and high qualitative swards, thereby facilitating themselves

Rabbits do have an important influence on vegetation, but nevertheless, they are not able to maintain a favourable vegetation structure themselves in IJzermonding and Ghyvelde (Chapter 2). When herbivores are able to maintain favourable grazing swards on their own, without the help of large herbivores, self facilitation (ARSENAULT & OWEN-SMITH, 2002) would be occurring rather than interspecific feeding facilitation. The results of this thesis however show that rabbits at the present density were not able to facilitate themselves sufficiently and therefore, we suppose that self facilitation will only play a secondary role in these coastal dune ecosystems.

Hypothesis 7: Large herbivores can modify vegetation composition. This has an influence on rabbits as they prefer certain plants species above others when foraging.

An alternative form of feeding facilitation (long term facilitation - KUIJPER *et al.*, 2008) might be present in our study areas: in the long term, large herbivores may not only alter vegetation structure (and eventually forage quality) but also alter plant species composition of the vegetation, which has been encountered in several of the Flemish dune reserves where grazing was introduced in the nineties (PROVOOST, 2005). This could lead to a long term form of feeding facilitation: medium-sized grazers would be facilitated by large grazers because other plant species emerge, which was shown in a herbivore assemblage of cattle and hares (KUIJPER *et al.*, 2008). Possibly, this type of feeding facilitation could also occur in assemblages of rabbits and large herbivores. Our study showed that the rabbits in the acid dune grassland system are selective grazers: they do not just eat what is available, but they do select for specific plant species, depending on the season (Chapter 6). Also, our results show that large herbivores influence plant species composition of dune grasslands (Chapter 2). This was shown more explicitly in IJzermonding than in Ghyvelde, but we should realize that this project lasted for only six years, while the long term facilitation in the study of KUIJPER *et al.* (2008) was observed in a 30-year period (see also PROVOOST (2005) for Westhoek and Houtsaeger Dunes). At least, in the coastal dune grasslands of our study, there is a potential for such long term feeding facilitation.

The main hypothesis: Feeding facilitation by creation and preference of short, dense, high quality vegetation?

The main hypothesis of this thesis supposes that interspecific feeding facilitation would occur when grazing by large herbivore species reduces vegetation height and stimulates grass re-growth, thereby enhancing the nutritional quality of forage for another species (ARSENAULT & OWEN-SMITH, 2002; VAN LANGEVELDE *et al.*, 2008), while rabbits would preferably forage in these created grazing lawns. The results of our research show that parts of this hypothesis can be affirmed (creation of short swards by large grazers, preference of rabbits for short swards, preference of rabbits for high quality forage and swards), while other parts (creation of dense high quality swards, preference for short swards because of quality considerations) could not be concluded. Also long term feeding facilitation by altering vegetation composition (KUIJPER *et al.*, 2008) could not be affirmed completely, although there is a potential that this type of facilitation could occur in the future. So, although mentioned anecdotically by e.g. WILLIAMS *et al.* (1974), WALLAGE-DREES (1982), OOSTERVELD (1983), DREES (1989) and DREES (1998), feeding facilitation between rabbits and large grazers is probably not present in the herbivore assemblage present in our study areas or could at least not be detected and our global main hypothesis about this type of feeding facilitation is not confirmed.

We could explain this by supposing that this type of feeding facilitation does not exist. However, feeding facilitation could be necessary for rabbit populations as they are not able to facilitate themselves and some mechanisms causing facilitation could effectively be detected, indicating that there are at least strong indications that the mechanism of feeding facilitation does exist in this grazer assemblage. We should therefore consider the option that the existence of feeding facilitation is possible, but that it could not be present in our study system, perhaps due to masking effects of interfering environmental factors, different grazer densities (large herbivores as well as rabbits), relatively low productivity of the dune grasslands under consideration, etc.

There are several reasons why feeding facilitation might not be detected. As feeding facilitation in herbivore assemblages is an indirect interaction, causal relationships are hard to prove and we could say that feeding facilitation is a hidden interaction. Also, we should be aware of the contrast between experimental results and field observations. In this study, the preference of rabbits for high quality swards and for short swards has been shown under simple, semi-controlled or entirely controlled experimental conditions (Chapter 3, 4), while field conditions (Chapter 3, second part) did not yield similar results. Also from this point of view, feeding facilitation could be considered as a hidden interaction. Also, the conditions for feeding facilitation might not be fulfilled in our study system. Under complex field conditions, many factors may be mixed up with the mechanisms of feeding facilitation, so that the interaction might not be possible.

First, the grazing behaviour of rabbits may be a trade-off of food quality considerations (Chapter 3), food availability, anti-predator considerations (IASON *et al.*, 2002; BAKKER *et al.*, 2009), social factors (LOCKLEY, 1976), food accessibility (VAN DE KOPPEL *et al.*, 1996) and distance from the burrow (DEKKER, 2007). Although rabbits might be selective for short swards, this could be masked on the moment that, for example, a high predation risk is perceived by the animals. Researchers should be aware that many factors influencing diet selection of rabbits could be operating at the same time under field conditions, which makes it impossible to understand causal mechanisms. Several types of facilitation (Chapter 1) could be operating at the same time, which makes it harder to detect them when not excluding confounding factors experimentally.

Second, the balance of facilitation and competition may depend on plant productivity (KUIJPER *et al.*, 2004; CHENG & RITCHIE, 2006; DEKKER & VAN LANGEVELDE, 2007). We showed already differences in the foraging behaviour of rabbits in Ghyvelde and Puyenbroeck, two areas which are different in plant productivity (Chapter 3). Also, the impact of herbivores on vegetation parameters was different for Ghyvelde and IJzermonding (Chapter 2), suggesting that also the creation of grazing lawns (MCNAUGHTON, 1984) might be different between both areas differing in both abiotic conditions and herbivore species, density and grazing regime. We could not detect the creation of grazing lawns in our study areas but this does not mean that the mechanism of grazing lawns does not exist: it might be occurring in other types of study areas. Therefore, confronting experimental data with field observations is indispensable: it may reveal confounding factors and finally allow researchers to model the feeding facilitation mechanisms along a gradient of productivity.

Third, as well the density of large herbivores as the density of rabbits might influence whether feeding facilitation occurs or not. The hypothesis that facilitation is stronger at lower rabbit densities (BAKKER *et al.*, 2009) could be confirmed by comparing the data of Ghyvelde with those of BAKKER (2003). Facilitation is occurring in the study area of the latter, with a lower rabbit density than Ghyvelde, where facilitation is not occurring (Chapter 3). However, this hypothesis is contrasting to the finding that there is facilitation in Puyenbroeck , an area with a much higher rabbit density.

Fourth, it is possible that facilitation is a seasonal phenomenon: there might be a temporal trade-off of facilitation and competition between grazers, which could explain why population effects of facilitation are hardly ever observed (ARSENAULT & OWEN-SMITH, 2002; DEKKER, 2007). The seasonal pattern of the rabbit diet selection (Chapter 6) is supporting the possibility of seasonal facilitation, although not yet present.

In conclusion, feeding facilitation is often a masked or even hidden interaction in herbivore assemblages, because of its indirect nature and because of the many confounding factors that are arising under field conditions and that might prevent facilitation. Therefore, experimental research under simplified conditions is indispensable to unravel causal mechanisms and the conditions in which facilitation can occur, while additional field research is still needed to link experimental data to field reality.

General conclusion

By combining experimental research with field data, we were able to discover some causal relationships (food quality as well as vegetation height influence foraging decisions of rabbits), meanwhile proving that more factors than these are involved in facilitative grazing interactions, so that the main hypothesis could not be unambiguously affirmed. From the combination of all our results, further questions are originating:

i) Is plant accessibility playing a role in rabbit diet selection?
ii) What is the role of predators in rabbit diet selection?
iii) Are short, dense, high quality swards ever realised in coastal dune areas?
iv) What is the role of habitat productivity?
v) What is the role of seasonality?

Experimental research on these topics is needed to gain further insight in these mechanisms, while combining the results with field data will still be indispensable for understanding the relationships and trade-offs between the different mechanisms.

Suggestions for further research

The field results from this study are originating from two coastal dune areas with a relatively low productivity. As productivity might influence the outcome of interactions in herbivores assemblages (CHENG & RITCHIE, 2006; DEKKER & VAN LANGEVELDE, 2007), similar research in other, more productive study areas would be very informative. Topics that should be investigated in these areas are the formation and nature of grazing lawns under different biotic and abiotic conditions, the ability of rabbits to facilitate themselves, the role of the often strongly fluctuating rabbit population sizes in time, the selection of foraging patches by rabbits and the seasonal aspect of these topics. When this kind of research could be executed in a standardized way along a gradient of productivity, predictive models about the effect of productivity on grazer interactions might become possible.

Further experimental research is necessary to understand causal relationships that are operating in the herbivore assemblage of the wild rabbit and large grazers. Especially, more attention should be given to the ability of rabbits to cope with a less or more complex vegetation structure, and their reaction to different degrees of perceived vegetation risk. These topics should be tested separately and under circumstances where there is no variation in food quality and sward height. Afterwards, incorporation of distance from the central place (burrow) in these kinds of experiments would add extra value.

Finally, combining these field data collected along a productivity gradient with experimental data unravelling causal relationships could lead to a coherent model about feeding facilitation and other types of facilitation in this grazer assemblage. Knowledge about the true nature of the interactions between rabbits and large herbivores could then be an interesting starting point for a global theory about feeding facilitation in herbivore assemblages in temperate grassland areas.

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Summary

This thesis focuses on the positive interaction 'feeding facilitation' which is predicted to occur in assemblages of large and small(er) herbivore species. The main hypothesis of the research is that introduced large herbivores facilitate rabbits (medium-sized herbivores) by modification of the vegetation. This modification involves creating short swards, creating denser (more productive) swards, creating swards that have a high food quality for rabbits and influencing vegetation composition. We expect that these modifications are not completely independent from each other: shorter swards are expected to have a higher food quality and to be more productive. Although several hypotheses about the causes and mechanisms of feeding facilitation can be mentioned, it is generally supposed that the high quality of swards previously grazed by large herbivores ('grazing lawns') attract medium-sized herbivores. These medium-sized herbivores would prefer the grazing lawns because of their need for high quality forage. This main hypothesis was tested during this research, with as model system: the wild rabbit (*Oryctolagus cuniculus* L.) and large grazers introduced in coastal dune grasslands. By conducting field observation, semi-controlled field experiments up to entirely controlled feeding experiments, we tried to test several aspects of the main hypothesis.

In Chapter 2, we focus on modification of vegetation structure and composition by grazing and digging effects of rabbits, when in combination with large herbivores. An exclosure experiment was carried out to measure the effect of rabbits and the combination of large herbivores and rabbits on vegetation structure and composition in two different coastal dune areas. In all cases, the combination of rabbits and large grazers as well as rabbits without large grazers were able to reduce vegetation height and litter cover. Additionally, rabbits created bare soil patches, giving extra structural diversity. Through these structural impacts, grazer activity maintained plant species diversity in only one of the study areas. The effect of rabbits on vegetation was smaller than the combined effect of rabbits and large grazers.

In Chapter 3, we tested whether the wild rabbit is foraging according to a unimodal functional response curve, which means that they would prefer a short to intermediate vegetation height for foraging rather than a high vegetation where food availability is higher. By performing two different studies in two study areas, we tested whether rabbits indeed prefer to graze in shorter vegetation, by comparing rabbit activity in short (grazed or mown) and ungrazed vegetation. Mown vegetation in the most productive grassland site proved to be preferred by the animals, as indicated by an experimental set-up. There were no indications that this was due to a difference in forage quality. In a more complex, natural situation, rabbits were not facilitated by large grazers: they did not prefer to graze in the shorter vegetation. We suggest that experimental studies might reveal phenomena that are masked under more complex field conditions, for instance due to variation in productivity of the grassland or the occurrence of self-facilitation.

In Chapter 4, it was tested whether rabbits are able to discriminate between plants of different nutritional value and whether they prefer the most nutritious. A feeding trial in which rabbits were offered two different types of grasses (fertilised and unfertilised) was executed under experimental conditions. The rabbits preferred the grasses with the highest protein percentage, when conditions were controlled for sward height/plant biomass. This observation is equivalent to results obtained in geese and provides experimental evidence about the capability of rabbits to select for plants with the highest nutritional quality.

Chapter 5 is a methodological study on microhistological faecal analysis. Microhistological faecal analysis is a widely used method in which the diet of the herbivore is qualified and quantified by identifying plant epidermal fragments which can be found in the pellets of this animal. A clear consensus on the accuracy of this technique is lacking. So we performed a feeding trial with rabbits to compare the known diet with the results obtained by faecal analysis. The main goal of the study was to find out whether the diet composition can be reliably derived from faecal analysis (despite problems of differential digestion). It is concluded that only the composition of grass diets can be estimated from pellet analyses, due to a higher digestion intensity of forbs. We recommend the identification of 150 epidermal fragments and quantification by area measurements.

In Chapter 6, we studied diet selection of rabbits at the plant species level: little is known about plant species selection in the field, the seasonal pattern of this selection, and the mechanisms of this possible selection. Therefore, we studied the diet composition of wild rabbits in an acid dune grassland, and compared it to the availability of food throughout the different seasons of the year. Rabbits proved to select indeed their food items: they do not just eat according to forage availability, and seasonality does influence the results. Plant quality did not prove to be the main trigger to select for certain species in field conditions. Although it has been proven that rabbits are able to select for the better quality of forage at the monospecific plant patch level, this study indicates that other triggers are at least equally important in determining the diet selection at the plant species level.

Finally, Chapter 7 synthesizes the results of the previous chapters. The results are commented within the framework of feeding facilitation. Vegetation structure was shown to be influenced by large herbivores: grazing results in short swards. These swards were not denser (which would be expected if overcompensation would occur). It was experimentally proven that wild rabbits prefer those short swards, although field observations showed that this is not the case in all study areas. The short swards created by large grazers did not have a higher nutritional quality than ungrazed swards. Rabbits are sensitive to food quality and prefer patches with high quality forage. Nevertheless, this was not related to sward height: the preference of rabbits for short swards is not necessarily related to forage quality issues. Also, we showed that rabbits in our study areas are not able to facilitate themselves. Long term feeding facilitation (by altering plant species composition of the vegetation) was not present yet, but could be possible in the long term. From all these results, we conclude that the main hypothesis could not be affirmed, although some causal mechanisms of feeding facilitation have been affirmed. We suggest that feeding facilitation is not necessarily absent, but is hard to detect or is not present under particular conditions. Experimental research is needed for further unravelling causal mechanisms about feeding facilitation and alternative approaches, while field observations remain necessary to gain insight into other variables (e.g. habitat productivity, predators, food accessibility, seasonality) that may shift the balance between the occurrence and absence of feeding facilitation.

Samenvatting

Het centrale thema van dit proefschrift is 'voedselfacilitatie', een positieve interactie die kan optreden in gemeenschappen van grote en kleinere herbivoren. Onze hoofdhypothese stelt dat grote grazers de vegetatie wijzigen op een zodanige manier dat middelgrote herbivoren erdoor aangetrokken worden. Mogelijke wijzigingen aan de vegetatie zijn: het korter maken van de vegetatiehoogte, het kwalitatief (op vlak van nutriënten) beter maken van de vegetatie, de densiteit van de vegetatie verhogen en de soortensamenstelling van de vegetatie beïnvloeden. We verwachten dat de verschillende wijzigingen aan de vegetatie niet onafhankelijk zijn van elkaar: kortere graszoden zouden een hogere voedingswaarde en een hogere productiviteit hebben. De middelgrote herbivoren zouden de korte graszoden verkiezen omdat ze een grote behoefte hebben aan hoogkwalitatief voedsel. De hoofdhypothese werd getest tijdens ons onderzoek in een modelsysteem van konijnen (*Oryctolagus cuniculus* L.) en grote grazers die geïntroduceerd werden in enkele kustduingraslanden. We combineerden veldobservaties, halfgecontroleerde veldexperimenten en volledig gecontroleerd experimenteel onderzoek om de verschillende aspecten van onze hoofdhypothese te testen.

In Hoofdstuk 2 onderzochten we de effecten van konijnen en de combinatie van konijnen en grote grazers op vegetatiestructuur en vegetatiesamenstelling in twee sterk verschillende duingraslanden. Zowel konijnen alleen als de combinatie van konijnen en grote grazers reduceerden de hoogte van de vegetatie en verhoogden strooiselbedekking. Bovendien zorgden de konijnen door hun graafactiviteit voor naakte bodem. Dit gaf extra structurele diversiteit. Ten gevolge van hun structurele effecten waren de herbivoren indirect in staat om de plantendiversiteit te behouden, hoewel dit slechts in één van de twee gebieden het geval was. Het gecombineerde effect van grote grazers en konijnen bleek groter te zijn dan het effect van konijnen alleen.

In Hoofdstuk 3 gingen we na of het graasgedrag van konijnen beantwoordt aan een unimodale functionele responscurve. Dit betekent dat konijnen een korte tot middelmatige vegetatiehoogte zouden verkiezen bij het foerageren, in plaats van een hoge vegetatie waarin de totale voedselbeschikbaarheid hoger is. We voerden twee onderzoeken uit waarin het gebruik van korte (begraasde of gemaaide) vegetatie door konijnen werd vergeleken met het gebruik van onbegraasde vegetatie. De experimentele opzet in het meest productieve studiegebied wees op een voorkeur voor gemaaide vegetatie. Er waren geen redenen om aan te nemen dat voedselkwaliteit hierbij een rol speelde. In een meer complexe veldsituatie bleken konijnen niet bij voorkeur te grazen in een door grote herbivoren begraasde vegetatie. Fenomenen die duidelijk zijn onder experimentele omstandigheden worden mogelijks verdoezeld in meer complexe veldsituaties, waarin bijvoorbeeld ook productiviteit of zelffacilitatie de situatie kunnen beïnvloeden.

In Hoofdstuk 4 hebben we getest of konijnen onderscheid maken tussen planten van verschillende kwaliteit en of ze bij het foerageren de meest voedzame planten verkiezen. Dit werd getest in een zuiver experimentele opzet waarbij konijnen bemeste en onbemeste grassen (eenzelfde soort) aangeboden kregen. De konijnen hadden een voorkeur voor de meest voedzame planten, met het hoogste eiwitgehalte, in omstandigheden waarbij zodehoogte of voedselbiomassa geen rol konden spelen.

Hoofdstuk 5 is een methodologische studie over het gebruik van fecesanalyse voor het bepalen van de dieetsamenstelling van wilde herbivoren. Epidermisfragmenten, afkomstig van geconsumeerde planten, die aangetroffen worden in feces worden geïdentificeerd en gekwantificeerd. Er bestaan een aantal onzekerheden over de juistheid van deze methode. Daarom voerden we een voederexperiment uit, waarbij het gevoederde (en dus gekende) dieet van een aantal konijnen vergeleken werd met de resultaten van de analyse van hun keutels. We zochten uit of er een goede overeenkomst was. Dit bleek enkel het geval te zijn voor een dieet dat enkel uit grassen bestaat (ten gevolge van het feit dat grassen minder goed verteren dan kruiden). Het is aan te raden om een 150-tal fragmenten te bekijken per keutelstaal en kwantificatie door oppervlakteschattingen van epidermisfragmenten geeft een beter resultaat dan tellingen van fragmenten.

In Hoofdstuk 6 werd voedselkeuze door wilde konijnen bekeken op het niveau van plantensoorten. De kennis over hun preferenties voor plantensoorten in veldsituaties is immers beperkt, evenals de kennis over seizoenale patronen van en redenen voor deze selectie. In een zuur duingrasland vergeleken we de dieetsamenstelling van het konijn met de voedselbeschikbaarheid in dit gebied gedurende de vier seizoenen van het jaar. Konijnen bleken inderdaad selectief te zijn in hun voedselkeuze: ze eten niet zomaar wat beschikbaar is. Seizoenaliteit heeft een invloed op de resultaten, maar plantenkwaliteit bleek niet relevant in dit opzicht. Uit dit onderzoek blijkt dat selectie van voedselplanten niet volgens dezelfde criteria gebeurt als voedselkeuze op andere niveaus.

Het laatste hoofdstuk, Hoofdstuk 7, synthetiseert de resultaten van de voorgaande hoofdstukken en kadert ze binnen de theorie over voedselfacilitatie. We toonden dat vegetatiestructuur beïnvloed wordt door grote grazers (verminderen van vegetatiehoogte). Er trad echter geen overcompensatie via extra groei op in deze zodes. Konijnen bleken een voorkeur te hebben voor dergelijke korte graszoden in experimentele omstandigheden, maar veldobservaties bevestigden dit beeld niet. De voedselkwaliteit van begraasde vegetatie bleek in veldomstandigheden niet beter te zijn dan die van onbegraasde vegetatie. Konijnen bleken wel gevoelig te zijn voor zoden met een hogere voedselkwaliteit, maar desondanks bleek deze eigenschap niet de reden te zijn waarom korte vegetatie verkozen wordt. Ook bleek dat de invloed van konijnen op vegetatie te beperkt is om zichzelf te faciliteren. Tenslotte was facilitatie op lange termijn (bij veranderende soortensamenstelling van de vegetatie) niet aanwezig, maar er is wel een mogelijkheid dat dit op langere termijn wel zal optreden. Wanneer we al deze resultaten gezamenlijk overschouwen, blijkt dat we onze hoofdhypothese niet konden bevestigen. Wél konden we een aantal onderliggende oorzakelijke mechanismen van voedselfacilitatie bevestigen. Het is dus niet noodzakelijk zo dat voedselfacilitatie niet bestaat: misschien is het gewoon moeilijk te detecteren of is het niet aanwezig in de omstandigheden waaronder wij gewerkt hebben. Verder experimenteel onderzoek kan nog meer informatie opleveren over de mechanismen van voedselfacilitiatie en alternatieve verklaringen voor facilitatie. Daarnaast blijven veldstudies echter noodzakelijk om inzicht te krijgen in andere variabelen (bijvoorbeeld productiviteit van het habitat, predatoren, voedseltoegankelijkheid, seizoenaliteit) die uiteindelijk zullen bepalen of voedselfacilitatie al of niet aanwezig is of kan zijn in bepaalde grazergemeenschappen.

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