The role of large herbivores in woodland regeneration patterns, mechanisms and processes

De rol van grote herbivoren in bosontwikkelingspatronen, -mechanismen en -processen

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inbo

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by / door

Jan Van Uytvanck

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Jan Van Uytvanck, 29 september 2009

Chapter 1. General introduction, aims and thesis outline

1.1 Introduction

Worldwide, present-day nature conservation is confronted with rapid land use change in vast areas of natural and semi-natural ecosystems. In densely populated areas, as is the case in most so-called developed countries, the pressure on the remaining open space and biodiversity is high (OECD 1998). However, there is a remarkable duality in the causes of this pressure. On the one hand, there is a tendency to an intensification of land use driven by an increase in scale of agriculture, industry and urbanisation. On the other hand worldwide, environmental and socio-economic changes are leading to increased levels of land abandonment (Benjamin *et al.* 2005). Understanding the development of plant and animal communities on former agricultural land will become increasingly important from a conservation, restoration and social perspective and will pose important scientific and policy challenges (Cramer *et al.* 2008). In Europe for instance, the Natura 2000 network provides ± 850 000 km² protected habitat (i.e. ± 20% of the European Union territory, European Commission 2009), often including traditional open agricultural landscapes that require appropriate and active conservation management. In contrast to these needs, large areas of former agricultural land are subject to afforestation, both spontaneously (2.6 million ha year^{-1}) and through planting (0.7 million ha year^{-1}) (FAO 2001). Decreasing biodiversity as a consequence of afforestation of traditionally open and half-open landscape types is found throughout Europe. Traditional low-intensity farming practices are widely recognized as a key-factor allowing high local biodiversity and their abandonment has generally been shown to have a detrimental effect on species richness by changing the competitive relationships between species and habitat conditions (Grubb, 1977; Huston, 1979; Bignal & Mc Cracken, 1996; Provoost *et al.* 2004). The rich European flora and fauna of low intensively used, open habitats depends mainly on semi-natural pastures (Bokdam 2003). At the same time, there is a strong and growing interest among conservation practitioners, scientists and policy makers in the restoration of native woodlands and the development of new wilderness on former agricultural land and in intensively managed forests. In the context of both the conservation of open landscape types and the development of new woodlands or wildernesses, management cannot pass over the important role that large herbivores, and their interactions with man, played and still play in a wide range of ecosystems (see Box 1.1).

Box 1.1 Large herbivores, man and landscape in a (pre-)historic timeframe

Many native large grazers are worldwide endangered and their occurrence is more and more limited to nature reserves, where their existence continues to be threatened by expanding human exploitation (Campbell & Borner; 1995; Prins & Olff 1998). E.g., in Europe, the aurox (*Bos taurus*) became extinct in 1627 (van Vuure 2003) and the wisent (*Bison bonasus*) remains in a small number of re-introduced and isolated populations in (semi-) natural conditions (Kivit *et al.* 2006). In a broader time scale, it is important to realise that at the end of the last ice age (Weichsel, 10 000 years BP) many large herbivores became extinct, although they are to be expected in interglacial periods such as the present Holocene (Bunzel-Drüke 2000). Among them were megaherbivores such as straight-tusked elephant (*Elephas antiquus)* and two rhinoceros species (Merck’s rhinoceros, *Dicerorhinus kirchbergensis* and steppe rhinoceros, *Dicerorhinus hemitoechus*), which were able to strongly affect vegetation and landscape structure due to their body size, physical strength and enormous food intake (Owen-Smith 1988). Also remarkable is the extinction of almost all grass-eaters (grazers). Among them are the above mentioned auroch and steppe rhinoceros, but also wild horse (*Equus ferus*) and European wild ass (*Equus hydruntius*) (Bunzel-Drüke *et al.* 1994 & 2002). With the decline or extinction of these grazers, one might also expect a decline of the ecological effects they exerted on the ecosystem. In this context, the recovery of the European landscape with closed forests after the last ice age may be a consequence of the absence or rarity of large herbivores, rather than their supposed minor role on landscape/forest development (Bunzel-Drüke 2000). The influence of modern man (*Homo sapiens*) on the functioning of the European Weichsel and Holocene landscape is probably crucial and ambiguous at the same time. On the one hand, man could be responsible for the extinction of many large herbivores (overkill hypothesis) that formerly shaped the landscape. On the other hand, the predominant influence of man on the historical landscape is exactly due to the widespread use of the descendants of native large grazers. Indeed, native large grazers have mostly been replaced by their domestic descendants (mainly cattle, or other livestock such as goats, sheep and ponies) (Prins 1992; Voeten 1999). Therefore, from a vegetation perspective, large areas continued to be grazed although the spatiotemporal dynamics and intensity of grazing pressure are now regulated by man (Bakker 2003). In this way, a large amount of native plant species related to open, grazed ecosystems may have persisted for many centuries in the European landscape, though nearly all native large grazers became extinct.

Since approx. the nineteen seventies, livestock is increasingly being introduced in many nature reserves in W-Europe to maintain or restore grassland biodiversity (Bakker & Londo 1998; WallisDeVries *et al.* 1998; Vera 2000). Regarding the scale at which grazers are used as a tool in nature conservation, it is important to understand the impact that herbivores may have on a parcel, a site, a landscape or a region. Over the last decades, evidence has accumulated that herbivores can exert a strong influence on vegetation development. Initially, large and medium sized herbivores were involved in traditional conservation management which refers to former agricultural use such as cattle or sheep-herding in heathlands or on embankments, summer-grazing in small parcelled grasslands or grazing the aftermath of meadows. These practices had rather predictable and often very good results due to the existence of a reference framework (a series of grassland and heathland types related tot former agricultural use, Bakker 1989) and the relatively small areas under management, making it easier to intervene and fine tune management measures. Nowadays, in a context of increasing land abandonment, but also an increased interest in restoration ecology, the challenge will be to manage larger areas in an ecologically and economically sustainable way. The principal idea is that large herbivores, whether wild or livestock species, can play a key role in restoring the functioning of ecological processes such as (cyclic) succession. Large herbivores have the ability to (temporarily) prevent, retard or reset vegetation succession, but they are also able to trigger or speed up succession. As a consequence, large herbivore activities also affect changes in community structure. However, it is still far from clear whether grazing management with large herbivores will be appropriate to come up to conservation managers' expectations (e.g. restoration of native plant and animal communities) in this new context.

In this thesis, we focus on woodland regeneration in relation to grazing management on former agricultural land. We will study the patterns that develop on former intensively used grassland (with a closed grass and herb vegetation), and former arable land (with bare soils), representing the two major land use forms in agricultural landscapes. We will investigate differences in the mechanisms and processes that play a role in woodland regeneration on both grassland and arable land. Therefore, the term "woodland regeneration", as stated in the title of this thesis, corresponds with the development of woody vegetations (from open types such as woodpastures to closed forests) outside existing forests, i.e. on former agricultural land.

The topic of woodland regeneration on non-forested land is an actual theme in conservation biology in Europe. In the Netherlands, pioneer research work has been conducted on both the elaboration of ecological theory and the practical issues of grazing management (WallisDeVries 1994; WallisDeVries *et al.* 1998; Olff *et al.* 1999; Vera 2000). In the British uplands, there is interest in the concept of 're-wilding' and large scale native woodland restoration in conjunction with extensification of agriculture (Worrell *et al.* 2002; Kirby 2003; Hodder *et al.* 2005). In Flanders (N-Belgium) low intensity grazing by semi-domesticated animals is currently explored as an alternative for active afforestation or spontaneous regeneration and the development of wooded pastures.

Central issues in the debate on woodland restoration are the ability of large herbivores to maintain (half-)open landscapes and their role in natural regeneration of woodland (Kirby *et al.* 1994; Vera 2000, Birks 2005; Mitchell 2005). Opinions on these topics remain contradictory and therefore, the translation to conservation management measures and policy is difficult and ambiguous. It thus appears necessary to conduct sound field research and experiments in order to gain insight into the patterns of woody species regeneration, the mechanisms at work and the natural processes that influence woodland regeneration on former agricultural land that is grazed by large herbivores. In the following chapters, these issues form the focus of attention.

1.2. Large herbivores and conservation management strategies

Given the nature of Europe's native large mammal populations, the choice for conservation strategies that protect or involve wild large herbivores seems to be obvious. The conservation of the pristine, evolutionary habitats of large herbivores should also offer the best conditions to conserve populations of wild plants and animals related to these habitats (Wilson 1999). WallisDeVries (1998) gives an overview of grazing systems as a function of land use practices. With growing intensity of land use, wild herbivores are replaced by domestic livestock. Nature conservation interests decrease with increasing human interference but are not restricted to natural grazing systems; also semi-natural landscapes and some cultural landscapes may be involved. Due to the vast loss of areas with natural habitats and most native large herbivore species in Europe, there are few possibilities to apply this "protection strategy" with absent or limited human interference. Therefore, suitable and feasible alternative strategies need to be found.

1.2.1 Grazing in tradional semi-natural landscapes

The first experiences with large herbivore grazing management refer to traditional pastoralism, which has only survived in economically marginal areas (Bignal *et al.* 1994). Site managers especially tended to use large herbivores on sites that were traditionally grazed by livestock, and that still bore evidence of this (Siebel & Piek 2002). As a consequence, a lot of experience is available on grazing in semi-natural landscapes such as heaths, dunes, calcareous grasslands (*Mesobromion*), *Molinietalia* and *Arrhenatherion* grasslands (Bakker 1989; Bakker & Londo 1998). These landscapes comprise "replacement communities", i.e. semi-natural communities derived from natural (mainly forest) communities due to felling and intensified grazing by domestic stock (Prins 1998). From a conservation point of view, the maintenance of these communities does not depend on grazing management alone (herding, low intensity seasonal grazing) and may be accompanied or replaced by mechanical measures (burning, hay making, sod cutting, tree cutting, litter removal…). While this strategy, conserving semi-natural landscapes and communities, is often discussed and criticized because of the lack of natural processes and the lack of wild herbivores, we think it is well worth keeping it in well defined areas. At present, these landscapes function as most important sources for wild plants and animals (Zobel *et al.* 1998). Second, leaving one strategy implies the application of another one that offers good chances for success.

1.2.2. Wilderness grazing

Such an alternative strategy could be naturalistic grazing, also called wilderness grazing (Baerselman & Vera 1989). This involves the re-introduction of native animals, such as beavers, moose, red deer, wisent and Heck cattle (to replace the extinct auroch) in large areas. The strategy was proposed in the early eighties in the Netherlands (van de Veen & van Wieren 1980; Vera 1986). The major challenges concerningthis strategy is to find out how the natural, wild landscape (e.g. in northwestern Europe) could look like if and when nature restoration takes place in the present agricultural and semi-natural landscapes. In other words, there is not yet a clear idea of spatial or temporal scales. Therefore, the choice for a certain conservation strategy is not only a question of how to define "nature", it is far more a question of (lacking) knowledge.

Although at first sight, naturalistic or wilderness grazing is a romantic approach without a formal definition, the concept offers some useful holds for conservation practice. The most important issue is the role of natural processes: there is no man-specified grazing density, herbivore population dynamics are driven by resource limitation. Rather than managing towards targets for habitat and species composition, direct management intervention is reduced to a minimum and the natural process is the aim (Hodder *et al.* 2005). Second, the wilderness approach to grazing has the potential to change the way that we practise large-scale conservation. Third, the wilderness concept claims that complete herbivore assemblages create open habitats as components of successional mosaics in the woodland domain. Such open habitats are analogous to open patches in the pre-farming woodland and are thought to have the capacity to provide conditions for a broad range of light demanding species, which are of conservation interest (Svenning 2002). Not all ecologists and conservation managers support this idea, and actually, experimental evidence to settle the question of suitability of wilderness grazing in Europe is not available (Bokdam 2003). However, we can use the ideal, but often impossible to achieve naturalistic grazing model as a guide to how we approach the management of real systems, that are limited in space and time and to which certain man-defined goals are assigned. Within the particular constraints, we can look for the option that is closest to the understanding of the natural situation, i.e. the option that involves the least human intervention and that approaches a system with no man-defined spatial and temporal limits.

1.2.3. Controlled low intensity grazing

In practice, problems will arise concerning the unpredictability of outcomes in naturalistic grazing. If the conservation of natural processes becomes the goal, it is then difficult to define targets different from the mere reconstruction of naturalness and to evaluate development of wild areas. The lack of defined goals is incompatible with the current management aims based on targets for habitats and species of conservation concern, which are guided by, for instance, the European Natura 2000 Network (Decleer 2007). It is not realistic to assume that biodiversity changes under naturalistic grazing can be forecast by describing the habitats and species found in the landscape that is assumed to emerge. Further, ecological as well as cultural factors impose limits to how natural a system can be.

E.g. reserve size and completeness (i.e. the range of habitats available), climate change, airborne pollutants, river embankments, will unavoidably affect the system. Therefore, a third conservation strategy (intermediate between mechanical management/traditional pastoralism and naturalistic/wilderness grazing) will often be applied. Controlled, low intensity grazing, using free-ranging livestock or wild herbivores in large areas, combined with a minimal mechanical management will often be the most realistic option. It has the advantages of being less labour-intensive and less expensive compared to mechanical management. Additionally, there may be opportunities for the marketing of animal products (Prins *et al.* 2000). Controlled low intensity grazing allows defining and evaluating conservation targets (e.g. native woodland development, the creation of nutrient poor open habitats or vegetation types) based on existing knowledge en experience. Vice versa, defining and evaluating conservation targets allows control and adjustment. If managers have insights into the grazing capacity of a certain area, controlled grazing might not necessarily exclude natural processes (Ebrahimi 2007). By introducing self-organising free-ranging large herbivores, controlled grazing may also mimic wilderness processes.

In this thesis, we approach the issue of conservation management strategies and landscape openness at the scale of present day, controlled grazing management in fenced, relatively small nature reserves on former agricultural land. The results however may allow to gain insights into mechanisms and processes that are, or have been, active in traditional wooded pastures as well as in lost or new wilderness landscapes. We aimed to contribute to the understanding of the factors that influence temporal and spatial patterns of the grassland-forest matrix in grazed conditions.

1.3 Grazing management in Flanders

In Flanders, purposeful grazing for conservation started in 1972, with a small sheep herd and a shepherd in the Kalmthoutse Heide near Antwerp (De Blust & Slootmaekers 1997). In 1976, there was a first grazing project with fenced animals (sheep) in De Gulke Putten, a small heathland reserve near Bruges (Stieperaere & De Meyer 1978). It was the first project in which grazing management and scientific evaluation were linked (De Meyer 1977) and it stimulated a lot of other conservation managers in the rest of Flanders. In the nineteen eighties low intensity grazing with free-ranging cattle and horses became more and more prevalent in Flemish reserves (Van Vessem & Stieperaere 1989; Eggermont *et al.* 1996). Nowadays, large herbivores are involved in the management of ± 300 nature reserves (±7300 ha). Grazing is often used to conserve well defined target species and vegetation types, situated in small scaled, parcelled cultural landscape types (±3800 ha). The use of free-ranging large herbivores to shape the landscape or develop mosaic vegetations in larger areas (semi-natural or (new) wilderness landscapes) is limited to ± 90 nature reserves and ± 3500 ha (pers. comm. Natuurpunt vzw, Stichting Limburgs Landschap, vzw Durme and Agentschap Natuur en Bos; Josten 2002). In some of these reserves, the restoration of native woodlands and wooded pastures is aimed. It is in such reserves that most of the study sites of this thesis are situated. The study sites are situated on former intensively used agricultural land and results may be of interest for the Western European lowland.

1.4 Conservation references for woodland regeneration on grazed former agricultural land

Structural heterogeneity in the vegetation is crucial for plant and animal diversity (Olff & Ritchie 1998; van Wieren 1998). There is no doubt that the loss of pristine forest habitats in Europe caused a substantial loss in biodiversity. This is particularly the case for animals such as large predators, but also for large numbers of insects related to old growth forests (Warren & Key 1991). On the other hand, actual diversity of a lot of birds, insects, plants, mosses and lichens is related to the presence of open or half open landscapes (e.g. Woodhouse *et al.* 2005). Therefore, insights into processes that affect structural heterogeneity of landscapes at different scales (Dufour *et al.* 2006), with spatial patterns of closed forest up to more open patches not dominated by woody plants, are important. Luoto *et al.* (2003) showed that grazing affects biodiversity at the landscape level. Landscapes where grazing disappeared due to the cessation of traditional farming lost 45% of their rare plant species within a time span of 20-40 years compared to landscapes with a remaining network of grazed patches. In this context, it is important to recognize that management measures aiming at fixed patterns of communities is fundamentally contrary to the intrinsic features of grazed ecosystems. Grazed ecosystems fluctuate *between* different stages, rather than fluctuating *around* one particular stage. Therefore, steering of processes is more appropriate for the preservation and the development of different stages in a grazed ecosystem than management of patterns. The most important processes for management are those that determine vegetation dynamics (Vulink & Van Eerden 1998). In the past 20 years, more attention has been paid to the development of landscapes with a high degree of self-regulation, i.e. with natural processes regulating vegetation dynamics. One of these processes is grazing.

Roughly spoken, grazing and browsing by large herbivores regulates the balance between forest and grassland on former agricultural land (Pépin *et al.* 2006). For management and restoration of woodlands, some reference views may help. For policy makers it is important to incorporate these views in global visions on woodland management and conservation in the future (Buysse *et al.* 2001).

1.4.1 Forest and wilderness

In literature, closed forest is rarely cited as a target habitat in relation to the presence or introduction of wild or domestic large herbivores. This is surprising because most forest ecologists agree that large herbivores in the end do not affect natural forest structure in an essential way (believe in a primarily bottom-up regulation model). Therefore, forest should not be excluded as a target habitat to achieve with low intensity grazing. E.g. the New Forest, often cited as an area under (too) intensive grazing for centuries, still remains for a substantial part "a forest". Peterken (1996) hardly pays attention to the role of large herbivores in his standard book "Natural woodlands: ecology and conservation in Northern temperate regions". He states that the cumulative effect of grazing on forest species composition is significant, but probably not substantial. In Flanders, pioneer work on forest development and grazing is done in Bos t' Ename near Oudenaarde (Tack *et al.* 1993; Van Uytvanck & Tack 2008) and in Altenbroek (Voeren, Van Uytvanck & Dewyspelaere 2008), but until now there's not yet a clear view on how these forests will evolve. In this thesis, early patterns, mechanisms and processes at work are treated.

In contrast to forests, large herbivore grazing becomes an important item in the new wilderness concept (Vera 2000). The term "new wilderness" for humanly redeveloped landscapes may sound like a contradiction. However, this contradiction only arises when one defines wilderness as pristine areas which are completely untouched by humans. However, it is also possible to define wilderness from a more subjective, psychological perspective, i.e. any natural area without discriminative human influences. On this basis, it is possible to refer to humanly redeveloped landscapes as wilderness landscapes (Van den Berg & Koole 2006). The idea of wilderness is about complete ecosystems that function without human interference (Van Wieren & Bakker 1998). Completeness refers to large areas that maintain all major habitats typical for a region. As almost all potential wildernesses are incomplete, reintroduction of species (large herbivores, beavers (*Castor fiber*) and also predators such as wolves (*Canis lupus*) and lynxes (*Lynx lynx*) is an important point of attention. To be able to restore wilderness areas, it is important to have a clear idea which key species should be present. In this context, large herbivores are thought to be key species in shaping plant communities and landscapes. It is assumed that large herbivores in wilderness ecosystems are able to maintain open areas. However, actual knowledge is limited to experiences (rather than well designed experiments or surveys) in a limited number of areas. This complicates sound foundations for the development of a reference framework with a broader applicability. Well known sites are the Oostvaardersplassen and the Veluwezoom in the Netherlands (Piek 1998), but these systems remain incomplete and knowledge on the key role of the present species is still insufficient.

1.4.2 Woodpasture landscapes

Woodpastures are found throughout Europe and represent a vegetation structure, rather than being a particular plant community confined to a certain region. This vegetation structure consists of open woodland with scattered trees or forest patches in a matrix of grassland, tall grasses and shrub (or heather). Large herbivores are part and parcel of the system, that is maintained by grazing. Healthy woodpastures should have a wide range of tree ages within them to ensure they survive in the future (Quelch, 2001). Often used terms, indicating more or less the same habitat, are woodpasture, wooded pasture and pasture woodlands (UK), Waldhude and Hudewälder (Germany), wastine/wastina (Flanders). The difference with the structurally similar new wilderness (although less determined) is that grazing is controlled (domestic animals, stocking rate determined by humans, fencing). Restoration of woodpastures becomes more and more prevalent because these systems are related to high species diversity (Pott 1998, Strandberg *et al.* 2005; Gillet & Gallandat 1996, Smit *et al.* 2005) and high cultural and esthetical values (Rozas 2004).

The origin of woodpastures goes back to the Neolithicum. By burning and logging forest parts and subsequent grazing by domestic cattle, forest vegetation locally disappeared and qualitative changes in the remaining forest took place. As a land use form, woodpasture remained important and practically unchanged from the Neolithicum till the late Middle Ages and the practice was locally continued till the 20th century. At present, woodpasture systems are rare in W-Europe and often restricted to mountainous areas (e.g. Massif Central, Jura, Alps, Central Spain, Vosges). Some well known lowland sites with high reference value are the New Forest in Hampshire (S-England) and the Börkener Paradies (NW-Germany).

Pott (1998) emphasizes that woodpastures were complex landscapes built through interaction of human activities and grazing animals. The management of woodpastures has always been characterised by low intensity grazing by domesticated herbivores, but simultaneously, traditional land use practices such as logging, coppicing, pollarding, hay-making, litter-collection and burning were part of the management (Pott 1998; Bakker and Londo 1998). Also planting of desired tree species was a usual practice. Flower (1980) mentions planting of European beech (*Fagus sylvatica*) in bundles of seven young trees, around which spiny species such as hawthorn (*Crataegus monogyna*), sloe (*Prunus spinosa*), holly (*Ilex aquifolium*) or roses (*Rosa* species) were sown. Rosén & Bakker (2005) point at high species diversity of the ängars and alvars in Öland (S-Sweden), where mowing, low intensity grazing (also of the aftermath), logging, tree thinning, burning branches and leaves and the spreading of the ashes were applied. At present, only grazing is still applied. This might overestimate the role of large herbivores in the present remnant woodpastures and the related biodiversity.

It is clear that the restoration of the historical woodpasture landscape, including all traditional practices will only be possible in a limited number of areas. The woodpasture landscape s.l. however may be a realistic reference landscape for many restoration projects on former agricultural land. For the establishment of woodpasture systems (s.l.), van Wieren & Bakker (1998) state that large areas and a long time span are necessary for the development of the complex of macrostructures such as grassland, fringe and tall herb communities, scrub and forest.

1.5 Woodland succession and grazing: theoretical and ecological framework

1.5.1 Landscape openness

Grazing systems are often ecologically very complex. There are numerous direct and indirect factors of large herbivore grazing or browsing that affect vegetation development and succession (de Bie *et al.* 1987; Cosyns & Hoffmann 2004). Concerning the role of large herbivores in succession from open habitats to closed forest, there are contradictory opinions. Experiences in low productive areas show that large herbivores in year-round grazing systems cannot prevent the succession of grasslands, heathlands and other unfertilised rangelands to woodland (Archer 1996; Pott 1998; Groot Bruinderink *et al.* 2000). On the other hand, evidence of the New Forest (Tubbs 1986), historical ecology concerning woodland use in N-Belgium (Tack *et al.* 1993) and long term grazing experience in some Flemish nature reserves, reveal the high capacity of large herbivores to suppress tree regeneration and maintain open habitats.

According to Vera (2000), park-like landscapes consisting of a mosaic of grassland, shrub and woody vegetation dominated the landscape since ± 8000 BP, i.e. long before the beginning of cattle breeding in the Atlanticum (ca. 6500-5000 BP), as a result of the interaction between the (now extinct) wild ancestors of cattle and horses (*Bos primigenius, auroch* and *Equus ferus, tarpan*) and the natural vegetation. In a process of cyclic succession, the landscape passes through all stages of vegetation. In this, the role of hoofed animals is crucial. E.g., floodplains in temperate lowlands naturally are subject to frequent floods in wintertime, resulting in fertile soils with a high production. It is likely that the native European large herbivores such as auroch (*Bos primigenius*) and tarpan (*Equis przewalski qmelini*) preferred these nutrient-rich habitats to forage. It is in such landscapes that a cyclic successional mechanism might have developed (Olff *et al.* 1999; Bakker *et al.* 2004). In the Junner-Koeland reserve in the Netherlands, cattle should have gradually replaced the original large herbivore fauna. Olff *et al.* (1999) propose that the resulting mosaic vegetation with floodplain grassland and hardwood floodplain forests may be the last remnants of a natural valley ecosystem that once occurred throughout the European lowland.

The central idea of Vera's assertion is that many shrubs and woody species, such as hazel (*Corylus avellana*) and oaks (*Quercus robur* and *Quercus petraea*) would have regenerated in open vegetation, not under a closed canopy, as is the case now. In the previous widely accepted theory, a closed forest covered the primeval landscape, assuming that large herbivores did not play a crucial role in succession mechanisms (see the above-mentioned believe in a bottom-up regulation of forest succession). Birks (2005) tentatively summarises the discussion: recent pollen studies (Mitchell, 2005) reject Vera's woodpasture theory. However, the use of current ecological knowledge to interpret incidence of species in the past (in particular *Quercus* and *Tilia*) and the lack of data of other taxa (in particular animals) may cause a considerable bias. Bradshaw *et al.* (2003) argues that, especially the lack of knowledge about population sizes of wild herbivores and herd behaviour, prevents a clear view of the primeval North-European landscape. We state that, whatever the role of large herbivores in natural ecosystems might have been, the historical evidence of occurrence and the well known reference views of park-like or woodpasture landscapes, sufficiently justify the need for conservation action, as is the case with closed forests and cultural landscape types with high conservation values such as heathlands.

1.5.2 Successional mechanisms

The key processes to be understood in grazed ecosystems that include woodland are plant strategies in coping with herbivory, herbivore selectivity and light requirements (Olff *et al.*, 1999). Olff *et al.* (1999) presented a descriptive model of long-term cyclic succession in which free ranging herbivores and alternating associational resistance and competition play a crucial role. This model is presented here in short. Core assumption is that unpalatable, thorny or spiny shrub species create safe sites for the establishment of palatable tree species in a palatable grassland matrix (i.e. associational resistance). In a next stage, the light demanding protective shrubs gradually disappear due to competition with overgrowing trees that were initially protected. In a last stage, gaps created by falling adult trees attract herbivores and enable the re-establishment of grassland in nutrient rich conditions. Establishment of woody species is temporarily prohibited through associational palatability (tree seedlings are eaten together with palatable grasses). Spatial asynchronisation of this cyclic mechanism causes shifting mosaics with patches of all structural vegetation types involved. Bokdam (2003) refined these mechanisms for nutrient poor ecosystems: the "resource-mediated successional grazing cycle" theory claims that free-ranging large grazers can act as driving force for successional woodland-grassland cycles. Bokdam (2003) found evidence that on sandy soils, cattle are able to cause nutrient depletion in grassland lawns and induce the invasion of dwarf shrubs (*Calluna vulgaris*) and trees (*Pinus sylvestris* and *Betula* spp.). Wood invasion and abandoning by the cattle induced replenishment of the soil nutrient pool. Further, the results showed that cattle induced grassland lawns in gaps in the heathland and woodland. They suppressed woody and tall herbaceous species in the woodland undergrowth and introduced grass seeds by dung deposition. The results showed the important role of the interaction between herbivory and plant resources in cyclic plant succession.

1.5.2.1 Plant strategies

Plant strategies in coping with herbivory are avoidance and tolerance (Rosenthal & Kotanen 1994). Woody species avoid herbivores by above or below ground growth to get out of reach, or they develop spines, thorns or glands that produce poisonous or unpalatable substances. Tolerance is found in species that regenerate fast after grazing damage, contain nutritional reserves in roots or rhizomes, or show a big physiological flexibility. In general, tolerant species are more palatable than species with defence mechanisms (Anderson & Briske 1995; Boege & Maquis 2005). Differences between avoidance and tolerance mechanisms are not only found between species, but also between different life stages of the same species. Typical avoidance strategies of species are found in grazed communities with both non-resistant and resistant (with defence systems) species. Instead of competition, facilitation is the dominant process (Callaway 1992). Individual plants positively affect individuals of another species by protecting them and reducing herbivory damage (i.e. associational resistance). Tolerance is mainly found in herbaceous species, though some tree species may also be categorized as tolerant (e.g. some *Salix* species). There are strong indications that tolerance and avoidance strategies exclude each other within a particular species (ecological trade-off system, Leimu & Koricheva 2006).

1.5.2.2 Alternating associational resistance and competition

In W-Europe, mainly facilitation of palatable tree species by thorny shrubs or scrub is supported with evidence (but see e.g. Bossuyt *et al.*, 2005). In productive areas where relatively large amounts of herbivores occur, grassland habitat may succeed for a long time because tree seedlings are consumed together with palatable grasses and herbs (associational palatability). Local disturbances (faeces, trampled soil, flooding) may induce the establishment of resistant herb species, which are temporarily avoided by large herbivores. In these patches, spiny shrubs may establish and grow out if herbivores are temporarily absent (e.g. due to migration, population crashes or diseases, Prins & van der Jeugd 1993). Different defence mechanisms may lead to associational resistance, facilitating tree establishment. In floodplains, characteristic vegetation patterns with grassland, tall herbs, scrub and forest may function in a cyclic successional system (Vera 2000; Bakker *et al.* 2004) with hawthorn (*Crataegus monogyna*) and sloe (*Prunus spinosa*) as facilitating species for trees. The same mechanism could be active on dry sandy soils with juniper (*Juniperus communis*) facilitating tree establishment (Kuiters 2002). Field observations revealed that *C. monogyna* and *P. spinosa* occasionally establish in unpalatable (e.g. *Juncus* spp.), spiny (e.g. *Cirsium* spp.) or prickly (e.g. *Urtica* spp.) vegetation patches (Olff *et al.* 1999). Common ash (*Fraxinus excelsior*) also establishes in grazed tall herb (e.g. *Epilobium hirsutum*), soft rush (*Juncus*) and tall sedge (*Carex*) vegetation patches (own observations). In nutrient-rich areas such as former agricultural land, also bramble (*Rubus* ssp.) seem to play an important role as facilitator. *Rubus* spp. combine spines and palatability. They are probably an important reserve food source (e.g. in winter, Garin *et al.* 2000) but in nutrient-rich areas, where forage alternatives (without spines) are available in all seasons, they might facilitate tree establishment. Some of these observations induced me to start up experiments and investigate the role as safe sites for tree establishment in this thesis.

In the presence of large herbivores, progressing succession is steered by alternating positive (associational resistance) and negative (competition) interactions between plant species. Small, unattractive plants such as tall herbs facilitate the establishment of taller unattractive plants such as spiny shrubs, which in turn take up the role of facilitating the establishment of palatable trees. In a next stage, shrubs dissapear through competition (for light and nutrients) with the outgrowing trees that were facilitated in the former stage. In Fig. 1.1, a more detailed description of successional mechanisms in grazed and ungrazed areas is given.

1.5.2.3 Herbivore selectivity

In general, associational resistance allows a palatable species to be proportionally less grazed by large herbivores if neighbouring unpalatable species have a higher relative abundance compared to the abundance of the palatable species. This means that in such circumstances individuals of palatable species will experience less biomass loss when they occur in relatively low abundance. The reverse effect may also occur: if palatable species have relatively higher abundances than unpalatable species, the latter will be proportionally more grazed. The functioning of these mechanisms strongly depends on the selective grazing capacities of the herbivores. Associational resistance will only be effective if herbivores are not able to distinguish between palatable and unpalatable species at a small scale or when herbivores are physically hindered by unpalatable species. Large grazers such as cattle and horses are more likely to fulfil these conditions than small species such as hares (*Lepus europaeus*), rabbits (*Oryctolagus cuniculus*), browsers such as roe deer (*Capreolus capreolus*) or herbivorous insects (Bakker 2003).

Fig. 1.1 summarizes the successional mechanisms discussed above. The sequence A→B1→A is the rule rather than A→B1→C which is an occasional event. Once established, shrubs may expand via clonal growth (e.g. *Prunus spinosa, Rubus* spp*., Ligustrum vulgare*), although young shoots also may need protection to grow out. A typical pattern is the occurrence of unpalatable forbs in fringe communities between scrub and grassland, which may be explained by mutual protection. In this way, the sequence A→B2→C, with clonal growth of scrub is enforced and often a fast going process. Subsequently, scrub patches that have become large enough act as safe sites for the establishment of palatable trees: C→D. Within 5-10 years, established trees will grow above the shrub layer (D→E) and start to out-shade them. This trees will not be able to regenerate in the same patch, once the shrub layer has disappeared. When trees die of old age, disease or storm (F→G), two different processes are possible. The branches of the fallen tree may temporarily provide protection against large grazers (branch-cage effect) and facilitate establishment of palatable trees (G→H). When dead trees decompose or get trampled, grasses and forbs will invade the nutrient rich gap. Such productive grassland patches will attract grazers, which prevent establishment of shrubs and trees. During this stage, associational palatability may delay the spreading of unpalatable species, prolonging the grassland stage. Only after disturbance, and/or when grazers temporarily neglect a patch, unpalatable herbaceous plant species may invade locally, and the cyclical turnover may start again. Without large grazers, gap-phase dynamics are active (Fig. 1.1d). In this type of cyclic succession, the phase with adult trees (A) is the longest; a grassland phase is absent and the prevailing vegetation structure is a closed forest (all according to Olff *et al.* 1999).

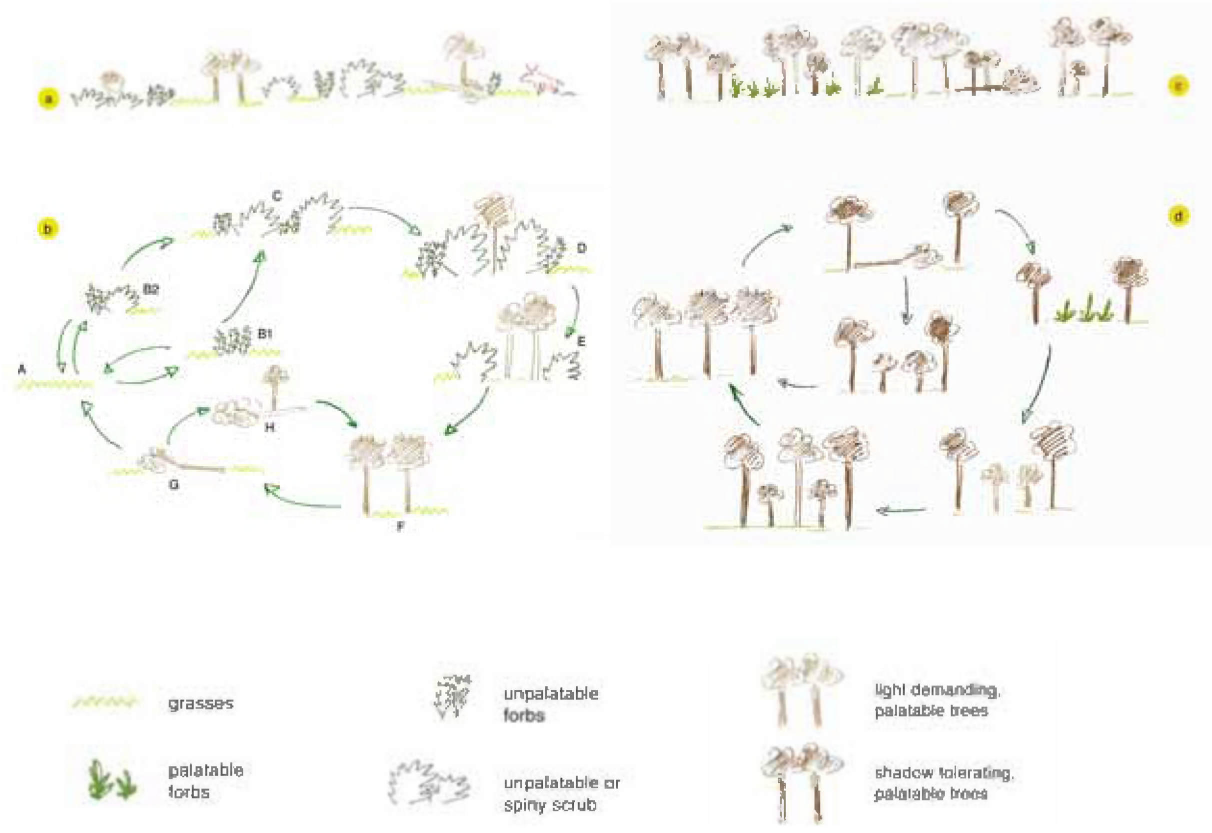

Figure 1.1: Expected spatial mosaic (a) as mediated by cyclic succession of plant functional types (b) in temperate woodlands with free-ranging large grazers, such as cattle and horses. Associational resistance to herbivory is an important mechanism causing this type of dynamics (see text). Without large grazers, a different spatial mosaic (c) and patch dynamics (d) would be observed. (after Olff *et al.* 1999).

1.6 Aims and questions

1.6.1 General aims of the thesis

Conservation biology should provide new insights into ecological questions and evidence based information for conservation. Results should be made clear to enable appropriate action by policy makers, conservation managers, volunteers, wardens (Pullin 2002; Maes 2004). In this thesis, we address several ecological topics in the field of plant-herbivore interactions. These topics refer directly to conservation and restoration management questions in areas where large herbivores are introduced. We studied interactions between large herbivores and woody species (trees and shrubs) in a context of nature restoration. The focus is on woodland regeneration processes on former agricultural land (arable land, grassland) that are included in nature restoration projects aiming at the development of mosaic landscapes (woodpasture landscapes) or/and woodland expansion. The scientific purposes of this thesis are imbedded in the theoretical and ecological framework that was described by Olff *et al.* (1999 ; former paragraph). We aimed at combining survey, experimental and elementary modelling studies to get an insight into different patterns, mechanisms and processes at work (see Box 1.2).

1.6.2 Main questions

- In which **patterns** does woodland regenerate on grazed former agricultural land?

What is the role of grazing related to the starting conditions of a site (arable land vs. grassland, vegetation structure, abiotic conditions) and the spatial configuration of the surrounding landscape (seed and propagules sources)? What are the effects of grazing on existing woodland vegetation, when included in grazing blocks with former agricultural land?

- Which **mechanisms** are at work in woodland regeneration on former agricultural land?

What is the role of associational resistance (safe sites), associational palatability, competition of surrounding vegetation and the functional plant strategies (related to herbivory) of establishing trees? What is the role of time gaps in grazing? Do they offer sufficient opportunities for woodland regeneration, and if yes, how does this interact with the other mechanisms at work?

- Which **processes** affect woodland regeneration?

What is the role of natural processes and disturbances such as flooding in the regeneration process of woody species. How does this interact with large herbivore activities such as grazing and soil trampling?

Does nutrient transport between different habitats occur as a result of selective habitat use and/or different foraging strategies of large herbivores? If yes, in what direction are nutrients transported? Is nutrient depletion, as a trigger for woody species regeneration, an active process on former agricultural land? What is the role of habitat proportion and structure in this process. Are vulnerable plant communities at risk of eutrophication in grazed mosaics? And how is this related to atmospheric deposition?

Box 1.2 Patterns, mechanisms and processes : definitions and examples

patterns	describe the spatial and temporal distribution of individuals, groups or actions. e.g. : frequencies of woody species in different vegetation types or in relation to distance to potential seed trees; presence or absence of grazing damage to seedlings; spatial and temporal distribution of cattle in different habitats; …
mechanisms	describe how several factors interact, leading to an explanation of observed changes. e.g. : protection by nurse plants allows growth of established trees, soil disturbance by herbivore hoofs triggers establishment of seedlings in gaps that experience decreased competition, …
processes	describe naturally occurring, coherent and observable actions and changes in a defined spatial and temporal framework. e.g. : the development of a woodpasture landscape in a grazed and temporarily flooded valley system; repeated grazing of trees; …

1.7 Thesis outline

Six different field studies are presented as individual chapters (2-7) in this thesis. None of these studies exclusively deals with either patterns, mechanisms or processes. In Fig. 1.2 and Table 1.1, we present how the different chapters are related to each other and which items are studied. We tried to comprise both the individual live stage cycle of a woody species and the cyclic successional pathway of a vegetation patch in grazed conditions, though it was not the aim to include all possible stages and interaction effects.

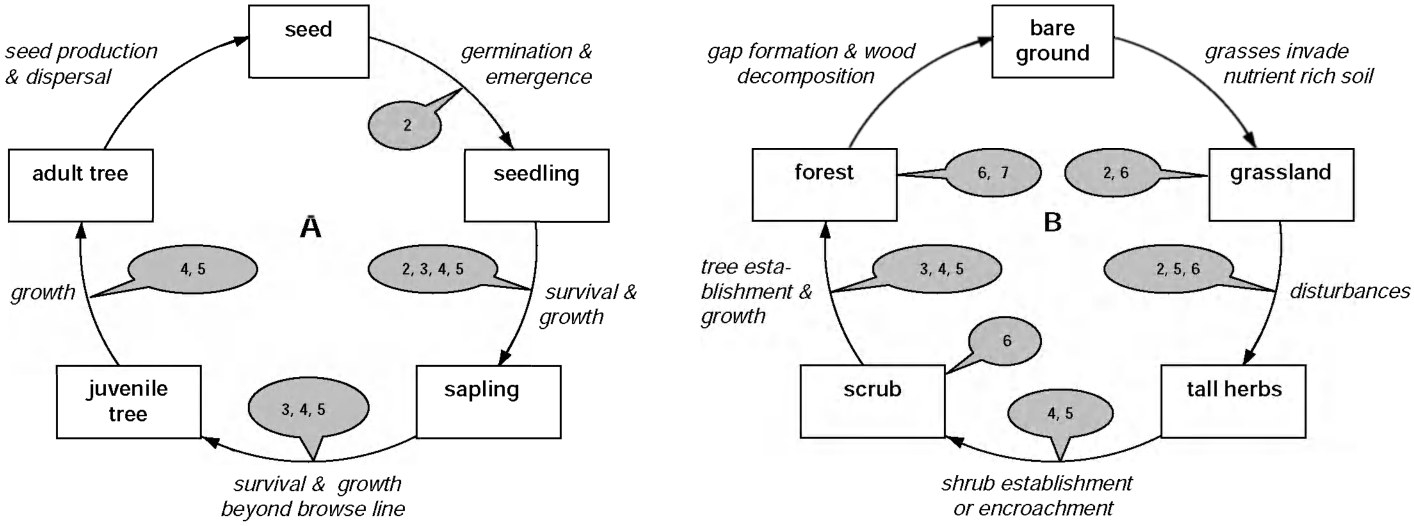

Figure 1.2: thesis outline: studied patterns, mechanisms and processes (balloons refer to different chapter numbers in this thesis). A: at the scale of an individual woody species, presented in a life stage cycle; B: at the scale of a vegetation patch, presented in a cyclic successional pathway. Rectangles represent stages, arrows represent transitions.

Table 1.1: short description of the studied patterns, mechanisms and processes affecting woodland regeneration (chapter 2-6) and forest ground vegetation (chapter 7).

	patterns	mechanisms	processes
chapter 2	vegetation structure vegetation gaps	competition	flooding soil trampling grazing & browsing
chapter 3	vegetation structure time gaps in grazing	associational resistance associational palatability	grazing & browsing
chapter 4	vegetation structure	resistance traits ~ herbivory associational resistance	seed dispersal grazing & browsing
chapter 5	vegetation structure	associational resistance	seed dispersal flooding soil trampling grazing & browsing
chapter 6	vegetation structure herbivore habitat use herbivore foraging strategy	habitat selection diet selection	nitrogen redistribution atmospheric deposition depletion-eutrophication grazing & browsing
chapter 7	vegetation composition	chemical defence	vegetation trampling grazing & browsing shrub encroachment

Chapter 2 deals with the earliest stages of woody species, the seed and emergence stage. We use *Fraxinus excelsior* and *Quercus robur* as model species in an experimental set up to investigate the role of vegetation structure (a successional sequence from grassland to scrub) and the interaction effects of grazing, flooding and trampling on emergence and early growth patterns. The studied species represent pioneer species with diverse recruitment strategies (differing in seed characteristics, dispersal vector and seedling light requirements) that establish naturally in grasslands with a natural disturbance regime (e.g. floodplains). This chapter describes the first bottleneck (after dispersal) in the recruitment of trees in grazed areas.

Chapter 3 examines the role of different vegetation structure types as safe sites for the survival and growth of establishing trees. Survival and growth represent a second and third bottleneck in the recruitment process of trees. In an experimental set up with exclosures, we use the same species and vegetation structure types as in chapter 2. Further, we evaluate the effect of time gaps in grazing. We analyse if this offers a window of opportunity for tree saplings to grow out beyond the browse line, which is an important threshold for woodland regeneration and/or woodpasture restoration.

Chapter 4 describes regeneration patterns of pioneer trees in woodland restoration projects on grazed former arable land. We examine the role of distance to source trees, but focus on differences between functional plant groups related to herbivory, i.e. trees with different resistance traits (tolerant, resistant, non-resistant). We investigate the facilitating role of an unpalatable or spiny herb and low shrub layer that quickly develops on the bare ground of ploughed fertile soils. We test if grazing by large herbivores affects the possibility of the different functional groups to grow beyond the browse line and consequently determines the species composition of pioneer woodland. We further examine if large herbivores are able to increase structural heterogeneity in early pioneer assemblages, which is often a goal in conservation management.

In **chapter 5** we study regeneration patterns of woody species invading grazed grasslands, using interaction effects of grazing, vegetation structure, soil moisture and natural disturbances such as trampling by large herbivores. We describe the spatial association of establishing trees and facilitating vegetation patches and the conditions that allow trees to grow beyond the browse line.

Chapter 6 deals with nitrogen redistribution in a grazed vegetation mosaic with grassland, wooded pasture and forest. We use nitrogen content of different diet classes, habitat use, foraging and defecation behavior, weight gain and nitrogen losses to explore four different habitat proportion scenarios and two different foraging strategies to calculate net nitrogen balances per habitat. We examine if the spatial redistribution of nitrogen among habitats by cattle could restore nutrient-poor conditions in preferred foraging habitats, and whether such translocation conversely could lead to a risk of extreme eutrophication in preferred resting habitats. We confront the results with atmospheric nitrogen deposition in a stressed environment and we discuss the conditions for the creation of nutrient-poor conditions in grassland that could trigger woody species regeneration.

In **chapter 7**, we examine if the introduction of large grazers is a good measure to decrease bramble cover in ancient forests. Expanding bramble cover as a result of increased atmospheric nitrogen deposition and altered forest management is considered as a threat for species rich forest ground flora. We study the effects of four years of large herbivore grazing on bramble cover, and on cover and flowering of a set of vernal flowering forest forbs. We further conduct experiments to investigate direct effects of grazing and trampling on forest ground flora.

Finally, in **chapter 8**, an integrated analysis and discussion summarizes the conditions for woodland regeneration on grazed former agricultural land. From this, we deduce a conceptual model for regeneration success on former arable land and grassland with different operating mechanisms. We discuss the integrated results in a context of conservation and restoration ecology and practical conservation management (grazing pressure, grazing regime, time gaps) and detect thresholds for grazing pressure. Further, we develop a conceptual management oriented state-and-transition model that describes the early successional pathways to be expected on grazed former agricultural land.

1.8 Study sites

Studies were conducted at 13 study sites (Fig. 1.3) and one greenhouse-gardencomplex. Detailed site descriptions of sites are given in the following chapters. In general, sites meet two criteria: (1) former land use was intensive agriculture (grassland or arable land), (2) soils are nutrient rich and fine textured (sandy loam, loam and clay). All sites are situated in the sandy loam and loam region of Belgium. Both interfluvial and floodplain sites are included. Some general characteristics of the study sites are given in Table 1.2.

Figure 1.3 a: situation of Flanders in Belgium and Europe, b: study site locations in Flanders and in the different Flemish ecoregions. Study sites: BR=Broekelzen; SU=Sulferberg; BE=Bos t' Ename; BB=Boembeke; BU=Burreken; HA=Hayesbos; TR=Trimpont; SB=Steenbergbos; MB=Moenebroek; BM=Boelaremeersen; RB=Rietbeemd; VA=Valier; AB=Altenbroek; GL-GA=Glasshous and Garden complex Wenduine. Flemish Ecoregions (in grey those containing study sites): 1=Coastal dunes; 2=Polders and tidal Schelde; 3= Pleistocene rivervalleys; 4=Cuesta region; 5=Campine region; 6=Western interfluvia; 7=Central Flanders transition zone; 8=Southwestern hills; 9=Southeastern hills; 10=Chalk-loam region; 11=Chalk region; 12=Gravel rivers.

At all sites, domestic large herbivores (cattle and/or horses) in low to moderate densities (< 1 animal unit ha^{-1}y^{-1}) were introduced for conservation purposes after the cessation of agricultural use. Conservation management aims were the restoration of woodpasture landscapes and/or woodland expansion. At all sites grazing occurs with free ranging large herbivores in fenced areas, most of them containing different habitats (e.g. former arable land, grassland, tall herb, old growth and pioneer forest).

Table 1.2: General characteristics of study sites and chapter reference. Type: F=floodplain, I=interfluvial slope; Soil type: Cl=clay, L=loam, SL=sandy loam; Present habitats: A=former arable land, G=grassland, T=tall herb, Sc=scrub Fo=forest, L=logged forest; Herbivore species: C=cattle, H=horses; Grazing type: S=seasonal, Y=year-round.

	type	soil	present habitats	herbivore sp. & density (AU $ha^{-1}y^{-1}$)	grazing type & no. of blocks	total grazed surface (ha)	chapter no.
grazed sites							
Boelaremeersen	F	Cl	G,T,Sc	C (0.4)	S (1)	9.2	2,3,5
Rietbeemd	F	Cl	G,T,Sc,L	C+H (0.4)	Y (1)	22.1	2,3,5
Moenebroek	F+I	L	A,G,T,Sc,F	C (0.4-0.6)	S (4)	27.9	4,5
Bos t' Ename	I	SL	A,G,T,Sc,F	C+H (0.3-0.4)	Y (1)	63.5	3,4,5,6,7
Steenbergbos	I	Cl	A,G,Sc,F	C (0.3)	S (1)	4.1	2,3
Burreken	I	L	A,G	C (0.8)	S (1)	2.1	5
Valier	F	Cl	G,Sc	H (0.6)	S (1)	1.5	5
Altenbroek	I	L	A,G,Sc,Fo,L	C (0.2)	Y (1)	44.5	4
Boembeke	F+I	L	A,G	C (0.5)	S (1)	5.6	4
Sulferberg	I	L	A,G,Fo	C (0.5-0.6)	S (2)	3.6	4
ungrazed sites							
Trimpont	I	L	A, Fo				4
Burreken 2	I	L	A, Fo				4
Broekelzen	I	L	A, Fo				4
Hayesbos	I	L	Fo				7

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New woodpasture landscape (Rietbeemd, Geraardsbergen, Belgium)

Chapter 2. Tree seedling emergence and natural disturbances

Van Uytvanck J, Milotic T, Hoffmann M. Interaction between large herbivore activities, vegetation structure and flooding affects tree seedling emergence. *Plant Ecology*, in press. DOI: 10.1007/s11258-009-9632-1. Reprinted with permission from Springer.

Abstract

Tree establishment in grazed vegetation mosaics involves a series of early bottlenecks, including seed dispersal, germination, seedling emergence, survival and growth. In a field experiment, we studied seedling emergence of two species with contrasting recruitment strategies, *Fraxinus excelsior* and *Quercus robur* , in five structurally different vegetations: grazed and ungrazed grassland, ruderal pioneer vegetation, soft rush tussocks, tall sedge mats and bramble scrub. In a simulation experiment, we studied the interaction effects of pre-emergence flooding (three weeks of inundation), trampling and grazing (simulated by clipping) of grassland vegetation on the emergence and early growth of both tree species in grass swards.

Seedling emergence was enhanced in low swards and sparse vegetation types. Despite different recruitment strategies, the interaction of flooding and trampling of swards enhanced seedling emergence of both species. Grazing of soft rush and tall sedges enhanced emergence of *F. excelsior.* Clipping grass swards increased early growth of emerging *Q. robur.* Our results support the hypothesis that natural disturbances of soil and vegetation create microsites for seedling emergence and reduce above ground competition. In grazed systems however, these results suggest a discordant relationship between successful seedling emergence and subsequent seedling growth/survival during the establishment process in structurally different vegetations.

Key words

competition for light; grazing; natural disturbances; tree recruitment and establishment; trampling; vegetation gap

2.1 Introduction

Grazed wetlands are often characterised by mosaic landscapes with grassland, scattered scrub, trees and forest patches (van der Valk & Warner 2009). Regular or periodic tree establishment is necessary for the continued existence of this structural heterogeneity (Olff *et al.* 1999). Herbivore activity and its interaction with surrounding spatial structure (e.g. Van Uytvanck *et al.* 2008b) and flooding are important ecological determinants for successful tree establishment.

Recruitment of woody species in grassland ecosystems is often episodic and disturbance-driven (Putman 1986; Grubb 1988; Clarke 2002). Generally, a dense grass canopy drastically reduces favourable microsites for germination and emergence and subsequent growth (above and below ground) and survival rate of seedlings (Eriksson & Ehrlén 1992; Davis *et al.* 1999; Mazia *et al.* 2001; Harmer & Robertson 2003; Setterfield 2002). Flooding in wetland ecosystems may cause die back or retard growth of grassland vegetation, temporarily reduce grass and herb competition and favour flood tolerant tree seedlings such as *Ulmus* species, *Quercus robur* and *Fraxinus excelsior* (Deiller *et al.* 2003; Kühne & Bartsch 2007). Additionally, local erosion and sedimentation following flooding (Van Splunder *et al.* 1995), but also soil trampling by large herbivores (Trimble & Mendel 1995), may create bare ground patches and small gaps in the grass layer. These may function as microsites for germination and emergence of woody species (Posada *et al.* 2000). In the absence or reduced presence of litter or roots (when destroyed or damaged by trampling) in the upper soil layers, radicles of germinated tree seeds may penetrate the soil easier avoiding early desiccation, which is a common failure for tree seedling establishment (Caccia & Ballaré 1998). Large herbivores locally create short grassland lawns by grazing. In these lawns, a reduced vegetation height allows partial release from competition for light, water, nutrients and space (Nilsson *et al.* 2002). As a consequence, lawns may provide additional opportunities for the establishment of woody plants (Jurena & Archer 2003; Vandenberghe *et al.* 2006; Hagenah *et al.* 2009). In contrast to tall and dense vegetation, short grazed lawns and gaps may also experience reduced seed predation by small herbivores such as rodents (Kelt *et al.* 2004). On the other hand, early and fragile seedlings in lawns may suffer from direct solar radiation and be less protected from heat and drought compared to seedlings emerging under the protection of surrounding vegetation (Berkowitz *et al.* 1995; Vandenberghe *et al.* 2006).

In the subsequent life stage, survival and growth of seedlings may be highly restricted by large herbivore grazing, even at low stocking rates (Van Uytvanck *et al.* 2008b). Therefore, increased opportunities for germination and emergence of woody species in microsites created by uprooting, trampling or grazing, may be followed by increased mortality of saplings by desiccation or grazing of favoured grassland lawns. In this paper, we study the individual effects and interactions of large herbivore activities (grazing, trampling), vegetation structure and flooding on tree seedling emergence and early seedling growth of *F. excelsior* and *Q. robur*. Both species are found in European lowland woodlands on a wide range of soil types with varying soil texture, pH, trophic and hydrologic conditions. Both are important pioneer species, invading grasslands where there are openings in the sward (Jones 1959; Wardle 1961; Rudinger & Dounavi 2008) and they are suitable model species for a broad range of palatable tree species that regenerate naturally in grazed grassland ecosystems. Recruitment strategies of *Q. robur* and *F. excelsior* significantly differ in (1) seed characteristics: seed mass (heavy vs. light), longevity (transient vs. short term persistent), dormancy (short vs. long); (2) dispersal vector (diplochory with animals as secondary dispersers vs. primarily wind and water), dropping off trees (fast vs. slow) and (3) seedling light requirements (strongly light demanding for survival vs. shade tolerant persistent seedlings).

We set up a field experiment to investigate the role of the vegetation structure and grazing on seedling emergence of *F. excelsior* and *Q. robur*. We elaborated on a previous study in which the seedling stage (survival and growth of planted individuals) of both species was investigated in five structurally different vegetation types: grassland lawn, ruderal pioneer vegetation on former agricultural land, tall *Juncus* vegetation, tall *Carex* vegetation and *Rubus* (bramble) thickets (Van Uytvanck *et al.* 2008b). We hypothesized that *F. excelsior* and *Q. robur* seedling emergence will respond differently to vegetation structure type and grazing effects. We hypothesized that the emergence of *F. excelsior* will be favoured in low or sparse (and therefore less competitive) vegetation types such as grassland lawns and ruderal pioneer vegetations. We hypothesized that grazing will enhance emergence of this species in tall vegetation types (*Juncus* and *Carex* vegetation) by creating gaps and reducing competition. Both hypotheses are based on the adaptive traits of *F. excelsior* seeds: they can reach favourable sites (short or sparse vegetation types) through wind dispersal and they are able to wait favourable conditions through dormancy (shortened vegetation or gaps after grazing). In contrast, we hypothesized that the emergence of *Q. robur* is independent on vegetation structure and grazing, because it has large seeds without innate features to escape unfavourable conditions. Large seeds have a large amount of nutritional reserves, which favours seedling emergence and survival (Winn 1985; Greiling & Kichanan 2002). Therefore, we hypothesized that competition of surrounding vegetation (whether influenced by grazing or not) does not influence emergence of *Q. robur* significantly. Second, we set up a greenhouse/garden simulation experiment to study the interaction of herbivore activities (grazing and trampling of grassland swards) and pre-emergence winter-early spring flooding on the emergence and early growth of seedlings of the same species. We hypothesized that disturbances (grazing, trampling and flooding) would decrease the ability of closed grassland swards to suppress emergence and early growth of seedlings. We hypothesized that grazing, pre-emergence trampling and flooding of grassland swards increase seedling emergence of *F. excelsior*. We hypothesized that these disturbances would not affect the emergence of *Q. robur*. We hypothesized that pre-emergence flooding enhances early growth of both species due to released competition.

2.2 Methods

2.2.1 Study sites and focal species

We conducted a field experiment on the emergence of *Fraxinus excelsior* and *Quercus robur* in three nature reserves on former agricultural land in the southern part of Flanders (N-Belgium): Boelaremeersen, Rietbeemd and Steenbergbos. All sites were grazed with cattle (0.4-0.5 AU ha^{-1}y^{-1}; at Rietbeemd two ponies were added to the herd). For a detailed description, past and current land use and grazing management of those sites, see Van Uytvanck *et al.* (2008b, **chapter 3**). We studied emergence in five structurally different vegetation types. We conducted a second, controlled experiment on the emergence of the same species under simulated natural disturbances in grassland, in a greenhouse and surrounding garden facilities in Wenduine (51°17'N; 3°03'E) in the western part of Flanders.

Q. robur and *F. excelsior* are two widespread, native, deciduous tree species with a similar distribution pattern in the European lowland (Jones 1959; Wardle 1961; Oberdorfer 1992). Both species occur in a broad range of woodlands and park landscapes (from hardwood floodplain forests to dry and well drained forest on rendzina soils). Vera (2000) states that in grazed landscapes, juveniles and young trees occur preferentially in protective vegetation patches such as thorny or spiny thickets. Both species are able to produce a large number of seeds but seed production of *Q. robur* shows great variability between years, while yearly seed production of *F. excelsior* is more constant. However, *Q. robur* and *F. excelsior* differ considerably in recruitment strategy: *Q. robur* has a transient seed bank; seeds exhibit very little dormancy but germination velocity is irregular and germination is spread over one year (Reyes & Casal 2006). Seeds are large and heavy, soon falling off and secondarily mainly dispersed by animals. Seedlings need sufficient light to survive, i.e. outside a closed tree canopy (Rackham 1980). In contrast, *F. excelsior* has winged, light seeds, that are fixed for several months to tree branches and are mainly dispersed by wind, and to a lesser extent by water and birds. *F. excelsior* has a short term persistent seed bank, restricted to 5-6 years, but with deeply dormant seeds that, in general, do not germinate until the second spring after dispersal (Thompson & Bekker 1997; Raquin *et al.* 2002; Willoughby *et al.* 2004). *F. excelsior* has shade tolerant persistent juveniles which are able to grow out quickly when light penetration increases (Tapper 1996).

2.2.2 Experimental design

For both experiments and model species, we acquired fruits (*F. excelsior*) and acorns (*Q. robur*), each from one particular tree nearby the study sites (50°46'N; 3°45'E) at the end of October 2005 and 2006. We stored seeds at 4°C until planting. We only used healthy fruits (visual inspection) of *F. excelsior* that obviously contained seed (>5 mm in length, considered to be viable, Gardner 1977), and healthy acorns (unaffected by insect larvae, worms or fungi). We did not remove seeds from the *F. excelsior* fruits, because the long dormancy period does not result in a significant difference in germination percentage (Gardner 1977) and because fruits are the dispersal mode.

In the field experiment, we studied the effect of grazing and vegetation structure on emergence. In the second week of November 2005, we planted 1584 seeds and fruits of *Q. robur* and *F. excelsior,* each, in five structurally different vegetation types (Table 2.1): short grassland (short, dense lawns), soft rush vegetation (*Juncus*; high tussocks), tall sedge vegetation (*Carex*; tall mats), ruderal pioneer vegetation (short, sparse vegetation on former arable land) and bramble (*Rubus*; tall thickets). Identification and location of the vegetation types in the field was mainly based on cover percentage of dominant species and former land use (in case of arable land) (Van Uytvanck *et al.*

2008b). We planted seeds in 44 plots, consisting of a grazed and an ungrazed (barb wire exclosure against large herbivores) subplot (2m x 2m). In ungrazed subplots, vegetation was taller, light penetration was decreased (Table 2.1). We divided each subplot in two parts (1m x 2m), randomly assigned to the species, and planted 18 seeds and fruits of *Q. robur* and *F. excelsior*, respectively, just beneath ground level in a fixed 3 x 6 grid. This resulted in 176 experimental units (appendix 2.1).

Table 2.1: Field experiment: number of plots in different study sites (BM = Boelaremeersen; RB = Rietbeemd; SB = Steenbergbos) and mean height, light penetration and soil moisture of different vegetation types. Soil moisture is estimated, using weighted Ellenberg values for moisture of the species in the vegetation; weighting factor is cover of the individual species.

	No. of plots			Vegetation height (cm)		Light penetration (%)		Soil moisture
	BM	RB	SB	ungrazed	grazed	ungrazed	grazed	(Ellenberg value)
grassland	5	4	4	54.6 (±19.0)	21.9 (±9.94)	84.0 (±18.0)	89.9 (±14.5)	6.3 (±0.2)
ruderal pioneer vegetation	-	-	4	66.2 (±1.4)	15.8 (±4.6)	73.3 (±29.6)	83.7 (±20.5)	5.1 (±0.3)
soft rush tussocks (*Juncus*)	5	4	-	92.2 (±11.6)	74.1 (±13.1)	58.1 (±21.2)	72.6 (±18.0)	7.0 (±0.1)
tall sedges (*Carex*)	5	-	-	97.2 (± 16.9)	71.3 (±18.4)	25.8 (±16.0)	53.8 (± 17.0)	8.8 (±0.1)
bramble (*Rubus*)	5	4	4	87.4 (± 26.6)	79.6 (±16.6)	21.5 (±23.9)	22.1 (±22.5)	6.4 (±0.3)

In the controlled simulation experiment, we studied the effects of grazing, trampling and winter-early spring flooding on the emergence and early growth of tree seedlings. In November 2006, we planted 2592 seeds and fruits of *Q. robur* and *F. excelsior,* each, in seed-bed plots in a cold greenhouse (average temperature November-January: 6.4 ± 1.8℃) with a natural light regime. Seed-bed plots consisted of previously sown grass swards (commercial grass mix, dominated by *Lolium perenne*) on a mixture of sterilised (heated at 180℃ in an oven to obtain homogeneous grassland swards in all plots) and nutrient rich potting compost and sand. In each plot (40cm x 42cm), 36 acorns or fruits were sown in a fixed 6 x 6 grid to account for seedlings that died and disappeared during the experiment. Plots were humidified to reduce desiccation of the seeds. In the first week of February 2006, we moved seed-bed plots with fully grown grass swards (cover = 100%) outside the greenhouse to apply the treatments.

Plots were arranged in a three way factorial, randomised complete block design (appendix 2.1). Treatments are combinations of 3 factors: species, trampling and clipping the seed-bed vegetation. One block consisted of eight plots. Within a block, one of eight possible factor combinations (i.e. clipping grass swards or not x trampling or not x two species) was randomly assigned to a plot. We established nine blocks, representing 9 replicas. Superimposed on this, a fourth factor (flooding or not) was applied, using a similar set of nine blocks. This resulted in a total of 144 experimental units.

We flooded one set of nine blocks with rain water for ca. three weeks (27/02/2007 – 22/03/2007), simulating winter-early spring flooding (water surface 15-20 cm above ground level of plots). The other set of nine blocks was not flooded. Because emergence of *F. excelsior* seedlings only starts after a second winter, we repeated the flooding regime in 2008 for the *F. excelsior* plots only. Clipping of the swards (simulating grazed conditions) and trampling started before emergence and immediately after flooding was finished. Trampling the swards was only applied once (in 2007) for *Q. robur*, twice (2007 and 2008) for *F. excelsior*. Repeated clipping (every week) kept the sward height between three and five cm. Unclipped vegetation resulted in a dense and high sward (average canopy height May-September = 25.1-38.4cm, Table 2.2) with bent grass stems and leaves and (in 2008) also litter production. We simulated trampling of the grass sward by large herbivores with a wooden cattle paw dummy (basal hoof surface = 49cm^2): we made 25 imprints in each trampled plot by pressing the dummy on the vegetation and soil with a weight of ± 100kg (i.e. ± 2kg/cm^2, a comparable pressure of an adult cow's paw). This resulted in complete trampling of the vegetation (canopy height reduced to 0-5 cm above ground level) and disturbance in the upper 5 (±2) cm of the soil in each trampling-subplot, creating hoof print gaps. We protected all plots from predation by birds using a fine meshed net. Plots were kept humid by rain; in dry periods by sprinkling.

2.2.3 Data collection

In both experiments, we recorded a seedling as emerged if it had developed cotyledons, an epicotyl shoot and at least one leaf. In the field experiment, we recorded emergence of seedlings four times per year: in April, June, August and September 2006 and in 2007. We measured soil moisture, light penetration and vegetation height (see Van Uytvanck *et al.* 2008b) in all plots in September 2006. Vegetation height was always lower in grazed plots than in ungrazed plots and light penetration was always higher in grazed plots (Table 2.1).

Table 2.2: Simulation experiment: grass sward characteristics after different treatments (values ± st. dev.)

	pre-emergence flooding				no flooding			
	clipped		unclipped		clipped		unclipped	
	untrampled	trampled	untrampled	trampled	untrampled	trampled	untrampled	trampled
Height (cm)								
May	kept between		25.6±25.0	22.8±20.3	kept between		26.3±12.5	26.0±11.3
September	3-5 cm		40.1±12.1	37.3±18.3	3-5 cm		39.3±18.2	37.0±15.8
Cover (%)								
May	97.5±5.1	71.3±12.1	96.1±4.8	66.1±14.7	100	96.3±3.9	100	95.9±2.6
September	100	100	100	100	100	100	100	100
Light penetration (%)								
May	98.9±3.1	99.2±2.2	63.4±7.1	87.1±9.4	99.0±2.0	97.6±4.9	62.6±6.6	52.2±8.1
September	99.6±1.4	99.1±2.7	71.3±8.3	68.4±9.1	97.8±1.9	99.3±0.9	68.7±8.4	69.9±9.8

In the simulation experiment, we recorded the emergence of seedlings two times during the growing season of 2007 (May and September) and repeated this for *F. excelsior* in 2008. We measured shoot height of all emerged seedlings in the last recording session in September. We measured vegetation height at the same time of emergence recording on four fixed points. At the same time we measured photosynthetic photon flux density (µmol photons $s^{-1}m^{-2}$) in all plots. We made these measurements at ground level (same four fixed points) and 50 cm (point without interfering vegetation) above ground level with a photometer (Skye Instruments LTD, SKP 200). We determined light penetration in the vegetation as the ratio between paired measurements at 0 and 50cm (Table 2.2).

A problem arose when, in May 2007, a substantial part of the acorns was dug out and predated or removed by the small rodent *Eliomys quercinus*, that had entered the experimental set up of the garden experiment. 45.7 % of the acorns were removed or predated. Three flooded acorn blocks were highly affected (predation > 70%). Therefore, we recorded the number of removed or predated acorns per plot to correct the percentages of emerged seedlings. *F. excelsior* seeds were not affected by predation.

2.2.4 Data analyses

We conducted statistical analyses using SAS 9.1. We used generalised linear mixed models (proc glimmix in SAS 9.1) to analyse the number of emerged seedlings in both experiments. Because of differences in germination velocity within one species, we used the total of emerged individuals as recorded after the final observations in both experiments, i.e. after 10 and 22 months for *Q. robur* and *F. excelsior,* respectively. Because we could not correct for differences in seed viability and germination rate between species, *Q. robur* and *F. excelsior* were analysed separately in both experiments. The response variable was log transformed to meet conditions for normality. Shoot height (simulation experiment) was analysed using general linear models (proc glm in SAS 9.1). AIC values for goodness of model fit were used to control, whether the model was improved or not after backward elimination of non-significant factors. Tukey-tests were performed to make multiple comparisons in all analyses.

For the field experiment, we entered study site and plot (nested in vegetation type) as random factors in the model. Vegetation type and grazing (yes or no) and their interaction were entered as fixed factors. For the simulation experiment, we entered flooding, trampling, clipping and all interactions as fixed factors.

2.3 Results

2.3.1 Field experiment: the role of vegetation structure and grazing on emergence

For both tree species, short and sparse vegetation types (grassland lawns and ruderal pioneer vegetations) showed significantly higher emergence percentages compared to soft rush, tall sedges and bramble thickets. For *F. excelsior*, grazing interacted with vegetation structure: comparable emergence percentages were found in short and sparse vegetation types and in grazed soft rush and tall sedges (Table 2.3; Fig. 2.1). For *Q. robur,* grazing did not affect seedling emergence significantly. Average emergence rate for *Q. robur* was 11.71 (±10.05) %; for *F. excelsior* it was 10.63% (±5.22). Random factors site (*Q. robur*: estimate = 0.024 ± 0.029 S.E.; *F. excelsior*: estimate =0.110 ± 0.114 S.E) and plot (*Q. robur*: estimate = 0.012 ± 0.011 S.E.; *F. excelsior*: 0.014 ± 0.012 S.E.) had a negligible effect on emergence, as compared to fixed effect factors, but were kept in the model as they are inherent to the study's experimental design. *Q. robur* emergence was restricted to the first year after acorn planting.

Table 2.3: Seedling emergence % - results of the GLM analyses. Independent factors in the field experiment: vegetation type (=veg), species and grazing; in the simulation experiment: clipping (the surrounding seed-bed vegetation), trampling and flooding.

	independent factor	nom. d.f.	den. d.f.	F value	Pr > F
Field experiment					
F. excelsior	veg	4	78	97.67	<0.001
	grazing	1	78	3.75	0.084
	veg*grazing	4	78	14.00	<0.001
Q. robur	veg	4	78	97.67	<0.001
	grazing	1	78	3.75	0.056
	veg*grazing	4	78	0.88	0.654
Simulation experiment					
F. excelsior	clipping seed-bed vegetation	1	64	9.14	0.004
	flooding	1	64	59.86	<0.001
	trampling	1	64	1.87	0.176
	clipping*trampling	1	64	2.25	0.138
	clipping*flooding	1	64	3.27	0.075
	trampling*flooding	1	64	9.17	0.004
	clipping*trampling*flooding	1	64	4.54	0.037
Q. robur	clipping seed-bed vegetation	1	64	32.08	<0.001
	flooding	1	64	7.83	0.007
	trampling	1	64	5.34	0.024
	clipping*trampling	1	64	2.66	0.108
	clipping*flooding	1	64	3.05	0.086
	trampling*flooding	1	64	11.11	0.002
	clipping*trampling*flooding	1	64	3.10	0.083

2.3.2 Simulation experiment: the role of natural disturbances on emergence and shoot growth

Cover and height of grass swards (initially = 100%) were reduced after trampling and flooding. In dry plots, cover was almost restored in May (Table 2.2), while in flooded plots recovery was delayed after trampling (cover in May < 72 %, with still visible hoof prints). Average canopy height of unclipped swards was comparable in flooded and unflooded plots, whether trampled or not, but variation in

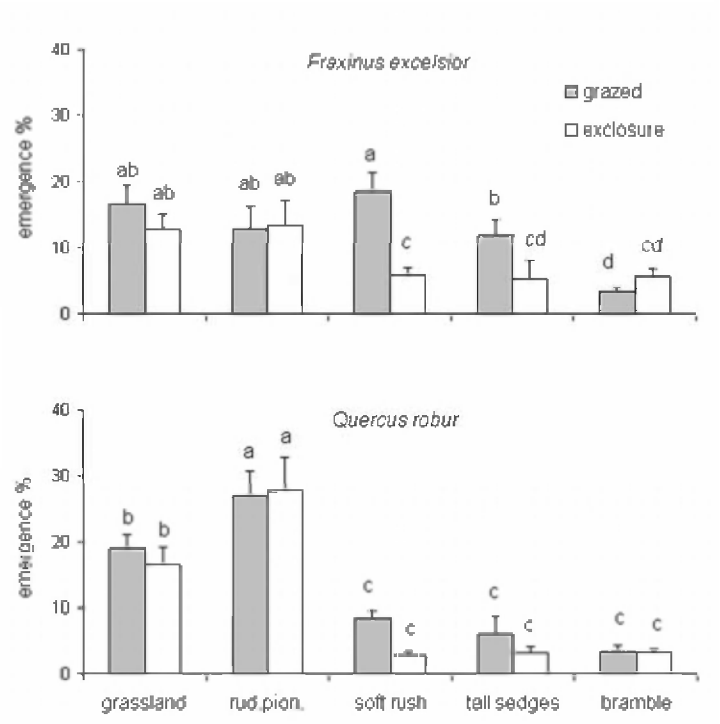

Figure 2.1: Emergence % (+ st. err.) of tree seedlings (*F. excelsior* and *Q. robur*) in five different vegetation types in grazed and ungrazed conditions (field experiment). Different letters indicate significantly different values (multiple comparisons with Tukey-tests, P<0.05) per species. Differences between species are not analysed.

height was higher in flooded plots, reflecting the presence of gaps and taller grasses in the sward. In September, recovery and vegetation height (including variance) were similar in both flooded and dry plots, whether plots were trampled or not (38.4 ± 15.6 cm). Light penetration was similar in all clipped plots (>95%). In unclipped plots, mean light penetration ranged from 52.2 – 63.4.3 % in May, except for flooded and trampled plots: 87.1 ± 9.4%. In September, light penetration was comparable in all plots.

For both *Q. robur* and *F. excelsior*, the interaction of trampling and flooding increased emergence (Table 2.3, Fig. 2.2) . Except for *Q. robur* in clipped plots, this effect was significant, but variation within flooding treatment was higher due to predation (see methods section). Clipping enhanced the emergence of *Q. robur* , while it had a complex effect on *F. excelsior*: no effect in flooded plots and a significant but small negative effect in trampled, dry plots (significant clipping*trampling*flooding interaction). Other interactions did not show significant effects. Average emergence rate for *Q. robur* was 18.13 % (±9.99); for *F. excelsior* it was 16.17 % (±10.24).

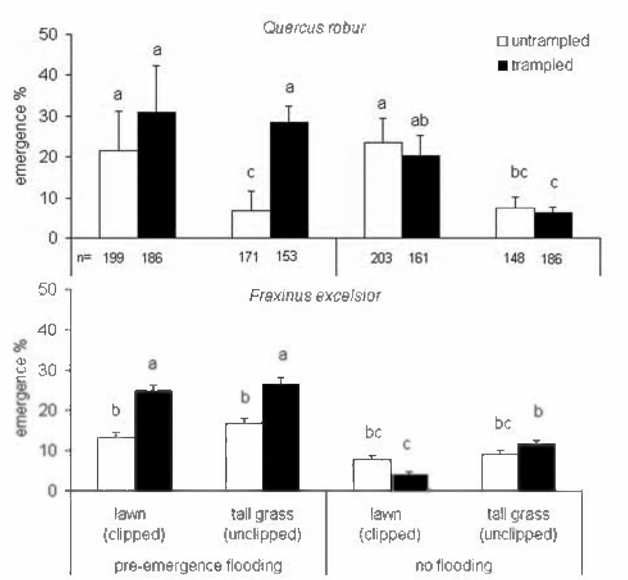

Figure 2.2: Emergence % (+ st. err.) of tree seedlings (*F. excelsior* and *Q. robur*) under simulated grazing (clipped plots have a short grassland lawn vegetation; unclipped plots have a tall herb vegetation), simulated flooding (inundation during 3 weeks; dry plots were not inundated) and trampling. Numbers below x-axis of *Q. robur* represent number of observations (n) after acorn predation (for *F. excelsior* all n = 324). Different letters indicate significantly different values (multiple comparisons with Tukey-tests, P<0.05) per species. Differences between species are not analysed.

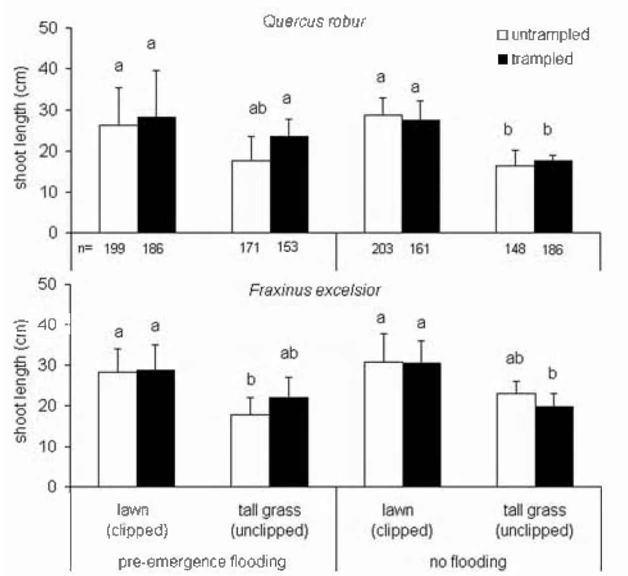

Figure 2.3: Shoot length (+ st. err.), after the first growing season, of emerged tree seedlings (*F. excelsior* and *Q. robur*) under simulated grazing (clipped plots have short grassland lawn vegetation; unclipped plots have tall herb vegetation), simulated flooding (inundation during 3 weeks; dry plots were not inundated) and trampling. Numbers below x-axis of *Q. robur* represent numbers observations (n) after acorn predation (for *F. excelsior* all n = 324). Different letters indicate significantly different values (multiple comparisons with Tukey-tests, P<0.05) per species. Differences between species are not analysed.

Shoot length of emerged seedlings after the first growing season was only significantly influenced by clipping the surrounding seed-bed vegetation (Table 2.4). In both species, mean shoot length in clipped grassland plots was higher (± 9 cm) than in tall grassland plots (Fig. 2.3).

Table 2.4: Shoot height - results of the GLM analyses. Independent factors: clipping (the surrounding seed-bed vegetation), trampling and flooding.

	independent factor	nom. d.f.	den. d.f.	F value	Pr > F
F. excelsior	clipping seed-bed vegetation	1	359	39.83	0.002
	flooding	1	359	2.33	0.162
	trampling	1	359	1.13	0.403
	clipping*trampling	1	359	0.46	0.500
	clipping*flooding	1	359	0.11	0.742
	trampling*flooding	1	359	0.01	0.932
	clipping*trampling*flooding	1	359	3.01	0.198
Q. robur	clipping seed-bed vegetation	1	243	37.24	<0.001
	flooding	1	243	2.16	0.248
	trampling	1	243	0.98	0.424
	clipping*trampling	1	243	1.66	0.374
	clipping*flooding	1	243	3.05	0.156
	trampling*flooding	1	243	0.05	0.830
	clipping*trampling*flooding	1	243	0.22	0.642

2.4 Discussion

We found two patterns that apply for both *Q. robur* and *F. excelsior*: (1) emergence and early growth were favoured in short or sparse vegetation structure types and (2) emergence was positively affected by the interaction of pre-emergence flooding and trampling. Grazing interacted with vegetation structure and enhanced emergence of *F. excelsior* in tall *Juncus* and *Carex* vegetations. For *Q. robur,* clipping (simulated grazing) grassland swards enhanced emergence. Our hypothesis that the effect of the large seed reserves of *Q. robur* would overrule the effects of disturbances and competition was rejected.

2.4.1 Vegetation structure and grazing interaction

In general, a closed grass canopy configuration is unfavourable for *F. excelsior* and *Q. robur* seedlings due to competition (Frost & Rydin 1997; Harmer & Robertson 2003). Though *F. excelsior* and *Q. robur* are relatively shade tolerant species with regard to emergence (Jones 1959; Tapper 1992; Crawley & Long 1995), emergence was significantly higher in vegetation types with high light penetration (grassland, ruderal pioneer vegetation and grazed soft rush and tall sedges vegetation) than in vegetation types with low light penetration (ungrazed soft rush and tall sedges, bramble thickets).

The high emergence of *F. excelsior* seedlings in grazed, tall herb vegetation is probably related to the creation of gaps due to herbivore activity rather than reduced vegetation height. Gaps offer increased light penetration but also better (buffered) soil humidity and temperature conditions for seedlings (Vandenberghe *et al.* 2006). Unpalatable vegetation types are not likely to be grazed but tall sedges are used as food supply in dry periods and grazing (between) soft rush tussocks occurs occasionally (Van Uytvanck *et al.* 2008b). For *Q. robur* seedlings, grazing of tall herb vegetation did not result in a comparable (significant) increase in emergence. In contrast, simulation of grazing grassland swards clearly enhanced the emergence of *Q. robur*, but vegetation height of the clipped grass lawns was much lower than the grazed vegetation types in the field experiment. Seedling light requirements of *Q. robur* are higher than for *F. excelsior* (Ziegenhagen & Kausch 1995), which might explain the lack of a significant effect of clipping on emergence of *F. excelsior* seedlings.

2.4.2 Flooding and herbivore disturbances

Moist conditions in disturbed soils are often a critical constraint for tree germination. Too wet conditions as a result of standing water in (hoof print) gaps may prevent or retard establishment (Nilsson *et al.* 2002). In particular on heavy soils (loam, clay), standing water in gaps may occur, but it was not the case in our simulation experiment. For both *Q. robur* and *F. excelsior*, the combination of trampling and flooding resulted in higher emergence. This combination seems to affect the structure of the grass sward more drastically than trampling dry swards: severe damage to grass roots and above ground grass vegetation most probably reduced above and below-ground competition and retarded sward recovery. Van Uytvanck *et al.* (2008a) found that the regeneration pattern of *F. excelsior* on grazed floodplains was enhanced in tall herb vegetation patches (compared to short grassland) on temporarily flooded soils. Subsequently, emerged seedlings in hoof print gaps were temporarily protected from grazing by unpalatable tall herbs via associational resistance (cf. Callaway *et al.* 2000; Smit *et al.* 2005). Gaps and vegetation removal are known to improve tree seedling emergence

(Agestam *et al.* 2003; Vandenberghe *et al.* 2006). Gap creation by soil trampling may also reduce seed predation (mainly by insects) compared to dense vegetations (Nystrand & Granstrom 2000; Smit *et al.* 2006). In a later life stage however, trampling of seedlings by large herbivores may be a main agent for mortality (e.g. in grazed mountain shrubland, Gómez *et al.* 2003).

Overgrowth of seedlings by neighbouring vegetation implies reduced light, space and nutrient availability (Vandenberghe *et al.* 2008). In our experiment, seedlings had the highest shoot growth in clipped plots, while there was no effect of trampling. Trampling had only temporarily effects : increased variation in sward height, reduction of sward cover and increased light penetration were found in May, but no more in September. Therefore, we suppose that trampling rather affects emergence than subsequent growth. Clipping increased light penetration during the whole growing season. Establishing seedlings tend to compete size-symmetrically with grass competitors early in development, but interactions between root and shoot competition could occur as woody seedlings become more dominant in the vegetation size hierarchy (Bloor *et al.* 2008). Clipping surrounding grass vegetation affects this competition and probably induces a size-asymmetric above ground competition for light (Weiner 1990). Our results suggest that emerging seedlings in clipped plots had a continuous advantage to grow under reduced light competition. Below-ground competition was probably low due to the nutrient rich seed bed plots and permanent water supply in our experiment. Under such reduced stress conditions, enhanced shoot or stem growth is to be expected (Davis *et al.* 1999; Husheer *et al.* 2006). However, the positive effect of a low surrounding grassland vegetation in our experiment may not be equally present in permanently grazed, natural conditions due to associated palatability (i.e. seedlings are eaten together with surrounding palatable grassland vegetation, Olff *et al.* 1999).

2.4.3 Seed-seedling conflicts in the recruitment process

In the light of definitive tree establishment, our results suggest that in grazed ecosystems, there is a discordant relationship between seedling emergence and seedling survival and growth in the process of tree recruitment. First, seed-seedling conflicts can occur in seed dispersal across a mosaic landscape, in which a trade-off mechanism optimises between predation avoidance (cf. *F. excelsior* with winged fruits) and nutritional support of the establishing seedling (cf. *Q. robur* with large and heavy seeds). Second, and similar to protective capacities of vegetation patches against grazing, environmental conditions (moist, light, nutrients) may favour the survival and growth of a seedling in some patches more than in others. Seed-seedling conflicts can occur during both of these phases of recruitment if (micro)sites or conditions that are suitable for seeds are unsuitable for seedlings (Schupp 1995). In grazed ecosystems, growth and survival of seedlings are highly restricted in vegetation types that favour emergence due to reduced competition (grassland, pioneer and tall herb vegetation). In contrast, unpalatable tall herb vegetation and scrub favour survival and growth due to associational resistance (Van Uytvanck *et al.* 2008b), but suppress emergence due to strong competition. Counteractive effects of grazing on different stages increase the complexity of the recruitment process and the uncertainty about the amount of individuals reaching the adult stage (Pérez-Camacho & Rebollo 2009). The complexity of tree regeneration in grazed ecosystems such as wood pastures was further elaborated by Smit *et al.* (2006), who found that removal of seeds was higher inside safe sites for seedlings than outside. Aggregated predation by different granivorous animals (insects, rodents, birds, cattle) were of great importance under nurse structures, that did not prevent but rather enhance seed predation. Therefore, safe sites that protect seedlings from grazing, by definition are not necessarily safe sites for seeds.

2.4.4 Conclusions

Interaction between large herbivore activities and flooding positively affects emergence of tree seedlings with variable recruitment strategies. However, the above mentioned conflicts represent just as much bottlenecks in the definitive establishment of woody species. They can have a major impact on the overall quantity of recruitment at the landscape level and on the distribution of recruitment among patches. Our data suggest that tree recruitment of palatable species in open landscape types, such as grazed floodplains, may start via microsite creation caused by large herbivore grazing and/or trampling. For subsequent growth and survival however, protective structures such as tall herb or scrub patches are needed. This implicates the need of a dynamic vegetation structure, offering changing patch qualities in space and time for tree species with different recruitment strategies (in our case *Q. robur* and *F. excelsior*). Large herbivores are able to regulate the balance between woody and herbaceous vegetation and maintain or develop fine-meshed (often mid-successional) mosaic landscapes including grassland, scrub, scattered trees and woodland patches (Bokdam & Gleichman 2000; Vera 2000; Hodder *et al.* 2005). The dynamic functioning of these landscapes may be explained by the shifting mosaics model of Olff *et al.* (1999), that is driven by large herbivores. We conclude that recruitment of native tree species assemblages may not only require an appropriate grazing regime, but also the restoration of natural disturbances, such as natural floods in floodplain areas.

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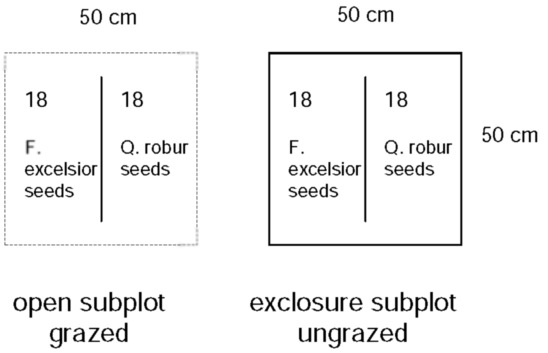
Appendix 2.1 :

Experimental set-up of the emergence-disturbance experiments in the field and in the greenhouse-garden-complex in Wenduine.

a. field experiment

Plot design



In 3 study sites, 4-5 replica's per structural vegetation type (5), were established, resulting in 44 plots with 3168 seeds

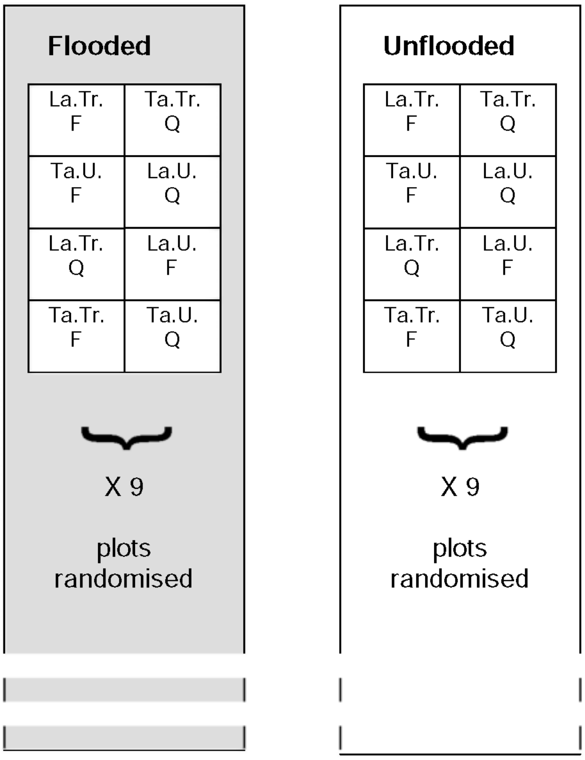
b. greenhouse-garden experiments

Preparation of seedbed plots.

Grass was sown in 144 seedbed plots in the greenhouse, before the planting of seeds (November 2006). Seedbed plots and were kept in the greenhouse till the end of February 2007.

Preparation of the grass swards in the greenhouse

Arrangement of the factorial randomised block design



Flooded

La.Tr. F	Ta.Tr. Q
Ta.U. F	La.U. Q
La.Tr. Q	La.U. F
Ta.Tr. F	Ta.U. Q

X 9

plots
randomised

Unflooded

La.Tr. F	Ta.Tr. Q
Ta.U. F	La.U. Q
La.Tr. Q	La.U. F
Ta.Tr. F	Ta.U. Q

X 9

plots
randomised

9 experimental blocks were flooded; 9 remain unflooded. Each block consists of 8 seed bed plots with three different treatments-species combinations. In each seedbed plot, 36 Q. robur acorns or F. excelsior fruits were planted.

La = Lawn (clipped) plot
Ta = Tall grass (unclipped) plot

Tr = Trampled plot
U = Untrampled plot

Fr = *Fraxinus excelsior*
Q = *Quercus robur*

Flooding treatment (3 weeks) was conducted in February-March 2007 and 2008 (for F. excelsior only). Clipping and trampliong treatments were conducted immediately after flooding.

emerged Quercus robur seedling in unclipped grass sward *uitvang(e), uitfange, uitfanck, utvang(he), uytvanck,…* :

n. m., part of the common, unreclaimed grounds.
zn. m., deel van de gemene niet ontgonnen gronden.

(Debrabandere 1993)

….. solitudinis seu deserti, quod teutonice vocatur *utfanc* vel *wostinia*

….. wildernesses or wastes, that the teutons (germanic people) called *utfanc* or *wostinia*
….. wildernis of woeste gronden, die de teutonen (germanen) *utfanc* of *wostinia* noemden.

(Charta Caroli Boni Comitis Flandriæ ann. 1119. apud Miræum tom. 1. pag. 680)

Chapter 3. Woodland regeneration via safe sites and time gaps

Van Uytvanck J, Maes D, Vandenhaute D, Hoffmann M, 2008. Restoration of woodpasture on former agricultural land: the importance of safe sites and time gaps before grazing for tree seedlings. *Biological Conservation* 141: 78-88. Reprinted with permission from Elsevier.

Abstract

Woodpastures (open, grazed woodlands with a mosaic of grassland, shrub and tree patches) are of high biological and cultural value and have become a threatened ecosystem In Europe. Spontaneous tree regeneration in the presence of large herbivores, is an essential process for management and restoration of this structurally diverse habitat. We examined the suitability of five vegetation types (grasslands, ruderal vegetations, tall sedges, rush tussocks and bramble thickets), grazed by large herbivores, for tree regeneration. We hypothesized that bramble thickets and tall herb communities operate as safe sites for palatable tree species through the mechanism of associational resistance. We set up a field experiment with tree seedlings in grazed and ungrazed conditions and recorded mortality and growth of seedlings of two palatable tree species (*Quercus robur* and *Fraxinus excelsior*) during three growing seasons. In the same experiment, we studied the effect of a two year's initial time gap before grazing.

Bramble thickets were suitable safe sites for survival and growth of seedlings of both species. Tall sedges, soft rush tussocks and ruderal vegetations with unpalatable or spiny species provided temporal protection, allowing seedlings to survive. Tree regeneration in livestock grazed grassland was highly constrained. Rabbits may undo the nursing effects of bramble thickets. The first year's survival is of major importance for the establishment of trees. Subsequent grazing affects growth rather than survival. A two year's initial time gap before grazing, had positive effects on survival, but did not enhance outgrowth of unprotected trees.

Key words

associational resistance; conservation management; low intensity grazing; large herbivores; tree regeneration

3.1 Introduction

Woodpastures are the product of a low intensity, often historical land use. Conservation values are high and include different species-rich vegetation types of heaths, wetlands, semi-natural grasslands, edge, bush and woodland communities (Pott & Hüppe 1991). Woodpastures are found throughout Europe and represent a vegetation structure, rather than being a particular plant community confined to a certain region. This vegetation structure consists of open woodland with scattered trees or forest patches in a matrix of grassland, tall grasses and shrubs (or heather). Large herbivores are part and parcel of the system, which is maintained by grazing. Healthy woodpastures should have a wide range of tree ages within them to ensure they survive in the future (Quelch 2001).

Since the beginning of agriculture in the Neolithic period, woodpasture has been the major form of woodland utilisation in Europe. Vera (2000) considers that this park-like landscape may be much older as a result of the natural interaction between wild large herbivores and vegetation development. Nowadays only remnants are left though, e.g. the New Forest in England and Börkener Paradies in Germany (Tubbs 1986; Pott & Hüppe 1991). On a smaller scale, woodpasture practices are maintained in the Alpine region in Austria, the Swiss and French Jura, the Vosges and the Massif Central in France and the dehesa ecosystems in Central-West Spain (Humphrey *et al.* 1998; Smit *et al.* 2006) or in nature reserves (e.g. Junner-Koeland in the Netherlands and a number of smaller remnants in Southern Scandinavia and the UK). In this respect, remnant woodpastures are not only important for their high biological values (Tubbs 1986; Vera 2000), they also represent landscape types with a high cultural-historical value (Rozas 2004; Manning *et al.* 2006). Heterogeneity in vegetation structure is an intrinsic feature of well developed woodpastures (Rosen & Bakker 2005) and is often linked with high floral and fauna biodiversity (Olff & Ritchie 1998; van Wieren 1998; Dennis *et al.* 1998).

The management of woodpastures has been characterised by low intensity grazing by domesticated herbivores, but other land use practices such as logging, coppicing, pollarding, hay-making, litter-collection and burning were also part of the management (Pott 1998; Bakker & Londo 1998). Despite this large variety of management measures, spontaneous regeneration of woody species seems not to be completely prevented. Understanding the operating mechanisms that lead to the typical vegetation and regeneration patterns is important with regard to conservation and restoration of woodpastures and the creation of comparably structured pastures today.

Recently, several efforts were made to restore or create woodpasture systems for conservation purposes in a broad range of nature reserves in Flanders (N-Belgium). In particular, former agricultural land, adjacent to existing nature reserves or forests, is often incorporated in restoration projects to create mosaic vegetations, with continuous gradients of open, half open to closed habitat types, i.e. from grasslands to forests. In the 1980s, this landscape type, with vegetation mosaics and continuous gradients, became a new frame of reference for conservation management in the Netherlands (Bakker & Londo 1998) and subsequently in the rest of Europe.

High stocking rates, as well as total abandonment may lead to a loss of spatial heterogeneity, and hence biodiversity (Kirby *et al.* 1995). A better understanding of the process of tree regeneration in grazed ecosystems is therefore essential for the continuation of a system that balances between suppression of woodland regeneration and succession towards closed woodland. In particular, little is known about shrub and tree regeneration under low-intensity grazing in restoration projects on former agricultural land (Olff *et al.* 1999). These early stages are important as they may determine the species composition and pattern of mid- and late-successional trees (Davidson 1993).

The key processes to be understood in grazed ecosystems which include woodland are plant strategies in coping with herbivory, herbivore selectivity and light requirements (Olff *et al.* 1999). Olff *et al.* (1999) presented a descriptive model of long-term cyclic succession, in which free ranging herbivores and alternating associational resistance and competition, play a crucial role. A core assumption in this model is that unpalatable, thorny or spiny shrub species create safe sites for the establishment of palatable tree species in a palatable grassland matrix. In a next stage, light demanding shrubs gradually disappear due to competition with growing trees. In a last stage, gaps created by falling trees, enable the re-establishment of grassland. Spatial asynchronisation of this cyclic mechanism causes shifting mosaics with patches of all structural vegetation types involved. Evidence for these succession mechanisms in the initial stages is accumulating (e.g. Callaway & Davis 1998; Callaway *et al.* 2000; Bokdam & Gleichman 2000; Rousset & Lepart 2003; Bossuyt *et al.* 2005), although experimental work is rather sparse (Callaway 1992, 2005; Rousset & Lepart 2000, Bakker *et al.* 2004; Smit *et al.* 2006).

As an alternative to associational resistance, temporal removal of large herbivores (either due tot a management decision or a natural crash in the herbivore population) may create a window of opportunity for woody species to establish (Peterken & Tubbs 1965; Olff *et al.* 1999; Mountford & Peterken 2003). Short time gaps (1-2 years) before grazing management often occur after cessation of agricultural use and before the start of conservation management. This may be the result of practical regulations or a carefully thought-out measure to trigger succession and tree regeneration. However the effects of such initial time gaps before grazing are poorly researched.

In this study, we focus on the initial stages of the establishment of woody species (mortality and growth of palatable tree seedlings of *Quercus robur* and *Fraxinus excelsior*) in grazed nature reserves on former agricultural land (no tree regeneration present before the start of conservation management). In general, we explore the interactions between grazing, time gaps and structurally different vegetation types in a broad geomorphological range on nutrient rich soils. We hypothesize that bramble thickets and tall herb communities act as safe sites during the establishment phase. We hypothesize that time gaps before grazing offer significant advantages to seedling survival and growth.

3.2 Methods

3.2.1 Study sites

We selected four nature reserves on former agricultural land for a field experiment in the southern part of Flanders (N-Belgium). Boelaremeersen and Rietbeemd are situated in the floodplains of the Dender basin and Bos t' Ename and Steenbergbos are situated on the drier slopes of interfluvial zones (Table 3.1). The former land use consisted of cattle breeding at all study sites and partly of arable farming (drier slopes).Grazing management started immediately after the cessation of agricultural use. In all sites, this was less than 10 years ago (Table 3.1). Since cessation of agricultural use, vegetation mosaics with patches of short grassland, tall herbs, tall sedges (*Carex sp.),* soft rush tussocks (*Juncus effusus*) and bramble thickets (4-600m^2) developed. On the drier slopes, the mosaics consisted of patches with ruderal pioneer vegetations (on former arable land), short grazed grassland and shrub thickets.

Table 3.1: Study site characteristics (seasonal grazing= May-October; Gr= grassland, Ju= *Juncus*, Ca= *Carex*, Ru= ruderal pioneer vegetation, Br= bramble, O= other; nat. regen.= natural regeneration with woody species; pat.= patchily and not connected, pat/con.= patchily and connected, cont.= contiguous; Ellenberg= Ellenberg indicator values L.O.I.= loss on ignition).

Study site	**Boelaremeersen**				**Rietbeemd**				**Bos t'Ename**				**Steenbergbos**			
Coordinates and size (ha)	50°78'N-3°88'E		9.2		50° 75'N-3°88E		22.1		50°86'-3°65E		39.5		50°78-3°78E		4.1	
Grazing type	seasonal (May-October)				year round				year round				seasonal (May-October)			
Starting year grazing management	2001				1997				1996				2001			
Large herbivores (nr. ha^{-1}y^{-1})	cattle (0.4)				cattle+horses (0.4)				cattle+horses (0.4)				cattle (0.5)			
Number of rabbit burrows ha^{-1}	0				0				0.7				2.3			
Soil type	clay				clay				sandy loam				clay			
Vegetation and soil type features	Gr	Ju	Ca	Br	Gr	Ju	Br	O	Gr	Ru	Br	O	Gr	Ru	Br	O
% cover per site	56.1	27.8	11.9	4.2	53.6	21.6	20.7	4.1	36.4	35.3	4.1	24.2	63.3	28.4	5.1	3.2
distribution	pat/con.	pat.	pat.	pat.	pat/con.	pat.	pat.	pat.	pat.	pat.	pat.	pat.	cont.	cont.	pat.	pat.
nat. regen. (saplings ha^{-1})	18.1	54.3	34.9	49.2	3.8	29.4	22.7	-	17.5	42.1	32.0	-	0.3	3.1	8.9	-
nr. of plots	5	5	5	5	4	4	4	-	4	4	4	-	4	4	4	-
soil moisture (Ellenberg)	6.2	6.98	8.78	6.40	6.23	6.95	6.45	-	6.23	5.13	6.03	-	6.38	5.05	6.43	-
pH (H_2O)	5.6	5.5	7.0	5.8	5.9	5.6	5.5	-	6.6	6.5	5.7	-	5.8	6.1	6.0	-
K (cmolc/kg)	0.18	0.18	0.21	0.22	0.19	0.16	0.26	-	0.49	0.38	0.37	-	0.40	0.34	0.16	-
Pextr. (mg/kg)	19.25	16.80	26.96	22.03	17.14	16.28	8.30	-	35.64	31.40	28.77	-	14.99	29.43	17.78	-
Ptot (mg/kg)	682.9	633.2	1463.1	860.8	684.1	737.1	557.6	-	729.4	406.8	643.5	-	426.2	451.6	451.3	-
L.O.I.-550°C (%)	10.12	11.11	13.56	10.74	10.81	9. 66	8.77	-	8.38	3.44	5.93	-	4.00	3.48	5.01	-
L.O.I.-900°C (%)	1.09	1.48	1.12	1.48	1.43	1.44	1. 21	-	0.96	0.60	0.74	-	0.88	0.93	0.90	-
NO_3^- (mgN/100g)	0.72	1.67	1.20	1.07	0.92	0.95	0.77	-	0.86	0.68	0.42	-	0.49	0.47	0.29	-
NH_4^+ (mgN/100g)	1.10	0.96	0.89	0.67	0.46	0.56	2.50	-	4.11	0.54	0.82	-	0.80	0.65	0.84	-

At all sites, sparse natural regeneration of woody species took place (sapling density is given in Table 3.1 (summarised personal data)). In the grassland, saplings of *Fraxinus excelsior*, *Quercus robur* and *Alnus glutinosa* are most frequent; on former arable land, sapling communities mainly consisted of *Salix caprea, Betula pendula*, *F. excelsior* and *Q. robur.* Scrub vegetation mainly consisted of bramble thickets (*Rubus sp.*) and small individual shrubs of spiny species (*Prunus spinosa* and *Crataegus monogyna*). Rabbits (*Oryctolagus cuniculus)* were present in sites on drier slopes and were nearly absent in floodplains. Occurrence of rabbits was estimated by counting burrows (autumn 2006). Burrows were only present in woodland and scrub vegetation. No roe deer were present in the sites.

3.2.2 Experimental design

We set up an experiment with seedlings of *Q. robur* and *F. excelsior* to study the interaction between vegetation type and grazing on the mortality and growth of seedlings (appendix 3.1). We established 56 plots, each divided in 2 adjacent subplots of 4 m^2. One subplot was grazed (from the beginning of May 2004 onwards), the other was protected from large herbivore grazing by a barbed wire exclosure (control subplots). Plots were established in five different vegetation types: short grassland (short, dense lawns), *Juncus* vegetation (high tussocks), *Carex* vegetation (high, no tussocks), ruderal pioneer vegetation (short, thin vegetation on former arable land) and bramble (high thickets) (Fig. 3.1).

Identification and location of the vegetation types in the field was mainly based on percentage coverage of dominant species and former land use (in case of arable land). *Juncus* plots were dominated by *Juncus effusus* (> 80%), *Carex* vegetations were dominated by tall *Carex species* (> 80%, dominated by *C. acuta*). Bramble vegetations consisted of various microspecies of the *Rubus fruticosus* group (>95%). In grassland plots *Poa trivialis* or *Holcus lanatus* were the dominant species. Ruderal pioneer vegetations were less homogeneous, with *Matricaria recutita, Poa annua, Conyza canadensis, Elymus repens, Sonchus oleraceus* and *Cirsium arvense* as most abundant species. In all study sites grassland formed the matrix, in which patches of the other types occurred, except for ruderal pioneer vegetations on former arable land, which were found in adjacent parcels. Plots were established in the centre of vegetation patches (> 100 m^2). The number of plots, cover percentages, and distribution of the vegetation types in each site are given in Table 3.1.

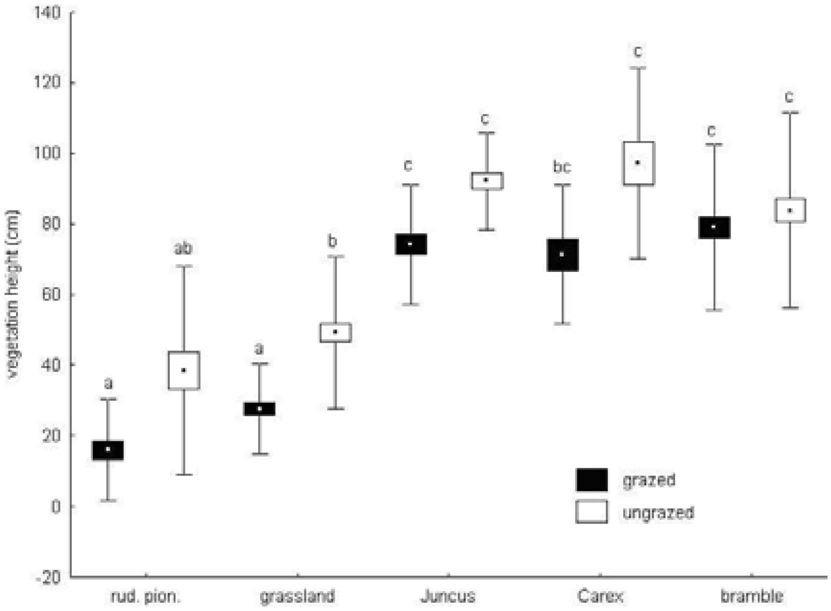


Figure 3.1: Average vegetation height (cm) of different vegetation types (divided in a grazed and ungrazed subset); point = mean, box = st. err., whisker = st. dev. Different letters indicate significantly different vegetation heights (Kruskal-Wallis Anova H(9, N=448) = 271.1; P<0.001; multiple comparisons: p<0.05).

In each subplot we planted 18 seedlings (nine *Q. robur* and 9 *F. excelsior*). We used 2 016 six month old seedlings, purchased from a commercial nursery. Seedlings had single stems with buds, but no leaves when planted (end of April 2004). Seedling length without roots varied from 20-40 cm. In each subplot, nine *F. excelsior* and nine *Q. robur* seedlings were planted in a grid, allowing each individual seedling to be located and measured repeatedly.

We measured seedling height (top apical shoot) monthly during the first year (May 2004 - April 2005). We recorded mortality (seedling disappeared or destroyed with dried out stem, apical shoot and buds) and damage, caused by large herbivore grazing, trampling, rabbit grazing and visual damage to leaves by insects, fungi and drought. Grazing damage by rabbits was easily distinguished from large herbivore grazing because rabbits cut seedling stems with a typical smooth, sloping sectional plane. Additional measurements of seedling height were done at the end of the second and third growing season in September 2005 and September 2006. In April 2006, poles and barbed wire of the exclosure subplots were removed to simulate a two year's initial time gap before grazing.

In September 2004, i.e. after the first growing season, we measured vegetation height at four fixed points fixed points in each subplot. Vegetation height was defined as the maximum height of the foliage at the point touched by a vertically held marked rod. It ranged from 15.8 (± 14.3) cm in grazed ruderal pioneer vegetations to 97.2 (± 27.2) cm in *Carex* vegetations (Fig. 3.1). Photosynthetic photon flux density (μmols photons $s^{-1}m^{-2}$) was measured on cloudless days in September 2004 at 40 cm (eight fixed points) and 150 cm (1 point without interfering vegetation) above ground level, using a photometer (Skye Instruments LTD, SKP 200). Light penetration in the vegetation was determined as the ratio between measurements at 40 and 150 cm. It was negatively correlated with vegetation height (Spearman rank order correlation: R= -0.54; P<0.001). Light penetration was significantly higher in ruderal pioneer vegetations, grasslands and grazed *Juncus* vegetation than in bramble and *Carex* vegetation (Kruskal-Wallis H(9, N=896) = 305.2; P<0.001).

To characterise each study site, one soil sample per vegetation type was taken in June 2004. This sample consisted of five bulked subsamples taken up to a depth of 10 cm. Inorganic nitrogen was determined as KCl-extractable nitrate (NO_3^-) and ammonium (NH_4^+). Resin-extractable phosphorus (P_{ext}) was analysed using the method of Lajtha *et al.* (1999). Total phosphorus (P_{tot}) was determined after microwave digestion of soils using ICP spectroscopy (Kuo 1996). Cation exchange capacity and concentrations of exchangeable cations (potassium) was determined according to the Silver-thiourea method (Pleysier & Juo 1980). Organic matter and carbonate contents were determined as weight loss after ignition (L.O.I.) at 550 and 900°C respectively. DCA-analysis of these soil characteristics revealed no clear distinction between vegetation types. Soil differences rather distinguished floodplains and drier slopes, the former having higher extractable NO_3^- -concentrations and higher organic matter content; the latter having relatively low P_{tot}-concentrations and higher extractable K-concentrations (Table 3.1).

Soil moisture was estimated using weighted averages of Ellenberg's indicator values (Ellenberg *et al.* 1992). Indicator values ranged from 5.1 to 8.8 (Table 3.1). With the exception of *Carex* plots (wet), all plots were in an ecological range from average moist to moist soils.

3.2.3 Data analysis

We used general linear mixed models to model growth (SAS 9.1, proc mixed). Generalised linear mixed models (SAS 9.1, proc glimmix) were used to analyse mortality. A random effects structure was build for both model types. A logit transformation was performed for binomial distributed data (mortality). In order to get insight in the complex data structure and the importance of the random factor "site", we initially analysed the first year's data of exclosure plots separately. Analyses of growth were performed for *Q. robur* and *F. excelsior* separately, because important differences in growth rate and regeneration capacity are intrinsic to the species (*F. excelsior* growing and regenerating faster). Negative growth means that an individual seedling is smaller than in the beginning of the experiment (time of planting). In the analyses, we entered 'vegetation type', 'treatment' (grazed vs. ungrazed), and 'species' (*Q. robur* vs. *F. excelsior*) as fixed factors. Time (1, 2 or 2.5 years) was included as a repeated measures factor. 'Site', 'plot' and 'individual seedling' were modelled as random factors, with individual seedling nested in plot and plot nested in vegetation type. The number of degrees of freedom was estimated using the method of Kenward & Roger (1997). Initial models included all variables and all interactions. Non-significant factors and interactions were gradually eliminated, using a backward procedure. AIC values for goodness of model fit (Akaike, 1987) were used to control, whether the model was improved or not after elimination. To control for independence of the two species, the experimental set up provided the same conditions in all plots. So, if there's any interaction, it will be the same everywhere. We could not test for independence statistically. Given the experimental set up and duration of the experiment, we didn't expect a significant influence. The influence of time gap treatment (2 years ungrazed, 1 season grazed) on mortality and growth was analysed in the same way, comparing it to the continuously grazed plots. Tukey-tests were performed to make multiple comparisons in all mixed model analyses.

3.3 Results

3.3.1 Random factors 'site' and 'plot'

We used a comparison of the control plot (ungrazed) data to assess the role of site and plot. We didn't find significant differences in mortality and growth in different vegetation types. In the mortality analysis, *Q. robur* had a significant higher mortality than *F. excelsior* ($F_{1,102}=5.45$; $p=0.02$). Random factors site and plot didn't contribute to the model in a significant way (covariance parameter estimates for site: $s^2 = 0.23$ (± 0.19 SE) and for plot: $s^2= 0.35$ (±0.28 SE)). Variation explained by random factors "site" and "plot" was constantly low (between 3.4 and 8.2%), compared to the residual variance.

3.3.2 Mortality

Depending on treatment (grazed vs. ungrazed), time (1-2.5 years), species and vegetation type, mortality varied between 0 and 75% (Fig. 3.2). The average mortality after 1 year was about 17.5% in ungrazed plots and 35.8% in grazed plots. Treatment and time were the most important factors, having significant interactions with vegetation type (Table 3.2). In ungrazed plots, mortality rate was highest in bramble plots, but for *Q. robur* not significantly higher than in other vegetation types; in grazed plots the highest mortality rate was found in grassland plots. In general, for both *Q. robur* and *F. excelsior,* mortality was higher in grazed plots. No significant differences in mortality, between grazed and ungrazed plots, were found in bramble (both species), *Juncus* and *Carex* plots (for *F. excelsior* only) (Fig.3.2).

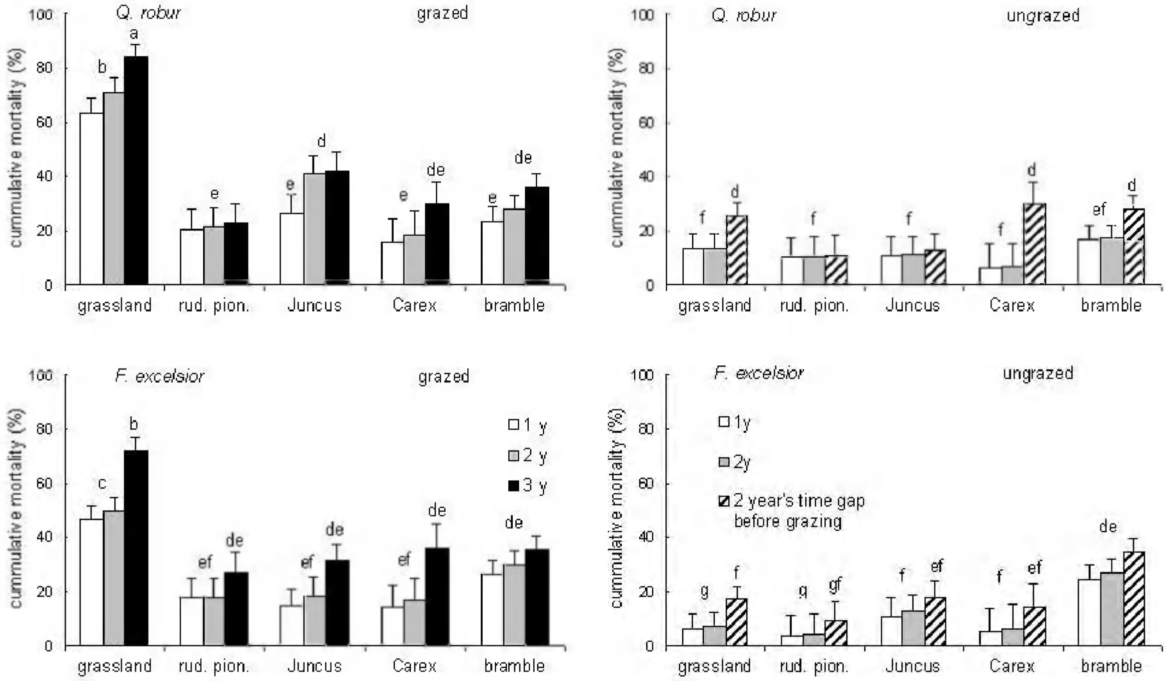


Figure 3.2: Cumulative mortality percentages (mean + st. err.) of *Quercus robur* and *Fraxinus excelsior* seedlings in different vegetation types, after 1st, 2nd and 3rd growing season and under different treatments. In the ungrazed plots, exclosures were removed after two years (time gap treatment). Grazing there started in the 3rd growing season (diagonal bars). Different letters indicate significantly different values (multiple comparisons with Tukey-tests, P<0.05).

Although mortality was higher in *Q. robur* than in *F. excelsior* (Fig. 3.2), differences in mortality frequency were low (on average less than 10%). This was the case for both grazed and ungrazed plots. Mortality generally increased with increasing grazing time. However, additional mortality in years two and three was always low compared to the first year's mortality (Fig. 3.2). In grazed plots, differences among vegetation types decreased with increasing grazing time, but not for grassland plots in which the mortality rate was significantly higher than in all other types.

Table 3.2: Results of the GLM-analysis with mortality (%) and growth as dependent and vegetation type (veg), treatment (treatm), time and species as independent variables. Time: 1 to 2.5 years (2 years for ungrazed plots).

	Factor	nom. d.f.	den. d.f.	F Value	Pr > F
Mortality	veg	4	19	1.29	0.308
	treatm	1	516	48.24	0.003
	time	2	516	66.79	<0.001
	species	1	516	4.17	0.042
	veg*treatm	4	516	16.07	<0.001
	veg*species	4	516	4.13	0.003
	time*veg	4	516	8.86	<0.001
	time*treatm	2	516	4.97	0.026
	treatm*species	4	516	5.06	0.025
Growth					
Q. robur	veg	4	97.5	5.59	<0.001
	treatm	1	2405	4.35	0.037
	time	2	2405	71.18	<0.001
	veg*treatm	4	2405	24.46	<0.001
	time*treatm	2	2405	114.35	<0.001
	time*veg	8	2405	6.93	<0.001
F. excelsior	veg	4	68.8	1.61	<0.182
	treatm	1	2405	7.94	0.005
	time	2	2405	103.95	<0.001
	veg*treatm	4	2405	41.23	<0.001
	time*treatm	2	2405	111.30	<0.001
	time*veg	8	405	3.89	0.004

Rabbit (*O. cuniculus*) grazing caused death of 6.3% of all seedlings in the experiment (= 1/5 of all died individuals). In the floodplains, 1.8% of all seedlings died due to rabbit grazing; on drier slopes, this was 12.9%. Mortality due to rabbits mainly occurred in bramble plots (87.5% of all cases). There were no differences due to rabbit grazing between *Q. robur* and *F. excelsior*, nor between grazed and ungrazed plots. Although rabbit densities and rabbit grazing damage differed considerably among sites, there were no significant differences in total mortality among sites after 2.5 years.

Drought caused death of 0.9% of all seedlings (= 2.6% of all died individuals) and was only observed in Steenbergbos and Boelaremeersen.

3.3.3 Growth

Average growth ranged from -17 to +52 cm for *F. excelsior* and from -36 to +52 cm for *Q. robur* (Fig 3.3). For both *Q. robur* and *F. excelsior*, treatment, time, vegetation type (not for *F. excelsior*) and the two-way interactions between these factors were significant (Table 3.2). Negative growth in this context means a lower seedling height compared to the height at the time of planting. Comparing both treatment types, a significant lower average growth was found in grazed plots, except for *Q. robur* in bramble and one year grazed *Carex* plots and for *F. excelsior* in bramble, *Carex* and one year grazed *Juncus* plots (Tukey-test: all P>0.05, Fig. 3.3).

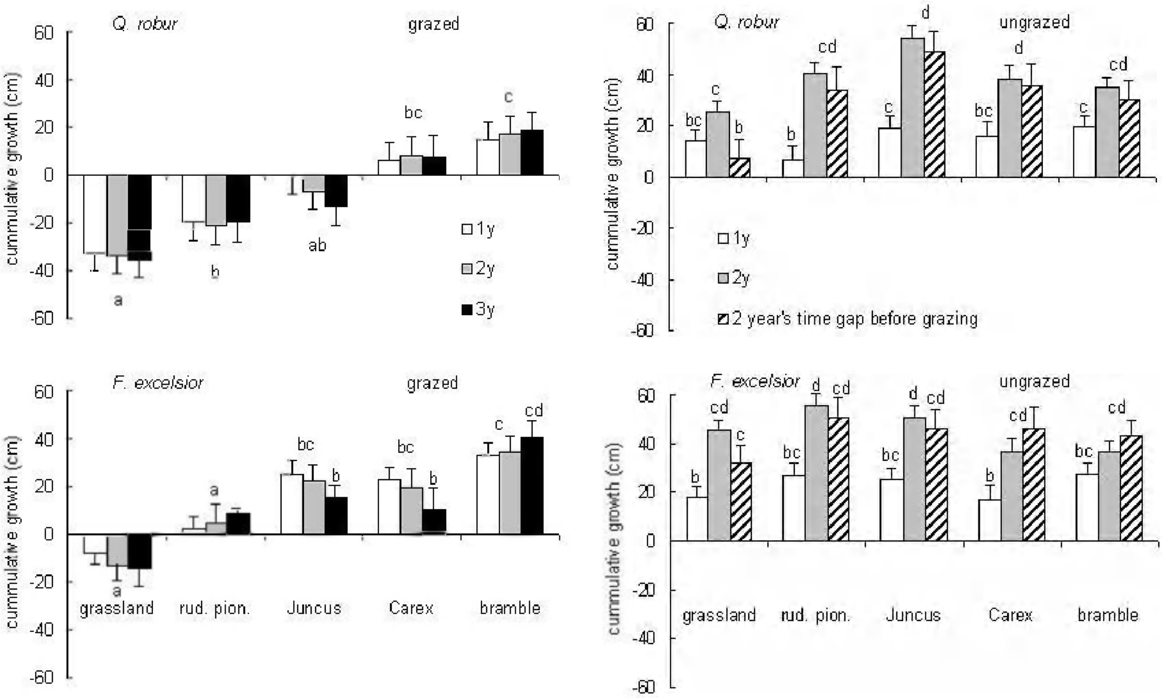


Figure 3.3: Cumulative growth (mean + st. err.) of *Quercus robur* and *Fraxinus excelsior* seedlings in different vegetation types, after 1st, 2nd and 3rd growing season and under different treatments. In the ungrazed plots, exclosures were removed after two years (time gap treatment). Grazing there started in the 3rd growing season (diagonal bars). Mean negative growth occurs when the mean height of seedlings is lower than the mean height at the starting point of the experiment. Different letters indicate significantly different values (multiple comparisons with Tukey-tests, P<0.05).

The effects of vegetation type were mainly significant in grazed plots. The highest average growth was found in bramble thickets for both species. For *Q. robur*, growth was on average negative in grassland, ruderal pioneer vegetations and *Juncus* vegetation (Fig. 3.3). For *F. excelsior*, average growth was negative in grassland and low in ruderal pioneer vegetation. *Carex* and *Juncus* vegetations took an intermediate position, with a significantly higher seedling growth compared to grassland and ruderal pioneer vegetation, but no significant differences with growth in bramble (except for 2.5 years grazed *Carex* and *Juncus* plots, Fig.3.3).

In ungrazed plots, growth increased with increasing time (data for two years only) in all cases (Fig. 3.3). In grazed plots, differences in growth among different grazing times were small. Even in vegetation types with a positive average growth in subsequent years, no significant differences were found among different grazing times (Tukey-test: all P>0.05).

The number of individuals able to grow, varied in different vegetation types. Percentages were low in grassland for both *Q. robur* and *F. excelsior* and in *Juncus* vegetation for *Q. robur* (± 9-24%) compared to other vegetation types (± 40-70%).

3.3.4 Time gaps

A two year's time gap before subsequent grazing (during 6 months) reduced mortality of seedlings in a significant way, compared to the 2.5 years continuous-grazing treatment, in grassland and ruderal pioneer vegetation for both tree species and in *Juncus* plots (for *Q. robur* only). Among other vegetation types, there were no significant differences, but in general, mortality was lower in the plots with a grazing time gap.

Time gaps resulted in a significant higher growth of *F. excelsior* (32.4 – 50.9 cm) and *Q. robur* (7.6 – 49.2 cm) compared to the 2.5 years grazing treatment (Fig. 3.3). However, no significant differences were found in bramble thickets (Tukey-test: all P>0.05). Among different vegetation types with the time gap treatment, differences in growth were small. A positive average growth after opening the exclosure plots was only observed in bramble and *Carex* plots (*F. excelsior*, on average 4.9 and 8.2 cm respectively). In grassland, average growth decreased significantly for *Q. robur*; in the other vegetation types growth came to a standstill for both tree species.

3.4 Discussion

Olff *et al.* (1999), Vera (2000) and Callaway *et al.* (2000) described the mechanism for diverse ecosystems, but stressed the need for an experimental basis. This study provides experimental evidence for the mechanism of associational resistance on nutrient rich soils in a broad geomorphological range. The mechanism was effective in both seasonal and year-round grazed ecosystems. Foraging on tree seedlings was largely restricted to spring and summer, when seedlings had nutritious buds and green leaves and, as a consequence, no differences were found between those two grazing management strategies (Van Uytvanck, unpublished data).

3.4.1 Bramble and tall herb patches as safe sites

Large herbivore grazing is an important factor causing death of seedlings. In grazed grasslands, survival of seedlings was highly constrained by grazing and browsing, whereas competition for light, rabbit grazing and, to a lesser extent, drought caused death in ungrazed circumstances. In natural and semi-natural circumstances, these stress factors were found to be equally or even more important than grazing (Frost & Rydin 1997; Pépin *et al.* 2006). In woodpastures in the Swiss Jura, desiccation of seedlings was the most common cause of seedling death (Vandenberghe *et al.* 2006). In the same area, Smit *et al.* (2006) found that grazing was the major cause of death, except for saplings planted near unpalatable *Gentiana* species. In the absence of grazing, we found the highest mortality of seedlings in the relatively dark bramble thickets. Kelly (2002) found that, in the absence of large herbivores, light was the limiting factor for tree regeneration (*Quercus petraea*) in grazed woodlands. Our results suggest a shift from light deficiency to large herbivore grazing damage as the limiting factor for survival of seedlings in grazed mosaic vegetations.

Highest mortality (± 90%) appeared during the first year, which corresponds with results of De Steven (1991a). In subsequent years, surviving seedlings became stronger and less vulnerable to grazing damage. As a consequence, the structural features and the ability of vegetation types to offer protection immediately after the germination of tree seedlings, is, to a large extent, determining the survival of these seedlings.

Large grazers prefer short vegetation types with sufficient nutrient rich leaves (WallisDeVries *et al.* 1999). Associational palatability caused a very low survival rate of tree seedlings in our grassland plots. Our results showed that in the unpalatable and taller *Juncus* and *Carex* vegetations, seedlings were able to become larger and, as a consequence, had more chance to survive. This corresponds with the findings that tall forbs and dense vegetations can facilitate the establishment of seedlings (De Steven 1991a; Kollmann & Schill 1996; Negi *et al.* 1996; Callaway *et al.* 2000). Unpalatable species can do the same (Callaway *et al.* 2005; Smit *et al.* 2005; Smit *et al.* 2006). Pollock *et al.* (2005) found that browsing intensity on saplings was negatively related to good quality biomass available for herbivores and adjacent vegetation height. In this study, *Carex* vegetations, which form high and dense vegetations, were only used as food supply in dry periods (Van Uytvanck, personal observations). Pelster *et al.* (2004) found reduced willow consumption in tall sedge vegetations higher than 20 cm. *Juncus effusus* is an unpalatable species with low nutritional value (Hopkins & Peel, 1985), which is only eaten in wintertime when food supply is low. On former arable land, a sparse vegetation with prickly or spiny and unpalatable forbs was present. It did not attract herbivores, but seedlings were easily perceptible. Prickly species (*Cirsium arvense* and *Urtica dioica*) began to dominate two or three years after abandonment. Associational resistance possibly explains why an increasing number of *F. excelsior* seedlings was able to grow in these vegetations types in year two and three. However, the studied tall herb and ruderal communities only offer growth possibilities to a certain extent. Kuiters & Slim (2003) also found rush tussocks and heather dwarf shrub patches, facilitating *Q. robur* and *Frangula alnus* regeneration in abandoned and grazed arable land on poor sandy soils. But even after 30 years, few saplings had grown beyond the browse-line.

In the presence of large herbivores, spiny shrub species are thought to be the most logical safe sites for trees (Callaway 1992; Callaway & Davis 1998; Castro *et al.* 2004). Kuiters & Slim (2003) demonstrated the important role of bramble thickets for tree regeneration in abandoned fields. Bakker *et al.* (2004) found *Prunus spinosa* to be an effective safe site for *Q. robur* in woodpastures. Clonal expansion by root suckers can form dense patches of spiny shrub thickets, which grow out in a horizontal direction. A comparable mechanism operates with *Rubus* species, which grow out with above ground suckers, forming large and dense thickets. Our experiments confirm the effective protection that bramble thickets provide against large herbivores. Single thorny shrubs such as *Crataegus monogyna*, may not provide sufficient shelter for seedlings and young trees because growth mainly follows a vertical direction. Moreover, thorns are only formed after one year. Although all woody species are vulnerable to grazing at the early establishment phase, the fast growth of *Rubus* sp. on former agricultural land enables the formation of protective thickets much earlier, compared to slower growing *Crataegus* species and *Prunus spinosa*. This is important because shrub thickets have to be large enough to function as an effective barrier for grazers (Gómez *et al.* 2001)

On the other hand, dense or high vegetation types are a safe and attractive environment for small herbivores (e.g. small rodents like voles), which consume seedlings and seeds (Ostfeld & Canham 1993; Weltzin *et al.* 1997; Hubbard & McPherson 1999). In our study, rabbits (*O. cuniculus*) were the most important small herbivores. In riverine floodplains Bakker *et al.* (2004) found that main differences in tree regeneration were due to differences in rabbit densities. *Q. robur* could not be protected by *Prunus spinosa* thickets against rabbits. High densities of rabbits are able to completely prevent regeneration of woody species in abandoned fields (Oosterveld 1983). Small herbivores like red-bellied pademelons (*Thylogale billardierii)* even select seedlings to consume in an unpalatable neighbourhood (Miller *et al.* 2007). At our study sites, rabbits were present in low densities, but burrows were concentrated in bramble thickets. Rabbits also preferred foraging on tree seedlings in the protective thickets above foraging in open vegetation types, where grazing damage was nearly absent. In bramble thickets, rabbit grazing was the major factor causing mortality of seedlings. However, it is still possible that in the end, seedling mortality in bramble thickets mainly depends on light conditions since in our study, after 2.5 years, there were no significant differences in mortality among bramble plots with high or low rabbit densities. Probably rabbit grazing only accelerates seedling mortality in bramble thickets, while mortality due to unfavourable light conditions, is a slower process.

Whether enhanced establishment in the protective vegetation types will finally lead to a desired woodpasture vegetation structure is not yet clear. Our experiment lasted three growing seasons, but the natural regeneration pattern of woody species in Bos t' Ename, Rietbeemd and Boelaremeersen (grazed since 1996, 1997 and 2001 respectively) generally confirms the role of the studied vegetation types: grown out trees in bramble patches, short grazed trees in *Juncus* and *Carex* patches. However, very low absolute numbers (< 5 ha^{-1}) of grown out trees were present (Table 3.1).

3.4.2 Time gaps

To maintain woodpastures, a balance between consumption and regeneration of tree species is needed. In woodpastures, regeneration capacity is an important feature for woody species. *F.*

excelsior regenerated faster and better (possibly after successive grazing events in one season) than *Q. robur.* As a consequence, *F. excelsior* is able to persist in vegetation types where grazing pressure is moderate (tall herb vegetation), whereas *Q. robur* clearly needs more protection. Only reduced grazing pressure or time gaps in grazing management may finally lead to successful establishment of palatable species outside shrub thickets (Mountford & Peterken 2003). Kuiters & Slim (2002) argue that browse-sensitive woody species such as *Quercus* species will successfully regenerate, only if temporal and spatial variation in browsing pressure can occur. If not, woodland regeneration on abandoned land may take several decades (Smit & Olff 1998; Harmer *et al.* 2001). Vera (2000) opposes this idea, assigning *Quercus sp.* to the browse-tolerant trees.

Because of the proven importance of the seedling's first year, a time gap before grazing had a large positive impact on seedling survival. However, a period of two years seems to be too short to enable a sufficient outgrowth. In Australian rangelands, sheep and cattle entered tall vegetations after the removal of exclosures. Damage to juvenile trees was due to sheep grazing, cattle trampling and breaking stems (Clarke 2002). Extending time gaps before grazing may be an option, but in species rich grasslands it carries the risk of biodiversity loss due to the strong expansion of tall herbs (Bakker *et al.* 2002).

3.4.3 Conclusions and applications for conservation management

Restoration of woodpastures, departing from agricultural land, starts with the process of woody species regeneration. Apart from dispersion (Kollmann & Pirl 1995) and germination (De Steven 1991b), establishment of woody species faces a double bottleneck: survival and outgrowth. The combination of safe sites and specific management measures can overcome these initial bottlenecks.

Moderate grazing induces the development of diverse vegetation pattern in a few years' time (Van Den Bos & Bakker 1990). In this pattern, bramble thickets play a key-role: they function as safe sites in which palatable tree species can survive and grow out. Tall herb and ruderal vegetations may be temporal safe sites for woody seedlings: survival is enhanced, but outgrowth will only be possible in periods with low or temporally absent grazing pressure. In homogeneous grassland, woodland regeneration is almost impossible, even with very low herbivore densities.

Initial time gaps before grazing may enhance regeneration success and accelerate the restoration process in woodpastures. However, conservation managers should find a good balance between the conservation of present grassland biodiversity and spontaneous regeneration of woody species.

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Appendix 3.1 :
Experimental set-up of the safe site & time gap field experiment

a. Plotdesign

Fraxinus excelsior (F) and *Quercus robur* (Q) seedlings were planted in a fixed grid in adjacent 2m x 2m subplots; one open subplot and one exclosure subplot.

2m

Q	F	Q
F	Q	F
Q	F	Q
F	Q	F
Q	F	Q
F	Q	F

open subplot
grazed

2m

Q	F	Q
F	Q	F
Q	F	Q
F	Q	F
Q	F	Q
F	Q	F

2m

exclosure subplot
ungrazed

In 4 study sites, 4-5 replica's per structural vegetation type (5), were established (spring 2004), resulting in 56 plots with 2016 planted seedlings.

Exclosures were removed in April 2006 (time gap = 2 years)

b. Examples

Grassland plot Bos t' Ename ± one year after planting; seedlings start growing beyond the grassland canopy in the exclosure subplot (June 2005.)

Plot in bramble scrub thicket in Rietbeemd (May 2005).

Hardly surviving and growing Homo sapiens, searching for saplings in a bramble scrub patch.

Chapter 4. Woodland regeneration on former arable land

Van Uytvanck J, Van Noyen A, Milotic T, Decleer K, Hoffmann M. Woodland regeneration on grazed former arable land: a question of tolerance, defence or protection? *Journal for Nature Conservation*, in press. DOI: 10.1016/j.jnc.2009.10.001. Reprinted with permission from Elsevier.

Abstract

Ecological restoration of native woodlands and wooded pastures on former agricultural land is an important topic in modern conservation practice. The introduction of large herbivores is increasingly used to achieve these aims. We investigated how grazing, resistance traits of plants (concerning herbivory) and associational resistance interact and affect the establishment pattern of woody species on abandoned arable land (N-Belgium, W-Europe). In these early successional tree assemblages, we tested whether grazing increased or decreased spatial heterogeneity, which is supposed to be a crucial factor for biodiversity.

With repeated measurements, 2-3 and 5-7 years after the cessation of agricultural use, we sampled 87 grazed and 56 ungrazed plots (314m^2) in 14 sampling areas (former arable land) on nutrient rich, (sandy) loam soils. We recorded established tree frequencies, related to grazing, time, resistance traits and unpalatable/spiny vegetation cover in the herb and low shrub layer. We investigated horizontal and vertical heterogeneity using variances in establishment frequencies and variances in frequencies of trees that were able to grow beyond the browse line, respectively.

We found massive colonisation of grazing tolerant and resistant woody species in early successional stages. Grazing decreased frequencies and height of the most abundant tolerant species (mainly *Salix caprea* L.). After 5-7 years, frequencies of defensive and tolerant species were equal, but the former (mainly *Betula pendula* Roth) were able to grow beyond the browse line. When the cover of unpalatable/spiny vegetation was high enough (>60% of plot size), it provided also suitable nurse sites for tolerant species to grow out. In early assemblages, grazing increased horizontal and vertical heterogeneity, resulting in intermediate successional stages. On the longer term, the mechanism of associational resistance will also allow non-resistant and tolerant species to grow beyond the browse line and promote forest succession and the order of species establishment and replacement.

Key words

Associational resistance; Browse line; Large herbivores; Low intensity grazing;

Pioneer tree assemblages; Resistance traits; Spatial heterogeneity;

Tree and shrub recruitment and establishment

Bushy growth of havily grazed Salix caprea in a fine meshed mosaic vegetation on former arable land (Bos t' Ename, Oudenaarde, Belgium).

4.1 Introduction

Worldwide, environmental and socio-economic changes are leading to increased levels of land abandonment. In non-tropical regions, large areas of former agricultural land are subject to afforestation, both spontaneously (2.6 million ha year $^{-1}$) and through planting (0.7 million ha year^{-1}) (FAO 2001). Given the extent of afforestation, the recovery of forest plant communities is an important topic both for managers and ecologists (Flinn & Vellend 2005). The intended and natural restoration of forest communities may also yield insights into fundamental questions on how plant species disperse and recruit and on how long it takes for forests to recover from the impact of former agricultural land use (Dupouey *et al.* 2002). Understanding the development of plant communities on abandoned arable land will become increasingly important from a conservation, restoration and social perspective and will pose important scientific and policy challenges (Benjamin *et al.* 2005; Cramer *et al.* 2008).

Since the nineteen eighties, there is an increasing interest (and debate) concerning the role of large herbivores into native woodland restoration. Central issues in this debate are the ability of large herbivores to maintain (half)open landscapes and their role in natural regeneration of woodland (Kirby et al. 1994; Vera 2000, Birks 2005; Mitchell 2005). The role of grazing animals in the past has probably been underestimated, both in the former wildwood, but also in ancient woods of all types (Goldberg et al. 2007). Opinions on these topics are contradictory and therefore, the translation to conservation management policy is difficult and ambiguous. Nevertheless, in temperate Europe, large herbivores (wild or livestock) already have been (re-)introduced in a large number of nature reserves (often on former agricultural land) to stop or retard succession, restore (semi-)natural disturbances, initiate patch dynamics or enhance the dispersal of propagules (Olff *et al.* 1999, Mouissie *et al.* 2005; Cosyns *et al.* 2006; Van Uytvanck *et al.* 2008b).

In this context, managers expect that large herbivores may also enhance spatial heterogeneity (both horizontally and vertically) and biodiversity in developing woodlands on former arable land, where undisturbed (ungrazed) secondary succession may lead to even-aged, uniform stands of wind-dispersed pioneer tree species (Rankin & Pickett 1989). However, literature on succession on grazed former arable land is scarce and almost restricted to nutrient poor soils (Van den Bos & Bakker 1990; Giourga *et al.* 1998; Kuiters & Slim 2003). In disturbed (ploughing or other ground works) and fertile, but ungrazed agricultural sites, usually convergent successional seres are found. For ecological restoration however, it is desirable that successional variability is high both within one locality and over a larger geographical scale (Prach 2003). A better insight into the nature of the early successional stages under the influence of vertebrate herbivory is important because at this stage, herbivore impact on species change is large, while it gradually declines when succession continues. Species diversity mainly depends on the competitive ability and survival of seedlings or saplings, not or to a lesser extent on its adult characteristics (De Steven 1991; Walker & del Moral 2003).

Our own and other author's observations on pioneer tree colonisation on bare, fertile soils reveal a very fast process that starts immediately after crop harvesting. It occurs simultaneously with the development of a herbaceous layer and results in massive establishment of mainly wind dispersed *Betula* L. spp. and *Salix* L. spp. (Osbornová *et al.* 1990; Dölle & Schmidt 2009). In general, successional changes on old fields have been interpreted in terms of competitive ability mediated by resource availability, in particular light and nutrients (Tilman 1985; Tilman 1994). It is to be expected that grazing and browsing by large herbivores and resistance traits of plants (i.e. escape, defence and tolerance, Boege & Marquis 2005) will affect competition among newly establishing plant species and, as a result also the establishment pattern of woody species (Smit 2002). Tolerance to herbivory can play an important role in explaining relative abundance patterns of plant species in grazed grasslands (Crawley 1990; del-Val & Crawley 2005). The ability to compensate for tissue loss may gain a competitive advantage over accompanying plant species, that die or regenerate only slowly after browsing (Grime 2001). The compensatory continuum hypothesis (Maschinski & Whitham 1989) predicts that the probability of compensation for herbivory (i.e. tolerance) increases with increasing resource levels and decreasing competition. This may be of particular interest for establishment on recently abandoned arable land, where grazing-tolerant tree species are favoured by high nutrient levels due to the after-effect of fertilizers in the soil and initially low competition due to the absence of a perennial herbaceous layer. On the other hand, there are some other mechanisms, which may explain establishment patterns of woody species. First, preferential grazing on dominant tree species suppresses their competitive ability, rather than their differential tolerance ability (Tahmasebi Kohyani *et al.* 2009). Therefore, the effects of herbivory should be related to its differential impact on competing species (Augustine & McNaughton 1998). Second, woody species with defensive traits (secondary metabolites, spines or thorns) may be favoured due to reduction or avoidance of grazing damage (Anderson & Briske 1995). It is suggested that tolerant and defensive plants should succeed equally well in grazed ecosystems (van der Meijden *et al.* 1988; Mauricio *et al.* 1997). Third, the mechanism of associational resistance allows non-resistant species to establish and survive. For woody species, protective vegetations consisting of spiny scrub, unpalatable or spiny tall herbs, rocks or hindering branches provide safe sites for regeneration (Bakker *et al.* 2004, Smit *et al.* 2005, Van Uytvanck *et al.* 2008b).

In this study, we focus on the mechanisms and patterns of early establishment of woody species on recently abandoned arable land. We expect that competition, herbivory, resistance traits and associational resistance interact and affect the early recruitment pattern, growth and heterogeneity of woody species assemblages, leading us to the following hypotheses.

(1) *grazing and resistance traits*: Large herbivore grazing or browsing decreases establishing tree frequencies and affects relative occurrence according to their resistance traits. We expect that tolerant or defensive species will dominate early succession patterns (i.e. in the first years after abandonment). If grazing continues we expect that, compared to grazing-tolerant species, relatively more individuals of defensive species will grow beyond the browse line.

(2) *grazing and associational resistance*: Protection by unpalatable or spiny plants in the herb and low shrub layer may interact with this process. With increasing cover of unpalatable/spiny vegetation, total frequencies of non-resistant trees and the relative frequencies of tolerant and non-resistant trees that grow beyond the browse line, will increase.

(3) *Spatial heterogeneity*: Variation in frequencies (horizontal heterogeneity) and in frequencies of juvenile trees that grow beyond the browse line (vertical heterogeneity) is higher in grazed than in ungrazed areas, as a consequence of differential grazing and browsing and the suppression of dominant species.

4.2 Methods

4.2.1 Study sites and sampling areas

We collected data in eight study sites in the northern part of Belgium, in which we marked out 14 sampling areas on former arable land parcels: nine grazed sampling areas and five ungrazed sampling areas (Table 4.1). All study sites were nature reserves situated in a hilly landscape, characterised by outcropping aeolian loam and sandy loam. The climate is temperate; annual precipitation is ±700mm.

At all study sites, former land use was arable farming combined with cattle breeding until recently (Table 4.1). After the cessation of agricultural use, nature management mainly consisted of low intensity grazing, mowing, conversion of tree plantations or spontaneous afforestation. Low intensity grazing with livestock (cattle, horses) was applied in newly established paddocks that contained former arable land, grassland and at two sites also forest (Table 4.1). In three study sites, one paddock was established; in Sulferberg and Moenebroek there were two and three separate paddocks, respectively. At four study sites (5 sampling areas) in the same region (Table 4.1), former arable land parcels developed spontaneously, in the absence of cattle or horses. These sites were used to establish control sampling areas.

Grazing management aimed at the development of species-rich vegetation mosaics, with woodland expansion on former arable land and grassland. Livestock, as a substitute for extinct wild large herbivores which occurred naturally in the European landscape, was introduced to guide this process. At all sites, natural regeneration of trees and shrubs on former arable land, started the first year after abandonment. Two grazing systems were applied at the study sites, both concerning free ranging animals in unpartitioned pastures (=paddocks): year-round, where animals remained in the paddock during the whole year and seasonal, where grazing is restricted to spring and summer (from the beginning of May till the end of October). In all paddocks, low intensity grazing started immediately after the cessation of agricultural use. Grazing intensity ranged from 78 to 225 livestock unit grazing days $ha^{-1}yr^{-1}$ and was fairly constant at every individual site. Cattle were used in all paddocks; at Bos t' Ename two Konik horses were added to the cattle herd. No other large vertebrate grazers or deer were present in the paddocks. Rabbits were present at most study sites in low densities (< 1 burrow per ha).

4.2.2 Data collection

We collected data on established tree frequency and height, treatment (grazing or not), time, resistance traits and cover of unpalatable/spiny plants in the herb and low shrub layer. In a preceding, exploratory study, we assessed the role of (distance to) potential seed sources, using a Global Positioning System (Garmin GPSMAP76). GIS (ArcGis9) analyses and Spearman Rank Order

Table 4.1: Characteristics of study sites and sampling areas. BE: Bos t' Ename; AB= Altenbroek; MB= Moenebroek; BB= Boembeke; SU= Sulferberg; BR= Broekelzen; BU= Burreken; TR= Trimpont. Grazing type: S= seasonal grazing (May-October); Y= year-round grazing. Large herbivores: C=Cattle; H=Horses. LU= livestock unit (1 LU = 500 kg body weight).

		grazed									ungrazed				
study sites / sampling areas		**BE1**	**BE2**	**AB**	**MB1**	**MB2**	**MB3**	**BB**	**SU1**	**SU2**	**BR**	**BU**	**TR**	**BE3**	**BE4**
coordinates	N	50°51′	50°51′	50°45′	50°48′	50°48′	50 °48′	50°50′	50°47′	50°47′	50°47′	50°48′	50°46′	50°5 1′	50°51′
	E	3°38′	3°38′	5°48′	3°52′	3°53′	3°52′	3°46′	2°46′	2°46′	2°44′	3°44′	3°46′	3°38′	3°38′
dominant soil type		sandy loam	sandy loam	loam	loam	loam	loam	loam	loam	loam	loam	loam	loam	sandy loam	sandy loam
size grazed paddock (ha)		61.5	61.5	44.5	2.3	12.0	7.8	5.6	1.9	1.7	-	-	-	-	-
size sampling area (ha)		1.6	3.8	3.2	0.7	0.6	0.9	0.9	0.6	0.6	1.0	0.6	0.8	1.2	0.4
forest adjacent to sampling area		yes	yes	yes	no	yes	yes	no	yes	yes	yes	yes	yes	yes	yes
grazing type (free ranging)		Y	S	Y	S	S	S	S	S	S		-	-	-	-
large herbivores		C+H	C+H	C	C	C	C	C	C	C	-	-	-	-	-
no. of LU grazing days ha^{-1} yr^{-1}		146	146	78	157	218	146	164	225	175	-	-	-	-	-
number of plots in sampling area		8	16	12	7	9	11	7	7	10	12	12	12	12	8
cessation of arable farming		2003	2003	1998	1993	1999	2002	2000	1994	1994	1999	1999	1999	2003	2003
sampling years															
first sampling		2005	2005	2001	1996	2001	2005	2003	1997	1997	2002	2002	2002	2005	2005
second sampling		2008	2008	2005	2000	2005	2008	2007	2001	2001	2005	2005	2005	2008	2008
% of habitat types in paddocks															
former arable land		17.5	17.5	15.5	30.4	17.3	23.6	16.1	56	48	-	-	-	-	-
grassland		41.6	41.6	11.8	69.6	82.7	76.4	83.9	44	52	-	-	-	-	-
forest		40.9	40.9	72.7	-	-	-	-	-	-	-	-	-	-	-

Correlation revealed that, in the ranges investigated (0-200m), and for the six woody species that established in more than 40 plots (*Salix caprea* L., *Salix alba* L., *Betula pendula* Roth, *Alnus glutinosa* L., *Quercus robur* L. and *Crataegus monogyna* Jacq., counting for more than 90% of all individuals), frequencies per plot were not correlated with distance to seed source (Van Uytvanck, unpublished data). Therefore, we did not include potential seed source distance in further analyses.

GIS (ArcGis9) analyses and Spearman Rank Order Correlation revealed that, in the ranges investigated (0-200m), and for the six woody species that established in more than 40 plots (*Salix caprea* L., *Salix alba* L., *Betula pendula* Roth, *Alnus glutinosa* L., *Quercus robur* L. and *Crataegus monogyna* Jacq., counting for more than 90% of all individuals), frequencies per plot were not correlated with distance to seed source (Van Uytvanck, unpublished data). Therefore, we did not include potential seed source distance in further analyses.

We sampled the study sites in the period 1997-2008. At each study site, we conducted field work during two different sampling periods: i.e. 2-3 years and 5-7 years after the cessation of agricultural use (Table 4.1). The first sampling period provided an insight in the initial establishment pattern of woody species. The combination with data of the second period allowed us to analyse time effects and the pattern in which those species were able to survive and grow (beyond the browse line) in ungrazed and grazed circumstances respectively.

At grazed study sites, we selected one sampling area per paddock (except for Bos t' Ename, where two spatially separated sampling areas were selected in the same paddock). At ungrazed sites, also one sampling area at a former arable land parcel was established. Grazed and ungrazed sampling areas were situated in different study sites, except for Bos t' Ename where two ungrazed exclosures, adjacent to the paddock, were used. A selected sampling area was always a representative surface of a former arable land parcel (at both control and grazed study sites), with one and the same crop history, exposition and micro relief. Therefore, not all sampling areas had the same size: surface ranged from 0.6 – 3.8ha. Per sampling area, we set out 2-4 linear transects (evenly spread and ranging between 60 and 200m length) on which we established circular plots with a radius of 10m. Transects, and the centres of plots along transects, were 20-40m apart. We tried to include the present variation of the whole sampling area.

We sampled the same 143 plots, 87 in grazed and 56 in ungrazed sampling areas, in both sample periods. We made one vegetation survey per plot, in which we estimated the cover of six functional vegetation classes in the herb and low shrub layer: bare ground, grasses, palatable forbs, spiny forbs, unpalatable forbs and spiny scrub. Among abundantly occurring plants, with a substantial biomass and cover, were *Holcus lanatus* L. , *Poa pratensis* L., *Poa annua* L., *Lolium perenne* L., *Lolium multiflorum* Lam., *Dactylis glomerata* L., *Elymus repens* (L.) Gould (grasses), *Trifolium repens* L., *Taraxacum* Wiggers *sp., Plantago major* L., *Sonchus oleraceus* L. (palatable forbs), *Cirsium arvense* (L.) Scop., *Cirsium vulgare* (Savi) Ten. (spiny forbs), *Eupatorium cannabinum* L., *Epilobium ciliatum* Rafin, other *Epilobium* L. *sp., Urtica dioica* L., *Matricaria recutita* L. (unpalatable forbs) and *Rubus* L. *sp.* (spiny scrub). We made measurements on woody species in both sampling periods. In each plot, we recorded frequencies of all established trees, i.e. individuals taller than 50cm or, if smaller (in case of heavily browsed individuals), with a stem diameter > 1cm (further listed as "juvenile trees"). We did this to assess establishment, rather than germination and emergence. By this we excluded large numbers of small tree seedlings in the first sampling period (up to 200 000 ha^{-1}, own observations) that appeared shortly after the cessation of agricultural use, but quickly disappeared afterwards. We recorded species and number of trees > 2.5m (i.e. beyond the browse line).

4.2.3 Data analyses (italic numbers between brackets refer to hypotheses)

(1,2) Grazing, resistance traits and associational resistance: we divided plots in five classes, based on the cover summation of unpalatable forbs, spiny forbs and spiny scrub: 0-20%, 20-40%, 40-60%, 60-80% and 80-100%. We used three biological databases to divide woody species into resistance trait classes that are related to grazing or browsing (sensu Boege & Marquis 2005; Table 4.2): (1) defensive species that avoid grazing and browsing through morphological or chemical defence mechanisms; there were no grazing avoiding species with escape traits (cf. Boege & Marquis 2005); (2) tolerant species with strong regeneration capacities after grazing damage; (3) non-resistant species lacking all former traits. We analysed data on the plot level, i.e. species composition, number of juvenile trees. For statistical analysis, we used generalised linear mixed models with repeated measures (SAS 9.1). We analysed the number of established individuals (log transformed to meet conditions for normality) with spiny/unpalatable vegetation cover (sum of percentages), treatment (grazing or not), and resistance trait as fixed factors. Sampling period (further stated as "time") was entered as repeated measures factor (with subject = plot). Site effects were treated by entering sampling area and plot (nested in sampling area) as random factors. To analyse the numbers of trees

> 2.5m, we used a GLM-analysis with the same fixed and random factors as for the total number of established individuals but without repeated measures (second sampling period).

(3) Spatial heterogeneity: as a measure of heterogeneity of a sampling area, we calculated the standard deviation on the set of tree frequencies per plot (after the second sampling period). For horizontal heterogeneity, we used the standard deviations on total frequencies per plot. For vertical heterogeneity, we used standard deviations on the frequencies of trees > 2.5m (i.e. grown beyond the browse line). We analysed different resistance trait classes separately, using a two way ANOVA (response factor = standard deviation). If there was a significant interaction mean frequency*treatment, we tested for differences in regression coefficients (regression mean frequency vs. standard deviation, with log transformation) between grazed and ungrazed sampling areas, using a t-test. This allowed us to conclude if (and in which cases) grazing affected heterogeneity.

In all GLM analyses, the number of degrees of freedom was estimated using the method of Kenward & Roger. We used AIC values for goodness of model fit to control, whether the model was improved or not after backward elimination of non-significant factors. We performed Tukey-tests to make multiple comparisons in all analyses. We corrected the models for stochastic variation in ungrazed vs. grazed plots.

Nomenclature follows Lambinon *et al.* (1998).

Table 4.2: Resistance traits (related to grazing) of woody species found in the sampling areas on former arable land (compiled from Van Duuren *et al.* 1997; Biesbrouck *et al.* 2001; Klotz *et al.* 2002). Between brackets: presence percentages in grazed and ungrazed plots, respectively (second sampling period).

tolerant		**defensive**		**non-resistant**	
Salix caprea	*(91,91)*	*Betula pendula*	*(79,82)*	*Acer pseudoplatanus*	*(25,25)*
Salix alba	*(47,38)*	*Betula pubescens* Ehrh.	*(10,7)*	*Fraxinus excelsior* L.	*(17,16)*
Salix fragilis L.	*(13,13)*	*Populus canescens* (Ait.) Smith	*(22,25)*	*Coryllus avellana* L.	*(18,14)*
Salix x rubens Schrank	*(5,2)*	*Alnus glutinosa*	*(44,16)*	*Quercus robur*	*(26,36)*
Sorbus aucuparia L.	*(16, 9)*	*Sambucus nigra* L.	*(23,32)*	*Larix decidua* Mill.	*(9,5)*
		Rosa canina L. *group*	*(15,9)*	*Prunus padus* L.	*(5,5)*
		Prunus spinosa	*(20,7)*	*Prunus avium* (L.) L.	*(10,16)*
		Crataegus monogyna	*(38, 46)*		

4.3 Results

Overall, we found large variation in juvenile trees frequencies. Random factors sampling area and plot were important in all analyses. Variation explained by sampling area ranged from 7.9 (total no.) – 13.9% (no. >2.5m) and by plot from 6.4 (total no.) – 17.1% (no. > 2.5m) respectively, compared to the residual variance. Therefore, and because random factors were inherent in the study's design, we did not remove them from GLM analyses.

4.3.1 Grazing, resistance trait, cover of unpalatable/spiny vegetation and time

In ungrazed plots juvenile tree frequencies were significantly higher than in grazed plots for tolerant species (both sampling periods) and for defensive species (first sampling period only) (Fig. 4.1). Grazing significantly interacted with time and resistance trait: five to seven years after abandonment (second sampling period), frequencies of defensive species had increased in grazed plots and did not differ significantly from ungrazed plots anymore (Table 4.3a; Fig. 4.1).

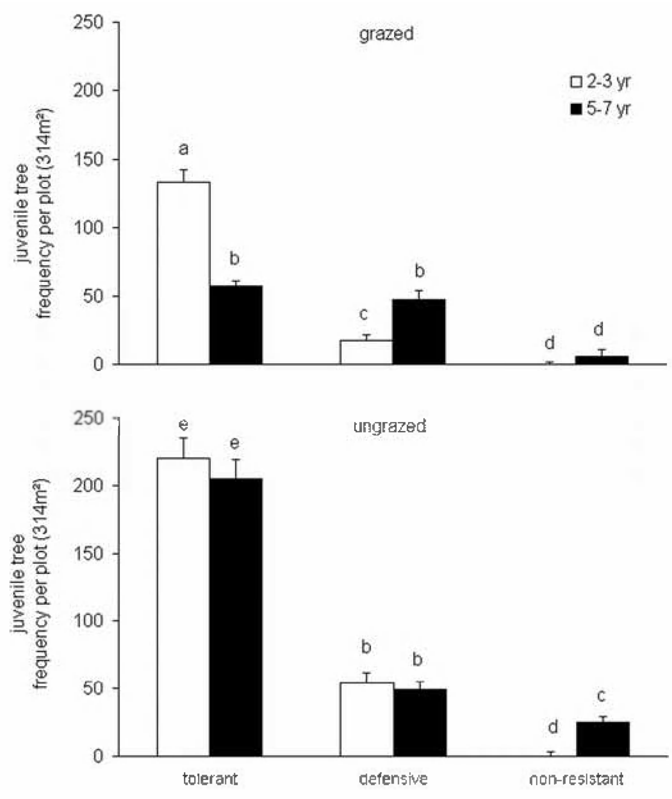

Figure 4.1: Frequencies of established juvenile trees related to their resistance traits and sampling period in grazed and ungrazed plots. Different letters indicate significant different frequencies across treatments (Tukey-tests P<0.05).

Tolerant species (mainly *S. caprea*) had higher frequencies than species from other resistance trait classes. However, in grazed plots, frequencies of tolerant species decreased over time and did not differ from frequencies of defensive species in the second sampling period. Non-resistant species had significantly lower frequencies but frequencies (mainly *Acer pseudoplatanus* L.) increased significantly in the second sampling period in the ungrazed plots (Fig. 4.1).

Table 4.3: Results of the GLM-analyses for juvenile tree frequencies. a: all established juvenile trees > 0.5m; b: established juvenile trees grown above the browsing line (2.5m). time = 1st or 2nd sampling period; cover unp/sp. = cover percentage of unpalatable and spiny vegetation in the herb and low shrub layer; resistance trait = tolerant, defensive or non-resistant.

response	effect	nom. d.f.	den. d.f.	F Value	Pr > F
a. frequency of established woody species > 0.5 m	time	1	789	0.88	0.348
	grazing	1	202	4.70	0.031
	cover unp/sp.	4	485	3.79	0.005
	resistance trait	2	690	29.55	<0.001
	time*grazing	1	784	3.76	0.029
	time*cover unp/sp.	2	522	0.93	0.082
	time*resistance trait	2	690	2.73	0.066
	grazing*cover unp/sp.	4	478	2.41	0.048
	grazing*resistance trait	2	686	4.43	0.012
	cover unp/sp.*resistance trait	8	724	5.44	<0.001
	grazing*cover unp/sp.*resistance trait	8	721	2.94	0.003
b. frequency of established woody species > 2.5 m	grazing	1	415	10.09	0.002
	cover unp/sp.	3	102	6.25	0.034
	resistance trait	2	419	8.89	<0.001
	grazing*resistance trait	2	415	3.93	0.020
	grazing*cover unp/sp.	3	415	5.27	<0.001
	cover unp/sp.*resistance trait	6	430	6.40	<0.001
	grazing*cover unp/sp.*resistance trait	6	415	2.95	0.008

Unpalatable/spiny vegetation cover in the herb and low shrub layer (sum of spiny scrub, unpalatable forbs and spiny forbs) had only a significant effect in grazed plots (Table 4.3a, Fig. 4.2): frequencies of established woody species increased with increasing unpalatable/spiny vegetation cover, except for the highest cover class (80-100%). In both lower cover classes (0-20; 20-40%) frequencies were significantly lower in the second sampling period. On average, total cover of unpalatable/spiny vegetation in grazed plots was similar in the two sampling periods: 63.7% and 60.3% respectively (Fig. 4.3) but the cover of the different classes changed over time. After 5-7 years in grazed plots, there was a strong decrease in bare ground and (biennial) spiny forb cover, a small

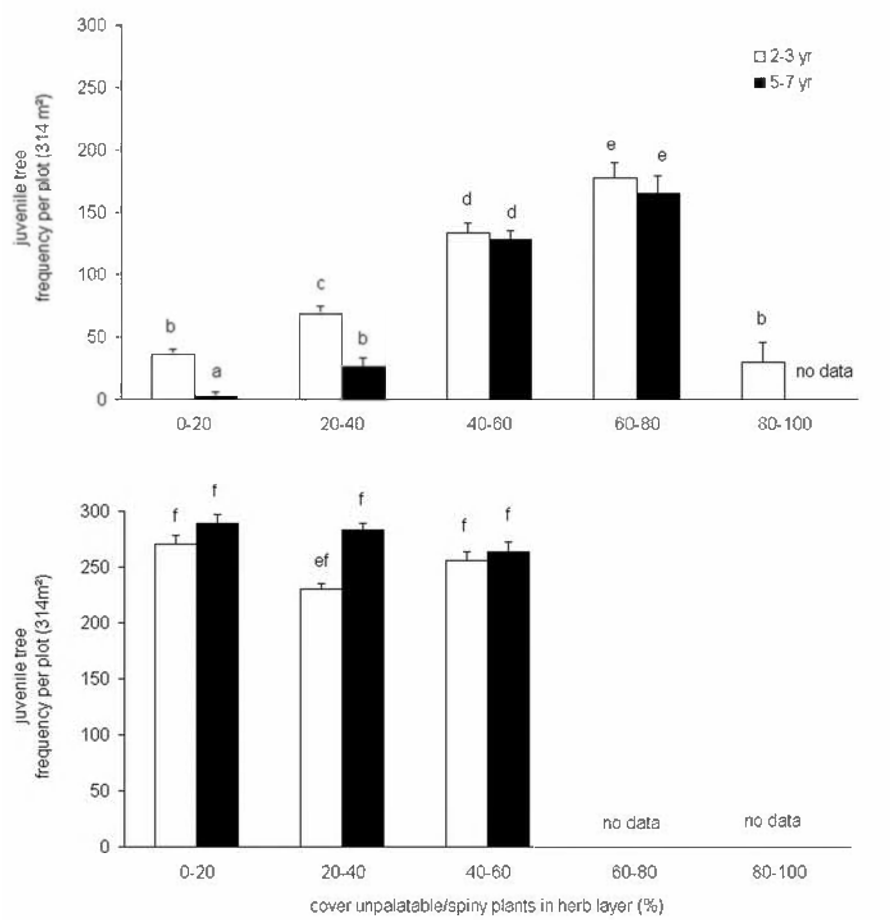

Figure 4.2: Frequencies of established saplings and juvenile trees related to cover of unpalatable/spiny plants and sampling period in grazed and ungrazed plots. Different letters indicate significant different frequencies across treatments (Tukey-tests P<0.05).

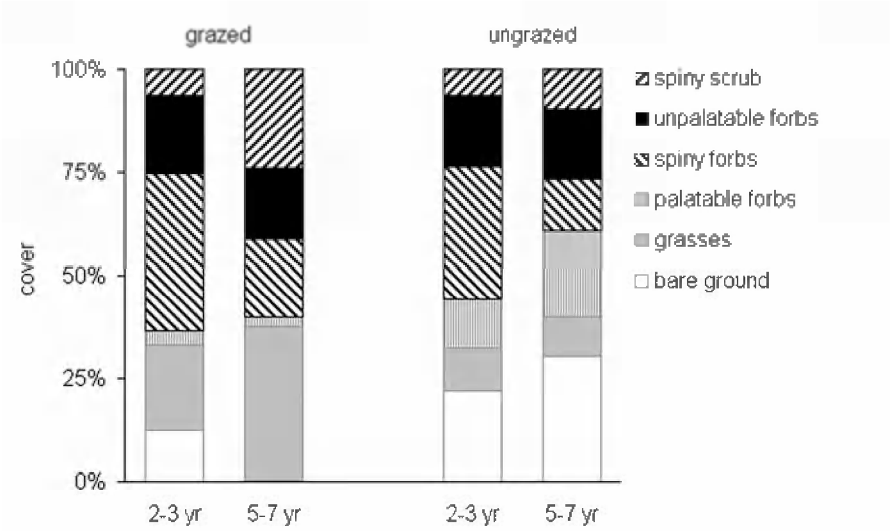

Figure 4.3: Cover of six functional vegetation classes in the herb and low shrub layer in grazed and ungrazed plots in two sampling periods 2-3 years and 5-7 years after the cessation of agricultural use.

decrease in unpalatable forb cover and a strong increase in perennial grasses and spiny scrub cover (mainly *Rubus* sp.). In ungrazed plots, the sum of spiny scrub, unpalatable forbs and spiny forbs cover decreased over time (55.7% after 2-3 years and 39.2% after 5-7 years) and there was an increase in bare ground (Fig. 4.3).

The number of trees that was able to grow out beyond the browse line (second sampling period only) was positively influenced by unpalatable/spiny vegetation cover (if cover > 60%) for tolerant tree species (Table 4.3b; Fig. 4.4). Unpalatable/spiny vegetation cover had a reverse effect on frequencies of defensive species: relatively more defensive tree species reached above the browse line when this cover was low. In ungrazed plots, more than 95% of all trees, were higher than 2.5m, whether they were tolerant, defensive or non-resistant (no figure given).

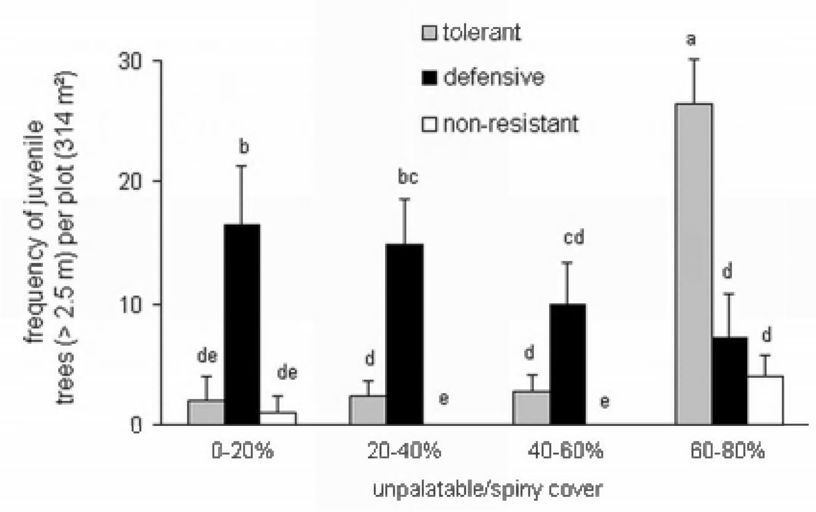

Figure 4.4: Frequencies of juvenile trees growing above the browse line (2.5m) in grazed plots after the second sampling period (5-7 years after the cessation of agricultural use), for different resistance trait classes and cover of unpalatable and spiny plants in the herb and low shrub layer. In ungrazed plots more than 95% of all juvenile trees > 2.5m (results not shown).

4.3.2. Spatial heterogeneity

We only analysed heterogeneity in frequencies for the tolerant and defensive species classes, because only these groups were present in all (14) sampling areas.

Horizontal heterogeneity: In general, we found higher standard deviations for frequencies in grazed plots (Fig. 4.5). We found a significant interaction mean frequency*treatment in the two-way ANOVA-analysis (F= 48.12; P<0.001) for tolerant species. There was a significant difference between regression coefficients (mean frequency vs. standard deviation) of grazed and ungrazed sites (t-test

P=0.008): we found higher differences in standard deviation (and so increasing differences in heterogeneity) between grazed and ungrazed sampling areas when mean frequencies increased. For defensive species, regression coefficients did not differ significantly (t-test P= 0.443): standard deviations were significantly higher in grazed than in ungrazed sampling areas (F= 16.49; P= 0.031), irrespective of mean frequencies.

Vertical heterogeneity: for both tolerant and unpalatable species, there was no significant interaction "frequency>2.5m"*treatment and regression coefficients did not differ significantly; t-test P = 0.111 and 0.402, respectively). In grazed areas standard deviations were significantly higher than in ungrazed areas for both resistance trait classes. For tolerant species F= 10.04, P= 0.018; for defensive species F= 23.04, P=0.006)

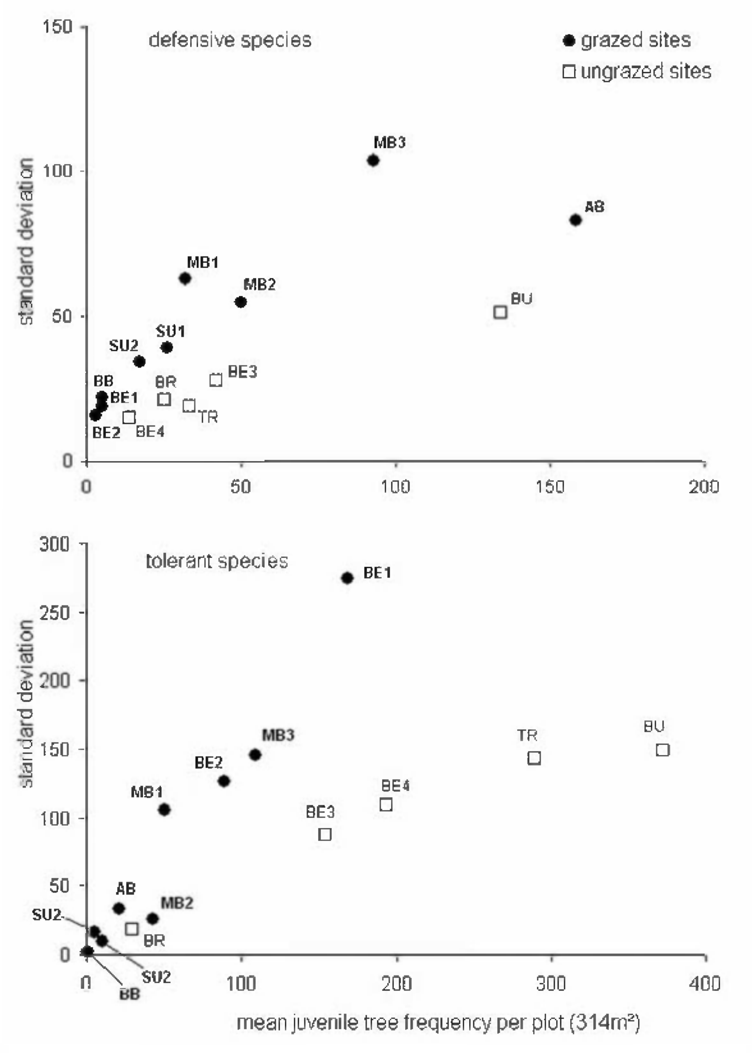

Figure 4.5: Variance in juvenile tree frequencies for different sampling areas: standard deviations related to mean plot frequencies (5-7 yr after the cessation of agricultural use). BE: Bos t' Ename; AB= Altenbroek; MB= Moenebroek; BB= Boembeke; SU= Sulferberg; BR= Broekelzen; BU= Burreken; TR= Trimpont.

4.4 Discussion

In general, we found a fast and massive colonisation of woody species. Although there were strong differences in grazing pressure, species composition, dominance and frequencies of established trees among different sites (important random effects) and results should be interpreted with caution, four general establishment patterns could be observed. First, grazing significantly reduced juvenile tree frequency, but this reduction was restricted to tolerant species, which were initially (first sampling period) most abundant in both grazed and ungrazed plots. Second, in grazed sites, increasing cover of unpalatable/spiny vegetation in the herb layer and low shrub layer increased tree frequencies. Third, increasing cover of unpalatable/spiny vegetation in grazed plots increased frequencies of tolerant tree species growing above the browse line, but had a reverse effect on frequencies of defensive tree species. In the ungrazed control plots, cover of unpalatable/spiny vegetation had no effect. Fourth, grazing increased spatial heterogeneity in the early tree assemblages.

4.4.1 Resistance traits

The reduction in frequency of grazing tolerant, abundant species such as *S. caprea*, as found in our study after the second sampling period, is probably related to life history stage: seedlings are less tolerant to repeated browsing than juvenile trees (Hódar *et al.* 2008) and their number may decrease over time. Competitively dominant tree species are likely to tolerate, rather than avoid herbivory because of the good regrowth opportunities owing to high nutrient availability in fertile soils (Olff & Ritchie 1998). Large herbivores prefer high productive vegetation types and therefore, repeated consumption of abundant species will influence the competitive relationships among the established tree species (Bilyeu *et al.* 2007). Altered interspecific competition through grazing may finally promote cover and growth of defensive species such as *B. pendula* as was seen after the second sampling period in our study. This resembles the mechanism of selective herbivory of dominant, grazing tolerant species in grasslands which induces shifts in species composition (Anderson & Briske 1995). The significantly higher browsing of tolerant species compared to defensive species suggests the validity of this mechanism also for woody pioneer assemblages. Augustine & McNaughton (1998) found that the maintenance of dominating but attractive species (for herbivores) only was to be expected under conditions of high levels of nutrient inputs or recycling and an intermittent temporal pattern of herbivory.

Species with morphological defence structures such as *C. monogyna* and *Prunus spinosa* L. were found in low numbers. This could be due to seed predation by rodents, slower colonisation rates of these (mainly) animal dispersed species and the absence of defence structures in the early seedling stage. Other possible reasons are the initial deficiency of attractive sites for rodents to hide seeds and for birds to rest and defecate consumed berries/seeds, or the deficiency of suitable micro sites to germinate in early successional communities (Smit 2002). However, the cover of low spiny scrub (mainly *Rubus* sp.) increased during succession on grazed former arable land and played an important role in protecting tolerant and non-resistant tree species to establish and for tolerant species to grow out.

4.4.2 Associational resistance

It is assumed that grazing is a major driving force behind the biotic facilitative process of associational resistance (Bokdam 2003). In this study, protective or facilitative vegetation types, whether spiny or unpalatable, had a positive effect on juvenile tree frequencies in grazed plots. In ungrazed plots, there was no effect and due to overshadowing of a dense juvenile tree layer, there was even an increase in bare ground in the second sampling period. Considering both sampling periods (2-3 and 5-7 years after abandonment), it is clear that in combination with grazing, the cover of unpalatable/spiny vegetation in the herb and low shrub layer needs to be high enough (> 40%) to have a substantial effect on the frequency of juvenile trees (x 3-4 compared to < 40% cover). When the cover of unpalatable/spiny vegetation was low (< 40 %), frequencies decreased after the second sampling period. Smit *et al.* (2005) found comparable results for unpalatable cover (>25%) and rock cover (>10%) in wooded pastures. Both deficient protective cover and greater appearance of juvenile trees above the canopy of the surrounding grass/herb vegetation, may have attracted herbivores and caused tree mortality. Vandenberghe *et al.* (2007) found that only one percent of large (height = ±40-60cm), but 25% of the small (height = ±12-19cm) saplings escaped browsing in wooded pastures in the Jura mountains. It is also likely that cattle easier penetrate small patches of unpalatable/spiny vegetation types than large patches (Gomez-Aparicio *et al.* 2005). The important role of bramble

(*Rubus*) scrub as a nurse structure on former arable land was previously shown by Kuiters & Slim (2003) and on nutrient rich flood plain grasslands by Van Uytvanck *et al.* (2008a).

In this study, growth beyond the browse line of tolerant species was significantly enhanced by cover of unpalatable/spiny vegetation but it had a reverse, but less pronounced effect on defensive species. Again, a significant nurse effect was only found when patch size of nurse vegetation was large enough (in this study > 60% of the plot size). Our results suggest that large protective patches have greater capacities to protect juvenile trees from browsing of the apical shoots, hence allowing them to survive and grow out. These findings contrast with Smit *et al.* (2006) who found that single plants of *Cirsium acaule* Scop. (spiny) and *Gentiana lutea* L. (unpalatable) offered protection for *Picea abies* (L.) Karst. sapling survival and growth. However, differences may be due to the better visual protection that high nurse plants offered to the relatively smaller *P. abies* saplings or due to a higher grazing pressure in our study sites, resulting in decreased protective capacities of nurse plants (Smit *et al.* 2007). The negative impact of unpalatable/spiny vegetation cover on frequencies of defensive trees in our study may be due to restricted germination or increased competition with seedlings of these species (Berkowitz *et al.* 1995). This may be particularly at stake for the abundantly present *B. pendula* which is highly light-demanding for germination and seedling growth.

4.4.3 Developing woodlands and spatial heterogeneity

Our results suggest an increase in horizontal and vertical spatial heterogeneity when developing woodland is grazed. Horizontal heterogeneity is expressed in a more variable frequency pattern of juvenile trees. In our study, we found increased horizontal heterogeneity for defensive and tolerant tree species. For the latter, this was only the case when frequencies were high. A possible explanation for this pattern is the mechanism of associated palatability (Olff *et al.* 1999): small seedlings (also of defensive species) are grazed together with surrounding palatable herb and grass species, causing different establishment patterns dependent on the present herb layer. So, differences in the composition and structure of the herb layer is reflected in higher horizontal heterogeneity of the early woody species assemblages. Our results suggest that in the absence of grazing, differences in the herb layer are less important. Without grazing, abandoned arable fields become reforested (Smit & Olff 1998; Harmer *et al.* 2001) often with a reduction of plant biodiversity that is probably related to a reduction of spatial heterogeneity. Such a reduction takes place in the area of the ecotone between closed forest and open land, consisting of grassland, tall herb, scrub and tree patches which may comprise a mixture of light-demanding early phase species and shade-tolerant late phase species (Staaland *et al.* 1998; Bonet 2004; Baniya *et al.* 2009). Productive environments, such as young forest on nutrient rich loam soils, feature intense light competition and thus have greater potential for herbivores to open the canopy and increase local colonisation rates of plant species (Olff & Ritchie 1998).

An increase in vertical heterogeneity is mainly due to the selective grazing on tolerant species. In our study sites, this resulted in a mosaic pattern of low *S. caprea* shrubs with strongly branched stems and outgrowing defensive species (mainly *B. pendula*). Schmidt (1988) found that in ungrazed old field succession on loam, a *Salix* layer up to three m in height was formed within three years. This was confirmed in our study: at ungrazed sites, developing woods were within 3-7 years in a dense thicket phase, mainly consisting of single stemmed *Salix species.* and *B. pendula* trees higher than 2.5m.

However, only low or moderate grazing pressure allows the development of structurally heterogeneous woodland. In Mediterranean regions, Giourga *et al.* (1998) found that high grazing pressure by goats and sheep almost prohibited recovery of old fields: in a range of 5-40 years after abandonment total plant cover, including woody species was below 25%. Kuiters & Slim (2003) found a cover of woody species < 2% on 27 years grazed old fields. It is likely that on the nutrient rich soils of our study sites, grazing pressure is a less dominant factor, determining the definitive establishment of woody species compared to nutrient poor systems. Grazing pressure differed considerably in our study sites. We found a grazing pressure threshold for regeneration is around 180 grazing days ha^{-1} y^{-1} (= ±0.50 AU ha^{-1} y^{-1}), given protective vegetation types develop. Kuiters & Slim (2003) found that 0.08-0.1 AU ha^{-1} y^{-1} (Iceland ponies) restricted successful regeneration on nutrient poor former arable land. Mountford & Peterken (2003) give (for grassland) maximum thresholds of 0.3 AU ha^{-1} y^{-1} for cattle and for ponies and deer 0.15 and 0.45 AU ha^{-1} y^{-1} respectively. Chatters & Sanderson (1994) recommend 0.1-0.2 AU ha^{-1} y^{-1} for woodpastures. All those stocking rates are far below common agricultural practice on nutrient rich soils (± 2 AU ha^{-1} y^{-1}).

It is not yet clear to what extent large herbivore grazing will affect spatial heterogeneity in later successional stages. In the (ungrazed) woodland regeneration process, *S. caprea* and *B. pendula* are among the pioneer species which persist longest in the forest (±70 years, Falinski 1998). Considering the importance of these species in succession on former arable land, grazing impact on vegetation structure might be important in a similar time span or be permanent in systems with shifting mosaics (Olff et al. 1999) and/or cyclic soil disturbances or timber harvesting creating pioneer circumstances (Van Uytvanck *et al.,* in press).

4.4.4 Conclusions

Our results indicate that on former arable land on nutrient rich, loamy soils, situated in a (patchily) forested matrix, succession to pioneer forests is a fast going process, even if large herbivores are present in low to moderate densities. Large herbivores influence the establishment of woody pioneers by suppressing frequencies and growth of species that are non-resistant or tolerant to grazing. The presence of ruderal ground vegetation with unpalatable and spiny species and *Rubus* scrub, commonly occurring on former arable land, interacts with this process and facilitates tolerant and to a lesser extent also non-resistant woody species to grow out. We found that grazing increased horizontal and vertical heterogeneity of the early woody species assemblages.

Considering the goals for nature management and restoration on former arable land, a choice for completely spontaneous succession, technical measures or a combination of both approaches has to be made (Prach & Hobbs 2008). Introducing large herbivores clearly corresponds with the latter choice and aims the recovery of semi-natural systems (cf. Machado 2004). Our results suggest that management should focus on an increase in spatial heterogeneity and the maintenance of, at least temporarily, mid successional communities (e.g. the restoration of wooded pastures, Mitlacher *et al.* 2002, Van Uytvanck *et al.* 2008b). Kumm (2003, 2004) argues that the re-creation of extensive pasture-forest mosaics provide an economically sustainable way (meat and timber production) of nature conservation. Managers should be aware of the facilitation process that tends to promote forest succession and the order of species establishment and replacement (Walker & del Moral 2003). Succession to forest may not be a conservation goal where intermediate stages are themselves desirable for biodiversity reasons (Hobbs *et al.* 2007).

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…

Dreunend over de bosaarde, alsof er op een gedempte pauk wordt geslagen, is er intussen iets dat nadert, heel dichtbij gekomen,- ook ik wilde er wel instinctief voor op de vlucht slaan, maar daar blijkt geen tijd meer voor te zijn, wat begin ik met mijn stramme benen en kortademigheid?

Ik ga achter een boom staan, met mijn rug ertegen, en hoor het luidruchtig snuivend iets, het beest, schuin achter me door de struiken breken, - gevolgd door een onlogisch plotselinge stilte die neerslaat als damp.

Een oerkoe, -stier, -os, dergelijk rund, wisent, bizon, jak, het kolossale lichaam oranjebruinrood behaard met lange franjes, hoorns die horizontaal uit zijn kop steken en dan loodrecht omhoog wijzen, uitlopend in levensgevaarlijke spiesen. Daar hebben losgetrokken takken met nog wat herfstloof zich omheen gedraaid, alsof hij een lauwerkroon draagt, aan de vacht kleven bladeren, takjes, dennennaalden, kleine steentjes, plakkaten zand, stukjes rode paddenstoel. Kop omlaag, steekwapens vooruit gericht, zo zie ik hem tevoorschijn stormen en dan onmiddellijk met een schok tot stilstand komen, bevriezend tot roerloosheid, mogelijk doordat hij even hard schrikt van mij als ik van hem, van mij heeft hij niks te vrezen, ik van hem des te meer.

Ik houd me achter de boom verschanst, al realiseer ik me dat de stam slanker is dan ik, natuurlijk heeft deze herkauwer me meteen gezien, in ieder geval zeker mijn hoofd, met gestremde adem kijk ik over mijn schouder naar hem.

Hij begint met een van de hoeven aan zijn voorkant door de dorre bladeren te roeren, schrapende geluiden over de grond, terwijl de bladeren knisperend verstuiven verliest hij mij niet uit het oog, wij kijken elkaar strak aan als hypnotiseurs en houden dat een aantal minuten die heel lang duren vol.

Van hoef tot hoornspies gemeten is hij een stuk groter dan ik, wat zal deze kolossaalheid wegen, een ton? - de neusgaten gesperd ademt hij grommend, het resoneert in het inwendige van zijn geweldige lijf, hij draagt een sluike pony, die gedeeltelijk voor zijn ogen hangt als rafelige vitrage.

Zeer op mijn hoede heb ik me losgemaakt van de boom, hij ziet me nu in mijn volle gedaante, nietig naast de zijne, ik verwacht dat hij me hoornschaterend zal uitjouwen, maar hij beweegt niet, behalve zijn aandoenlijk trillende kniegewrichtjes, ik beweeg ook niet.

Men moet dit dier, vermoedelijk een paar van deze dieren, pas onlangs in het bos hebben losgelaten, eerdere kennismaking ermee heeft niet plaatsgevonden. Het zal wel ergens goed voor zijn ze hier te laten rondstruinen, tot stervensschrik van alle boom- en struikbewoners zolang deze niet aan de vreeswekkende allochtonen gewend zijn.

In de druilerige halfstilte, versneden door zijn gehijg, hoor ik verder alleen insecten, - die hebben het op zijn natte oogranden en neusingangen voorzien, het beest schudt geïrriteerd zijn kop en manen, wat begin je met zulke ontzaglijke, uit je hersens gegroeide steekwapens tegen een strontvlieg? Weinig geïnspireerd zwaait hij nu en dan zijn staart tegen het lijf, zich tegen het geniepige gedierte teweerstellend als een soeverein tegen lasterpraatjes.

Ik begin achteruit te schuifelen, wat ik dacht onhoorbaar te zullen doen, maar er breekt dor hout onder mijn schoenzolen, waar ik mijn voeten zet kraakt het, ritselt het, verschuiven er dingen. Hij, mij onafgebroken aanstarend schopt dan weer met zijn hoef of hij een Harley-Davidson aantrapt, zijn hele lijf, kop naar beneden, spant zich, hij gromt alsof ik hem met een rode lap op stang jaag, maar in plaats van mij na een korte aanloop met zijn bajonetten te doorboren begint ook hij zich stapje na stapje achteruit te bewegen, terug de bosjes in, opgewoelde grond en bladeren als branding rondom de haarflossen aan zijn poten.

Dan schiet door mij heen:

Dit bos is het labyrint waarin ik hopeloos ben verdwaald, al ken ik iedere boom, struik, regenplas en zie ik verderop mijn witte huis. En dit monster, zijn vacht in de kleur van roestig bloed, dit snuivende, stampende, door vliegen omgonsd gevaarte, tevoorschijn gestoven als plotselinge rukwind om paniek en angst voor zich uit te jagen, is de mensetende Minotaurus, dit met stervend eikenblad gekroonde ondier is: de Dood, - in zijn zwarte vochtige ogen zie ik mezelf als verkleind gespiegeld.

…

Uit: Datumloze dagen, Jeroen Brouwers (2007)

Chapter 5. Woodland regeneration in grassland

Van Uytvanck J, Decleer K, Hoffmann M, 2008. Establishment patterns of woody species in low intensity-grazed pastures after the cessation of intensive agricultural use. ***Forest Ecology and Management*** **256: 106-113.** Reprinted with permission from Elsevier.

Abstract

Low intensity grazing with large herbivores is increasingly used to steer the development of mosaic landscapes (including woodpastures) and woodland expansion on former agricultural land. We studied the establishment and early growth pattern of woody species in grasslands (formerly used for high intensity summer cattle grazing) in which a low intensity grazing regime was set up between 4 and 11 years ago. We tested whether the developing vegetation patches, consisting of tall herbs and scrub, influenced this pattern. Further, we investigated if increased susceptibility to trampling disturbance by large herbivores on wet soils in floodplains influenced sapling establishment.

Formerly intensively used grasslands were studied at six sites, four in floodplains and two in interfluvial zones on nutrient rich, heavy soils. In total, we sampled 141 plots with grassland, tall herb or scrub vegetation and recorded individuals of established tree and shrub species, their height, diameter, browsing damage and distance to potential seed sources. In the floodplains, we recorded soil disturbance by large herbivores (hoofprints) in wet and moist soils.

Sapling frequencies, with *Fraxinus excelsior* as the most common species (>50 % of all individuals), were 2-3 times higher in tall herb and scrub patches compared to grassland. Only the spiny *Crataegus monogyna* established in higher frequencies in grassland plots. In floodplains, we found 2-3 times more established woody saplings in wet tall herb plots, compared to moist and wet grassland plots and moist tall herb plots.

Browsing reduced sapling height in grassland and tall herb patches. Only in scrub patches did average height (± 2.5m) reach above the browse line. We found 30-70% less browsing damage to saplings in scrub compared to damage in grassland and tall herb plots. In particular, damage to the apical shoot was strongly reduced.

Our findings show that tree establishment occurs in spatial association with non-grassland patches that offer protection against browsing. Fast growing spiny shrubs like *Rubus sp.* offer protection for palatable saplings, allowing establishment and growing out beyond the browse line.

Alternatively, increased establishment occurs in wet tall herb patches, where soil disturbance by moderate trampling favours germination and, subsequently, saplings are protected at least temporarily against browsing by unpalatable tall herbs.

Key words

Fraxinus excelsior; facilitation; grazing; herbivory; tree establishment;
woodland expansion and development

Establishment of Crataegus monogyna in grassland and Fraxinus excelsior and Alnus glutinosa in bramble scrub patches (Moenebroek, Geraardsbergen, Belgium).

5.1 Introduction

Low intensity grazing with large herbivores is increasingly used in conservation management (WallisDeVries *et al.* 1998; Bakker & Londo 1998; Bakker *et al.* 2006). The principal idea is that large herbivores, whether wild or livestock species, can play a key role in restoring the functioning of ecological processes. Native woodland regeneration on formerly intensively used grassland is an important item in this view. In the British uplands for example, there is interest in the concept of 're-wilding' and large scale native woodland restoration in conjunction with extensification of agriculture (Worrell *et al.* 2002; Kirby 2003; Hodder *et al.* 2005). In this context, Holl & Smith (2007) argue that targeted grazing management is needed to restore the ecological functioning of such landscapes, with a range of representative habitats (including different woodland types), communities and species at different scales. In Flanders (N-Belgium) low intensity grazing by semi-domesticated animals is currently explored as an alternative for active afforestation or spontaneous regeneration and development of woodpastures.

It is generally known that the regeneration of woody species is strongly affected by large herbivores, but local site differences, including spatial effects, palatability and competitive capacities of ground vegetation, grazing intensity, herbivore and tree species, will influence the interactions between herbivores and woody species (Kramer *et al.* 2006). Large herbivores like deer are able to regulate the balance between woody and herbaceous vegetation where agricultural land has been abandoned (Pépin *et al.* 2006), but also free ranging domestic large herbivores may maintain or develop fine-meshed (often mid-successional) mosaic landscapes, including grassland, scrub, scattered trees and woodland patches (Bokdam & Gleichman 2000; Vera 2000; Hodder *et al.* 2005). However, livestock herbivory has a complex relationship with tree regeneration (Sankey *et al.* 2006). On one hand, large herbivores have been found to retard or prevent tree regeneration in wooded landscapes, dehesas and wood-pastures (Putman 1986; Moser *et al.* 2006; Linares 2007; Vandenberghe *et al.* 2007). On the other hand, large herbivores may enhance woody species establishment, e.g. in grassland vegetation in temperate regions (Kirby *et al.* 1994; Olff *et al.* 1999; Vera 2000), but also in logged forests with a palatable understory (e.g. bamboo, Darabant *et al.* 2007). Generally, a dense and closed grass canopy reduces favourable sites for germination and limits growth and survival rate of established saplings (Davis *et al.* 1999). The lack of suitable germination sites in grassland may be a limiting factor for seedling recruitment (Mazia *et al.* 2001; Setterfield

2002). Grazing, trampling and uprooting by large herbivores can open dense grass swards, increase favourable microsite availability, temporarily decrease competitive ability of grasses and favour the germination and establishment potential of woody species (Posada *et al.* 2000; de Villalobos *et al.* 2005; Vandenberghe *et al.* 2006).

Woody species establishment in open and grazed landscapes occurs in a non-random spatial pattern: saplings are often found in close association with plants or vegetation patches that facilitate their establishment (Smit *et al.* 2005) and provide a certain level of protection against herbivory. The latter seems to be crucial for successful establishment of woody species in grassland ecosystems. Saplings may be protected against browsing by unpalatable, thorny or spiny species (often shrubs), which provide safe sites, although higher seed removal by predators under these shrubs may limit establishment (Smit *et al.* 2006). Moreover, browsing damage by small rodents, finding cover against predators in dense ground vegetation, may increase (Ostfeld & Canham 1993). Facilitating shrubs usually have the ability to establish in open sites, produce attractive fruits, discourage growth of competitive grasses, ameliorate soil conditions, sprout readily after browsing damage and compete relatively little for water and nutrients (Vieira *et al.* 1994). The process of facilitation in grazed ecosystems is known as associational resistance (Huntly 1991; Olff *et al.* 1999). It is determined by a large spectrum of variables, such as herbivore selectivity, herbivore density, palatability of facilitating plants, plant strategies and light requirements of the facilitated trees and their surrounding, facilitating plants (Callaway 1992; Rousset & Lepart 2000). Evidence is growing that it is a key process in the dynamics of grazed ecosystems that include woodland (Olff *et al.* 1999; Bokdam 2001; Bakker *et al.* 2004). Olff *et al.* (1999) presented a descriptive model of long-term cyclic succession, in which low intensity grazing by free ranging herbivores, associational resistance and competition play a determining role in succession.

In this paper, we focus on the early stages of tree establishment in grasslands, that were formerly used for high intensity summer grazing, in which the grazing regime was recently altered to a low intensity grazing regime. We studied the tree establishment pattern and the developing spatial associations in six nature reserves on former intensively used agricultural grasslands. We investigated whether the frequencies of the five most common woody species colonising these grasslands were influenced by presence and distance to potential seed sources. Negative correlations were expected. Further, we explored tree establishment on soils with different sensitivity to large herbivore trampling.

We hypothesized that on wet soils, which are more susceptible to trampling disturbance, the opening of the sward by this trampling offers better establishment conditions. We hypothesized that, ultimately, the protective capacities against browsing of the present structural vegetation types will determine the establishment pattern of newly colonising woody species.

5.2 Methods

5.2.1 Study sites

A survey study was conducted in 6 nature reserves situated in the Western and Central Hills Ecoregion in the southern part of Flanders (N-Belgium, Fig. 5.1, Table 5.1). The climate is temperate (annual precipitation: ± 700mm). The rather small (10-100 ha) nature reserves are located in a hilly landscape, composed of the upper courses, floodplains and interfluvia of two medium-sized lowland rivers (Schelde and Dender). At all sites, a mosaic of grassland and woodland is present. Four sites are situated in floodplains, two sites are situated on the slopes of interfluvial zones. Due to local embanking and damming of the rivers, the floodplain nature reserves are only flooded after heavy rainfall during winter. Flooding periods only last a few days, with a maximum length of two weeks. Soil types range from moist sandy loam and loam to wet clay (Table 5.1).

Before the start of low intensity grazing, all sites consisted of homogeneously structured, short grazed pasture, without any woody species, tall herb or scrub patches. The moist to wet grasslands are moderate nutrient rich to nutrient rich types: *Alopecurion pratensis, Cynosurion cristati, Lolio-Potentillion* and, due to manuring, impoverished communities that are dominated by the palatable species *Poa trivialis* or *Lolium perenne*. Tall herb patches are numerous and are mainly dominated by unpalatable species such as *Juncus effusus, Carex acutiformis* and *Epilobium hirsutum.* Moreover, these species and the also abundant *Phalaris arundinacea* have rhizomes allowing them to spread fast in grasslands where grazing pressure has been decreased. Tall herb patches occur on moist and wet soils, but are more numerous on wet soils. At all sites, sparse natural regeneration of woody species is present. Saplings of *Fraxinus excelsior*, *Quercus robur*, *Salix alba* and *Alnus glutinosa* are most frequent. Scrub patches mainly consist of fast growing bramble thickets (*Rubus sp.*). Individual shrubs of spiny and thorny species include *Prunus spinosa*, *Crataegus monogyna* and *Rosa canina.*

Forest patches are mainly of the *Stellario-Carpinetum* (slopes) or the *Fraxino-Ulmetum* (floodplains) type.

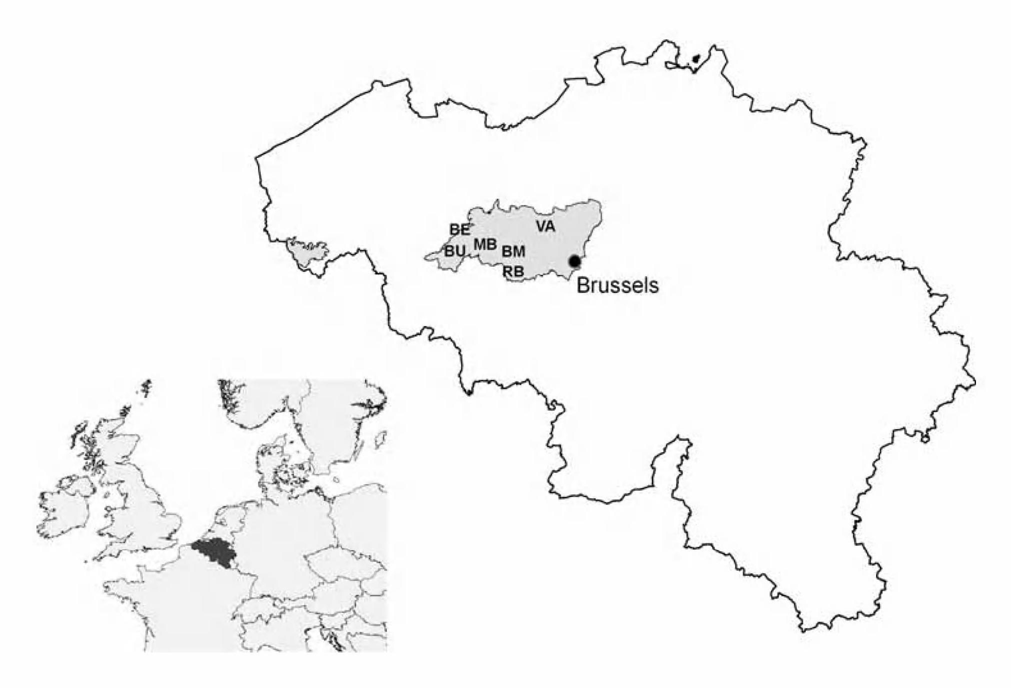

Figure 5.1: Location of the study sites in Europe (left) and in the Western and Central Hills Ecoregion in Belgium (right): BE= Bos t' Ename; BU= Burreken; MB= Moenebroek; BM= Boelaremeersen; RB= Rietbeemd; VA= Valier.

Two grazing systems are applied, both concerning free ranging animals in unpartitioned pastures: year-round, where animals are in the pasture during the whole year and seasonal, where grazing is restricted to spring and summer (from the beginning of May till the end of October). Low intensity grazing, as a conservation management measure on the formerly intensively used grasslands, started between 4-11 years before data collecting in this study. Average grazing period after cessation of agricultural use is 6.3, 6.9 and 7.5 years for grassland, tall herb and scrub plots. respectively. Actual grazing blocks may also include forest, logged forest and former arable land (Table 5.1). Grazing pressure is rather high (≥ 0.4 animal units (= AU) ha^{-1}y^{-1},i.e. ≥ ±200 kg herbivore biomass ha^{-1}y^{-1}, cf. WallisDeVries 1998) and fairly constant on all sites, allowing the development of a varied vegetation structure. Cattle are used on all sites, except in Valier, where horses are grazing. In Bos t' Ename and Rietbeemd, respectively, two horses and two ponies are added to the cattle herd (Table 5.1). No other large vertebrate grazers or deer are present on the sites. Rabbits are present at low densities at Bos t' Ename and Burreken and almost absent on the others sites.

Table 5.1. Study site characteristics. BE=Bos t' Ename; BU=Burreken; BM=Boelaremeersen; RB=Rietbeemd; MB=Moenebroek; VA=Valier. Study site type: IS= interfluvial slope; FI= Floodplain. Grazed vegetation types: G= Grassland; F= Forest; A= Former Arable land; L= Logged Forest; Grazing type: S= seasonal grazing (May-October); Y= year round grazing. Large herbivores: C=Cattle; H=Horses. AU= Animal Units (1 AU=450-500 kg).

study site	**BE**	**BU**	**BM**	**RB**	**MB**	**VA**
coordinates	50°86′N 3°65′E	50°80′N 3°70′N	50°78′N 3°88′E	50°75′N 3°88′E	50°84′ N 3°84′N	50°88′N 4°10′E
type	IS	IS	FI	FI	FI	FI
dominant soil type	sandy loam	loam	clay	clay	loam	clay
grazed area (ha)	63.5	2.1	8.9	22.5	5.8	1.5
grazed vegetation types	G F A	G F A	G F L	G L	G A	G
sampling area (ha)	2.0	1.5	4.0	2.5	1.5	1.5
grazing type	Y	S	S	Y	S	S
large herbivores	C+H	C	C	C+H	C	H
AU $ha^{-1}y^{-1}$	0.4	0.8	0.4	0.4	0.5	0.6
start grazing management	1996	2000	2001	1997	1994	1997
number of plots and proportion (%) of sampling area (between brackets)						
grassland	6 (32)	9 (80)	27 (53)	5 (43)	7 (37)	10 (67)
tall herb	6 (39)	0 (4)	24 (38)	7 (24)	12 (48)	0 (4)
scrub	8 (25)	4 (13)	3 (7)	6 (28)	1 (13)	6 (27)
other	0 (4)	0 (3)	0 (2)	0 (5)	0 (3)	0 (2)

5.2.2 Data collection

Field work was carried out between May and August 2005. At each study site, we selected one sampling area (0.5 - 4 ha, Table 5.1) where a mosaic of grassland, tall herb and scrub patches appeared. In the sampling area, we established 2-5 linear transects (evenly spread and ranging between 100 and 300m length) and laid out plots with a radius of 10m.Transects, and the centres of plots along transects, were 20m apart. Plots were assigned to one of three structural vegetation types (grassland, tall herb or scrub) on the basis of a visually estimated percentage cover of plant species and vegetation height. We made one vegetation survey (decimal scale Londo, 1984) per plot. Vegetation height was measured at four fixed points in each plot. We used a vertically held marked rod. Vegetation height was the maximum height of the foliage at the point touched by the rod. For grassland (height < 50 cm), tall herb (height >50 cm) and scrub (>50 cm), average vegetation height was 13.6 (± 8.4) cm, 72.3 (± 18.7) cm and 86.2 (± 27.8) cm, respectively.

Plots, in which one of these three structural vegetation types did not exceed a cover of 80% were excluded. We sampled 141 plots: 64 in grassland, 49 in tall herb and 28 in scrub patches. Proportion of these vegetation structures in the sampling area were measured using a geographical information system (Arcview2) and aerial photographs (AGIV 2006 Eastern Flanders; Table 5.1). In each plot, we recorded sapling number, height and diameter (30 cm above ground level). Only individuals taller than 40 cm or, if smaller, older than 1 year (in case of heavily browsed individuals), were recorded (to assess survival and establishment, rather than germination and emergence). Presence or absence of browsing damage in general and browsing damage to apical shoots in particular were recorded. Whether the stem was branched or not at ground level, was recorded as a measure for grazing damage in an early stage. In floodplain plots, we recorded presence or absence of hoofprints, causing visible soil disturbance. Soil moisture regime of all plots was estimated based on the vegetation survey by using weighted averages of Ellenberg's indicator values (Ellenberg *et al.* 1992). We divided plots in a moist and a wet category (weighted averages of Ellenberg's indicator values between 5.00 - 6.60 and 7.00 - 8.99, respectively). The position of potential seed sources (i.e seed producing trees) within a range of 30 m around the grazed area (Table 5.1) was recorded for all woody species present in the sampling area, using a Global Positioning System (Garmin GPSMAP76).

5.2.3 Data analysis

We used generalised linear mixed models (SAS 9.1, proc glimmix) to model established sapling frequencies, presence of browsing damage and stem branching. We performed a log transformation for Poisson distributed data (frequencies) and a logit transformation for binomial distributed data (browsing damage and stem branching). We used general linear mixed models to analyse sapling height and stem diameter (SAS 9.1, proc mixed). Both proc glimmix and proc mixed cope with unbalanced data.

In order to determine the general tree regeneration pattern, a first analysis was made with all species pooled together. Subsequently, we analysed the regeneration pattern of species present in at least 20 plots. This was the case for the five most frequent species. Analyses on sapling height and diameter and browsing damage were performed for *Fraxinus excelsior* (more than 50% of all individuals) only. In these analyses, we additionally tested the effect of grazing period after cessation. Impact of soil moisture (moist or wet) was analysed for the floodplains only (no wet plots were available on the interfluvial slope sites), excluding scrub plots (only present in moist plots). For this analysis, frequencies of all species were pooled together.

In the analyses, we entered structural vegetation type, species, grazing period after cessation of agricultural use and moist category as fixed factors. Site and plot were modelled as random factors. The number of degrees of freedom was estimated using the method of Kenward & Roger (1997). Initial models included all variables and interactions. They were simplified using a backward procedure, using AIC values for goodness of model fit (Akaike 1987), to eliminate non-significant interactions. Site was always retained in the model, because of its inherent role in the survey's design (including both floodplains and slopes). Tukey-tests were performed to make multiple comparisons in all mixed model analyses.

For the five most frequent species, the distance to the nearest potential seed source was calculated from the centre of each plot in which the species was present. For this, we used Arcview3.2. The correlation between frequency per plot and distance to the nearest seed source was tested using the Spearman Rank Order Correlation.

5.3 Results

5.3.1 Sapling frequencies and height

We recorded a total number of 595 established saplings belonging to 15 different woody species. *Fraxinus excelsior* was the most frequent species at all sites (305 individuals). *Fraxinus excelsior, Quercus robur, Alnus glutinosa, Salix alba* and *Crataegus monogyna* were present in more than 20 plots. In 38 plots, no woody species were recorded (28 grassland, 6 tall herb and 4 scrub plots). Distance to the nearest potential seed source was negatively correlated with sapling frequency per plot for *Fraxinus excelsior, Quercus robur* and *Crataegus monogyna.* No significant correlation was found for *Alnus glutinosa* and *Salix alba* (Table 5.2). A series of species, of which numerous seed trees were present in the examined zone surrounding the sampling areas, did not establish (e.g.

Sorbus aucuparia, *Castanea sativa* and *Salix cinerea*) or established in very low numbers (e.g. *Carpinus betulus* and *Corylus avellana*).

Table 5.2: Correlation between established woody species frequencies and distance to the nearest potential seed sources (R=Spearman rank order correlation coefficient).

Species	R	p	analysed range (m)	nr. of sites	nr. of plots
Fraxinus excelsior	-0.78	<0.01	0-200	6	75
Quercus robur	-0.90	<0.01	0-160	5	30
Alnus glutinosa	-0.62	0.561	0-180	4	22
Salix alba	0.19	0.655	0-460	4	28
Crataegus monogyna	-0.95	<0.01	0-200	4	25

The effect of structural vegetation type on the frequency of established woody species (all species together) was significant (Table 5.3): frequencies in scrub and tall herb were 2-3 times higher than in grassland (Fig. 5.2). We found no significant differences in total sapling frequencies between tall herb and scrub. For the five most numerous species combined, structural vegetation type (F=7.13; p<0.001), species (F=6.47; p<0.001) and the interaction between structural vegetation type and species (F=3.48; p<0.001) had significant effects on frequency: higher frequencies for *Fraxinus excelsior*, *Quercus robur*, *Alnus glutinosa* and *Salix alba* in tall herb and scrub; only *Crataegus monogyna* had higher frequencies in grassland (Fig. 5.2). Sapling height and stem diameter of *Fraxinus excelsior* were significantly higher in scrub patches, but differences in mean values were rather small (Table 5.3). Grazing period (in the range of 4-11 years) after agricultural cessation had no effect on sapling diameter and height (p= 0.13 and p= 0.58, respectively), but there was a significant interaction with vegetation type for diameter (p<0.001) and a trend for height (p=0.07). The positive effect of increasing grazing period was most expressed in tall herb plots for both diameter and height.

When investigating the effect of moisture conditions (only in the floodplain study sites), overall sapling frequency depended on structural vegetation type (F=12.63; p=0.02), moist category (F=5.28; p<0.01), species (F=4.43; p=0.05) and the interaction between structural vegetation type and species (F=1.74; p=0.03) and showed a clear pattern: higher frequencies in wet plots and in tall herb (Fig. 5.3). A significant effect of species and the interaction between species and structural vegetation type was due to the deviant establishment pattern of *C. monogyna*, which established significantly more in grassland than in tall herb vegetation. No significant interactions between structural vegetation type and moist (p=0.76) and between moist and species (p=0.15) were found. We found hoofprints in all wet plots and in 45% and 32% of moist grassland and moist tall herb plots, respectively.

Table 5.3: Mean (± st. err.) values for frequency, height, diameter and three browsing damage parameters of established saplings (plots = circles of 314m²) per structural vegetation type and results of the GLM-analyses (effect of structural vegetation type). Different letters indicate significantly different mean values (post hoc Tukey-tests).

	mean (± st. err.)			GLM - analysis			
Response	Grassland	Tall Herb	Scrub	Num df	Den df	F value	Pr>F
All species							
sapling frequency per plot	2.23 (±0.54)a	5.94 (±1.74)b	4.76 (±1.40)b	2	135	47.90	<0.001
Fraxinus excelsior							
sapling frequency per plot	0.91 (±0.21)a	2.49 (±0.81)b	3.32 (±1.13)b	2	71	53.2	<0.001
sapling height (m)	1.72 (±0.27)a	1.93 (±0.26)a	2.42 (±0.27)b	2	301	10.52	<0.001
sapling diameter (cm)	2.10 (±0.28)a	1.99 (±0.27)a	2.60 (±0.27)b	2	301	10.20	<0.001
browsing dam. (general) (%)	84.9 (±4.6)a	79.5 (±4.9)a	47.7 (±7.5)b	2	301	21.64	<0.001
apical shoot damage (%)	56.5 (±11.1)a	52.8 (±10.9)a	10.9 (±4.8)b	2	301	24.35	<0.001
stem branching (%)	50.1 (±12.0)a	50.1 (±11.5)a	31.3 (±10.1)b	2	301	5.28	0.005

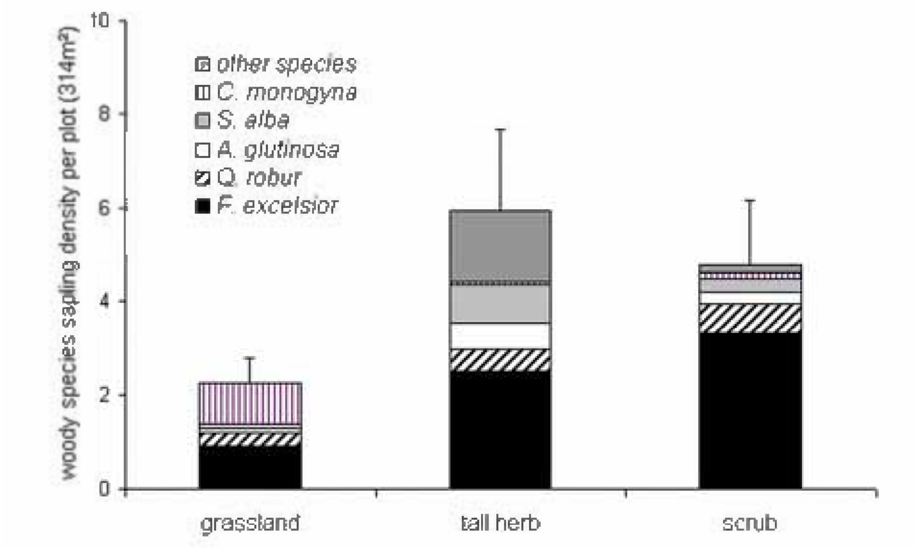

Figure 5.2: Densities of established woody species saplings in three structural vegetation types.

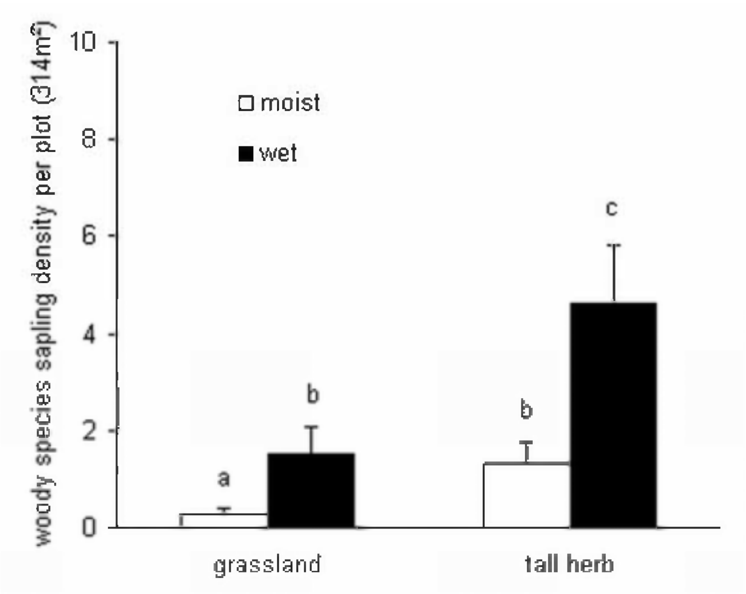

Figure 5.3: Densities of established woody species in moist and wet vegetation patches in floodplains. Different letters indicate significantly different frequencies (post hoc Tukey-tests).

5.3.2 Browsing damage

Overall, high numbers of established *F. excelsior* saplings were browsed (48-85%). In scrub patches, a significantly lower percentage of saplings showed general browsing damage, apical shoot damage and reduced stem branching (Table 5.3; Fig. 5.4). Differences between grassland and tall herb patches were not significant.

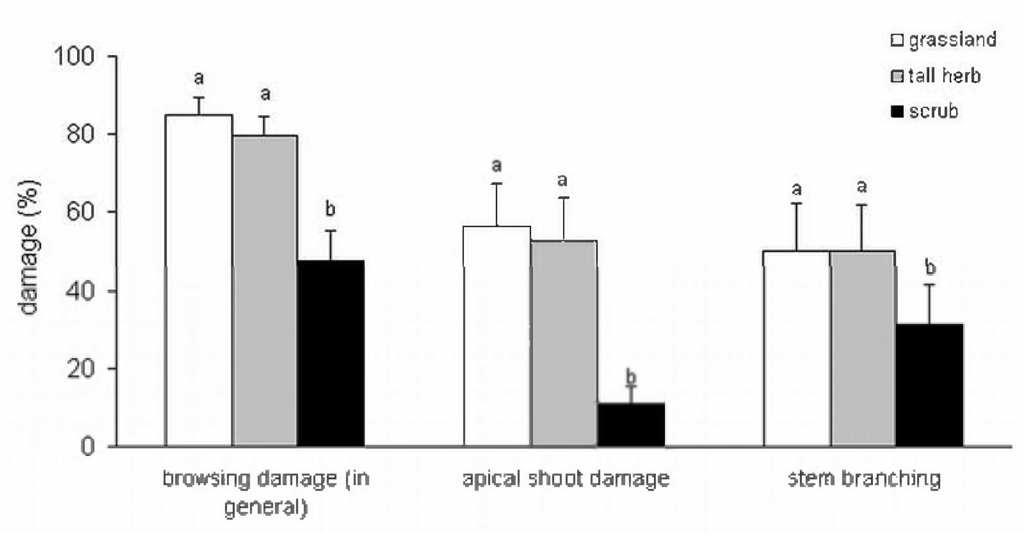

Figure 5.4: Browsing damage pattern of established *F. excelsior* saplings in three structural vegetation types. Different letters indicate significant differences between vegetation structures for a particular browsing damage type (post hoc Tukey-tests).

5.4 Discussion

Palatable saplings were found in close association with scrub, tall herb vegetation and wet patches. Compared to dry grassland patches, scrub and tall herb patches must have provided protection against browsing and wetter patches must have offered better establishment conditions.

5.4.1 Woody species assemblages

Sapling communities are good indicators of the dynamic trends of woodlands, since they represent the result of recent dynamics of a given site and indicate how forest expansion will evolve (Duarte *et al.* 2006). We expected in our study sites a colonisation of grazing tolerant species like spiny shrubs (Archer *et al.* 1988), unpalatable saplings or a combination of protective scrub and palatable saplings (Bakker *et al.* 2004). We mainly found palatable saplings, established in tall herb and scrub patches.

In general, 4 to 11 years after the cessation of intensive grassland summer-grazing, establishment of woody species was sparse, but nevertheless present in all sites. It is known that on nutrient rich and productive sites, it takes 30-45 years before woody species on former open vegetation types become dominant (Smit & Olff 1998). Closed grassland swards inhibit regeneration of woody species (Harmer *et al.* 2001) and retard succession. Woody pioneer and wind dispersed species like *Betula pendula* and *Salix caprea*, which often dominate the early stages of abandoned arable land (Finegan 1984) on heavy soils, occurred in negligible numbers. In contrast, mainly animal and water dispersed species of mid-successional or mature forest stages were numerous. The most frequently found *F. excelsior* is, to a certain extent, also a wind dispersed species, but it has rather heavy seeds. Walker *et al.* (2000) found contrasting establishment patterns for *F. excelsior* and *Q. robur,* the latter being widely dispersed by animals over a newly developing woodland, the former being dispersed at short distance from seed trees. In our study sites, increasing distance to seed trees was correlated with decreasing frequencies of both *F. excelsior* and *Q. robur,* and also of *C. monogyna.* However, sapling numbers decreased gradually with increasing distance to potential seed source and no clear edge effects (i.e. saplings only found in the direct vicinity of the seed trees in adjacent forest; Walker *et al.* 2000) were found. In floodplain sites, seeds of *Alnus glutinosa* probably were mainly spread through inundations. In this study, we were only able to examine rather small areas, with a maximum distance of 460m between plot and potential seed source and 200m between plot and forest edge. In these small areas, the presence of seed sources, wind, inundations, plant-herbivore interactions and even distance to seed sources seemed to be important factors influencing establishment patterns of woody species.

5.4.2 Herbivory and vegetation structure

In early successional stages in a low productive environment, woody species establishment is limited by abiotic stress, rather than by herbivory or competition (Menge & Sutherland 1987). In the productive former agricultural landscapes that we studied, top-down control by large herbivores, as predicted by the exploitation ecosystem theory (Oksanen *et al.* 1981), failed. Van de Koppel *et al.* (1996) showed that in productive environments, high plant standing crop decreased foraging efficiency of herbivores (hares, rabbits, geese), leading to the development of dense vegetations. A comparable mechanism of decreased foraging efficiency may have occurred in tall herb patches in our study sites. However, the development of these patches was primarily due to to the reduction of grazing pressure after the cessation of agricultural use. Consequently, grazing activities structured the vegetation, leading to the development of short grazed patches, dense tall herb patches with mainly unpalatable and spiny species, and scrub. A varying herbivore pressure across such a mosaic with different successional stages, will affect the establishment of woody species. Our findings of increased establishment of palatable saplings in tall herb and scrub (mainly bramble, *Rubus sp.*) patches correspond with the findings of Kuiters & Slim (2003) on woody establishment on former arable land grazed by horses. In a previous study (Van Uytvanck *et al.* 2008) high percentages of seedlings of *Q. robur* and *F. excelsior* survived in tall herb patches of *Juncus* or *Carex* species and these tree species were also able to grow out beyond the browse line in bramble patches. These experimental findings accord to the observational results of this study, but this mechanism was now proven to work for other palatable species as well. However, one of the five common species, *C. monogyna* (palatable but spiny) established mainly in open grassland. This confirms findings of Benjamin *et al.* (2005), who stated that selective browsing may even favour regeneration of spiny shrubs in intensively grazed pastures. Large herbivore grazing or mowing are able to reduce competition between saplings or less-preferred woody saplings like *C. monogyna* and the surrounding vegetation (Archer 1995; Löf *et al.* 2004; Vandenberghe *et al.* 2008).

Damage by herbivores to established saplings depended on the different structural vegetation types. Fewer seedlings were browsed in scrub patches. This was most clearly expressed in damage to the apical shoot of the saplings. The mechanisms here may be related to associational resistance (for scrub), to associational palatability (for grassland, Olff *et al.* 1999) and to neighbour contrast susceptibility (for tall herb, Bergvall & Leimar 2005), meaning that scrub patches offer direct protection to saplings through impenetrable and spiny branches, that in grassland saplings are eaten together with palatable grasses and that in tall herb (mainly unpalatable and hence less visited) palatable saplings, growing out above the canopy, may attract herbivores like cattle. In such a case, selective browsing by cattle in an unpalatable patch may explain the damage pattern of palatable saplings and their apparent inability to grow out beyond the browse line. Kuiters & Slim (2003) also found that established saplings in soft rush and heather patches could not escape from browsing.

However, we believe that direct regeneration in tall herb patches, without a scrub stage, may be an operative short cut in the cyclic vegetation succession in low intensity grazed ecosystems as proposed by Olff *et al.* (1999). First, a few individual saplings in our study sites had already grown out beyond the browse line. Our data cover only 4-11 years. So, it may be only a question of time before forest patches develop. Second, a temporal decrease or absence of grazing pressure (managed or natural) may lead to a sudden and fast growth of already established saplings in tall herb patches (Van Uytvanck *et al.* 2008). Kuiters & Slim (2002) argue that browse-sensitive woody species such as *Quercus* species need a window of opportunity to regenerate. As a consequence, we expect that species that are less browse-sensitive (e.g. *F. excelsior* and *A. glutinosa* have better regeneration capacities after browsing than *Quercus* sp., moreover, *A. glutinosa* is less palatable) will play an important role in woodland development in grazed floodplains, in particular via regeneration in wet tall herb patches, which seem to provide favourable microsites for germination.

5.4.3 Trampling damage and microsites for germination

Gaps or openings in swards, created by trampling or uprooting, may be considered as microsites, allowing partial release from competition and provide opportunities for establishment of woody plants (Jurena & Archer 2003; Vandenberghe *et al.* 2006). Mercier *et al.* (2001) and Staaland *et al.* (1998) showed that imperfect soil drainage on wet soils slowed down the colonisation of woody species in ungrazed, abandoned agricultural land. In the rather intensively grazed sites in this study however, the opposite happened. Wet soils are more susceptible to soil damage by trampling and the increase in suitable germination sites could have favoured establishment. This establishment may logically happen when grazing disturbance is moderate, e.g. in poorly used or unpalatable vegetation types, like tall herb patches (which developed more numerous on wet than on dry soils). Increased desiccation of seedlings in gaps, a major cause of seedling mortality in mountain wood-pastures (Vandenberghe *et al.* 2006) is unlikely to happen in trampled zones in wet tall herb patches in floodplains. Nevertheless, moist conditions in disturbed soils are often a critical constraint for tree germination and establishment success may differ over time (Nilsson *et al.* 2002).

Recruitment of woody species in livestock grazed systems is often episodic and disturbance-driven (Clarke 2002). Disturbances in floodplains like local erosion and sedimentation caused by inundation (Van Splunder *et al.* 1995), dung deposition (de Villalobos *et al.* 2005) and local enrichment may induce natural recruitment of woody species via tall herb and scrub patches, developing in grassland lawns (Olff *et al.* 1999; Vera 2000). Our data suggest that woodland development in floodplains additionally may start via microsite creation caused by large herbivore trampling in wet tall herb patches, which subsequently offer (at least temporal) protection to saplings via associational resistance. We started germination experiments with simulated disturbance regimes in floodplain grasslands to further explore this mechanism.

5.4.4 Conclusions and implications for management

The development of new woodlands in low intensity grazed grasslands is strongly determined by vegetation structure. The pattern of spiny scrub and unpalatable tall herb patches developing in such grasslands reflects future woodland patches, although a temporal decrease in grazing pressure or a temporal absence of large herbivores may be needed for successful outgrowth of established woody species in tall herb. Moderate soil disturbance, caused by trampling, probably is important to create sufficient microsites for germination in tall herb patches. However, local disturbances like flooding, availability of seeds, distance to seed trees and differences in soil moisture influence the regeneration pattern and these factors may interact with large herbivore grazing. Longer time series of woody species development after the cessation of agricultural use are needed to confirm our findings.

Although there’s evidence that large herbivores increase biodiversity in productive ecosystems (Proulx & Mazumder 1998; Bakker *et al.* 2006), the use of large herbivores for woodland expansion at productive sites on former agricultural land is a relatively unknown tool in conservation management. More experience and a process-based approach are needed to fine-tune grazing management strategies and to quantify multiple interaction processes of inhibition and facilitation (Sankey *et al.* 2006). In this study on nutrient-rich soils, we found that a grazing pressure below 0.5-0.6 AU $ha^{-1}y^{-1}$ allows rather fast tree establishment when protective vegetation structures are available. When spiny scrub develops in the grasslands, an even higher grazing pressure will not prevent woodland regeneration. However, in the short term (20-30 years), woodland expansion steered by grazing will only realise open woodland types, but as goal in itself or as a pathway to more closed forest types, these open woodlands may refer to more natural (Rotherham 2007) or attractive park-like landscapes with a high conservation value (Piek 1998).

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Browsed but surviving common ash (Fraxinus excelsior) sapling in a soft rush tussock (Boelaremeersen, Geraardsbergen, Belgium)

Juvenile Fraxinus excelsior trees growing out in a grazed wet tall herb patch (Boelaremeersen, Geraardsbergen, Belgium)

Chapter 6. Large herbivores as nutrient removers

Van Uytvanck J, Milotic T, Hoffmann M. Nitrogen depletion and redistribution by free-ranging cattle in the restoration process of mosaic landscapes: the role of foraging strategy and habitat proportion. *Restoration Ecology*, in press. DOI: 10.1111/j.1526-100X.2009.00599x. Reprinted with permission from Wiley-Blackwell.

Abstract

In a mosaic landscape in N-Belgium (W-Europe), consisting of forest, grassland and wooded pasture on former agricultural land, we assessed nitrogen redistribution by free-ranging cattle (±0.2 animal units $ha^{-1}y^{-1}$). We examined if the spatial redistribution of nitrogen among habitats by cattle could restore nutrient-poor conditions in preferred foraging habitats, and whether such translocation conversely could lead to extreme eutrophication in preferred resting habitats.

We used nitrogen content of different diet classes, habitat use, foraging and defecation behavior, weight gain and nitrogen losses in the actual situation to explore four different habitat proportion scenarios and two different foraging strategies to calculate a net nitrogen balance per habitat. An atmospheric deposition of 30kg N $ha^{-1}y^{-1}$ with varying interception factors according to the habitat types, was included in an integrated nitrogen balance.

All scenarios showed a net nitrogen transport from grassland and wooded pasture to forest habitat. We found that nitrogen redistribution strongly depends on habitat proportion. Nitrogen losses from preferred grassland habitat can be high, given its proportion is small. Depletion is only to be expected at excretion free areas and probably is of minor importance to trigger establishment of woody species. In general, nitrogen transported by cattle was much lower than input by atmospheric deposition, but grazing can compensate for high N inputs in excretion free areas and maintain grassland types that support critical loads of 20-25kg N $ha^{-1}y^{-1}$. In none of the scenarios, N transport by cattle resulted in the exceeding of critical nitrogen loads to vulnerable forest ground vegetation.

Key words

atmospheric deposition; critical nitrogen loads; grazing management; habitat and diet selection; large herbivores

6.1 Introduction

The use of free-ranging large herbivores (cattle, horses, sheep) as a tool for conservation management in Europe is increasing (WallisDeVries *et al.* 1998). Targeted grazing with wild or livestock species, can play a key role in restoring the functioning of ecological processes in a wide range of landscapes, habitats (including former agricultural land), communities and species (Holl & Smith 2007). The creation of open nutrient-poor habitats by large herbivores, as part of shifting successional mosaics or the restoration of highly productive sites (e.g. heavily fertilized agricultural land), are important conservation items (Bakker & Berendse 1999; Pywell *et al.* 2007; Prach & Hobbs 2008). For conservation managers, a prediction of the magnitude and the direction of nutrient redistribution (e.g. nitrogen) by large introduced herbivores in a certain area is important. This knowledge may help determining stocking rate, herbivore species, area and nature of different habitat types to include (or exclude) for grazing. For instance, horizontal nutrient transport by free-ranging cattle may affect vulnerable vegetation types such as forest ground flora. The aim of this paper is (1) to assess to which extent nitrogen redistribution by free-ranging-cattle in a nutrient-rich mosaic landscape, results in the creation of nitrogen-poorer conditions or, alternatively, in the eutrophication of habitats, and (2) to assess the role of habitat proportion and foraging strategy of cattle on nitrogen redistribution.

Selective foraging, habitat use and migration influence nutrient distribution: e.g. nutrient depletion in heathland (Bokdam 2003) and semi-arid savannah (Augustine *et al.* 2003) and nutrient redistribution in riparian systems, (Jacobs *et al.* 2007) and humid grasslands (Moe & Wegge 2008). Bokdam (2001) found clear indications that in nutrient-limited ecosystems, cattle grazing caused alternating dwarf shrub-grassland transitions as a result of alternating lawn depletion and increased nutrient availability after dwarf shrub mortality. Mechanisms determining succession patterns in low-intensity grazed, nutrient-rich areas refer to associational resistance (Van Uytvanck *et al.* 2008b) as a driving force, but little is known about underlying processes such as nutrient redistribution by free-ranging cattle.

Effects of cattle grazing on nitrogen flows are variable, complex and sometimes opposed in time (seasonal differences) and space (Bailey *et al.* 1996). They are induced by diet choice, intake rate, body retention, excretion, nitrogen losses from excreta and habitat use. Indirectly, grazing affects primary production, litter production and the relative availability of forage species and habitats (Semmartin *et al.* 2008; Van Langevelde *et al.* 2008). Habitat use and foraging of free-ranging cattle and other large herbivores play a crucial role in understanding nitrogen flows in an area, because they link all above mentioned factors. The foraging pattern may result from decisions made by the animals at different spatial and temporal scales (Bailey *et al.* 1996). Non-cognitive mechanisms act at smaller spatial scales: foraging velocity decreases and intake rate increases in areas of abundant and high quality forage. Cognitive mechanisms include selection of feeding sites, based on spatial memory, to improve foraging efficiency. The ability to distinguish and remember (the quality of) different feeding sites enables decision making on a larger spatial scale. Both mechanisms refer to matching behavior. Matching is an aggregate response pattern that has been observed in several species, among which cattle: the preference for plant communities is related to relative quantities of preferred species and nutrient abundance (Senft *et al.* 1987a). However, as a consequence of searching and recognition costs and other advantages (e.g. shelter, water) certain habitats offer, the foraging pattern will not be a straightforward response to resource distribution (WallisDeVries & Daleboudt 1994). Cognitive and non-cognitive selection mechanisms do not exclude each other but they may interact in a different way with habitat proportion. The use of spatial and temporal memory may increase or decrease foraging in a particular habitat, though its proportion is small or large respectively.

The magnitude of nitrogen redistribution and potential depletion in grazed areas needs assessment within the larger scope of air-borne nitrogen pollution, which has caused the deterioration of many sensitive ecosystems (Sala *et al.* 2000). Critical loads (Achermann & Bobbink 2003) are often exceeded (Neirynck *et al.* 2007) and restoration management measures for a wide range of ecosystems in Europe will be needed to mitigate or compensate the negative impact of high deposition. In low productive systems, Kooijman & Smit (2001) found that grazing by large herbivores reduced nitrogen availability and compensated for high atmospheric nitrogen deposition. However, very little data are available on the interaction of free-ranging cattle on nitrogen redistribution and atmospheric deposition in predominantly nutrient-rich mosaic landscapes.

In this paper, we test the hypothesis that a net nitrogen transport occurs from preferred grazing habitat (grassland) to less preferred habitats (forest and wooded pasture), having lower nutritional value, but showing a relatively higher occupancy. Second, we simulate the impact of different habitat proportion scenarios (equal proportion, grassland dominance, forest dominance and wooded pasture dominance) on nitrogen redistribution among these habitats. We hypothesize that nitrogen depletion is possible to occur in excretion free areas of preferred foraging habitat with a more pronounced effect when the preferred habitat proportion is small. Further, we explore the effects of two different foraging strategies, related to non-cognitive and cognitive foraging mechanisms respectively. In the former, we assume that the observed foraging pattern corresponds with the velocity cattle transit a certain habitat: slower movement through areas of greater nutrient abundance, and therefore dependent on the present habitat proportion. In the latter strategy, we assume that the observed pattern corresponds with a strong selection, based on the qualities of a habitat, and therefore independent of its proportion. We hypothesize that by using the latter strategy, higher nitrogen losses will be found. In these scenarios, we assess the chances for restoration of nutrient-poor conditions or the risks of habitat eutrophication over time, by calculating the seasonal and yearly proportion of excretion free area per habitat. Finally, we assess the relative impact of nitrogen redistribution by cattle in an environment with a high atmospheric nitrogen input. Results are discussed in the framework of succession in grazed mosaics and the restoration of low productive grasslands vs. the danger of increased nitrogen input on vulnerable forest ground vegetations.

6.2 Methods

6.2.1 Study site

The study was carried out in the nature reserve Bos t' Ename (50°86' N, 3°65' E; ± 100 ha) in N-Belgium (Fig. 6.1). The climate is temperate; annual precipitation is ±700mm. The study site is a mosaic of forest, grassland and developing wooded pasture on sandy loam soils. The forests mainly consist of *Carpinion* and smaller parts of *Alno-Padion* vegetation. In both forest types, vast areas with species-rich ground flora (with abundant *Anemone nemorosa, Hyacinthoides non-scripta, Primula elatior, Vinca minor*) are present. Grasslands are moist to wet and composed by moderate nutrient-rich (*Molinio-Arrhenatheretea*) to nutrient-rich vegetation types (dominated by *Poa trivialis* or *Lolium perenne*). The developing mid-successional wooded pastures are situated on former agricultural land. *Salix caprea*, often heavily grazed, is the dominant tree species. Saplings and juvenile trees of *Betula pendula*, *Fraxinus excelsior*, *Quercus robur* and *Alnus glutinosa* occur frequently; scrub patches of

Rubus sp., *Prunus spinosa*, *Crataegus monogyna* and *Rosa canina* occur occasionally. The ground flora in the wooded pastures consists of a mix of short and tall grassland and ruderal forb patches. Given the temperate climate, the moist and loamy soils and the neglible presence of *Leguminosae* (*Trifolium repens* occurring occasionally and locally in grassland habitat), we assumed nitrogen leaching and biological fixation to be very low in all vegetation types.

Figure 6.1: Study site Bos t' Ename. a: location in Europe and Belgium (asterisk); b: map of the grazed landscape mosaic, distinguishing three global habitat types and water supply sites.

In Bos t' Ename, cattle have free access to forest (25.16 ha), adjacent grassland (27.16 ha) and developing wooded pasture (9.92 ha) since 2004. Small dust roads and paths lacking vegetation are found in forest (0.35 ha) and wooded pasture (0.15 ha). Drinking water sites (ditches, brooks, ponds, springs) are available throughout the year and spread over the whole area (Fig. 6.1). A small herd of the hardy "white-red cattle breed of East-Flanders" was introduced in the area for year-round grazing. This herd consisted of 11 animals: one bull, five cows, two heifers and three calves. Mean individual live weight was 516 (± 219) kg. Though herd size tends to a minimum, grazing pressure is rather high (± 9000 kg/km^2), exceeding grazing pressure in most natural ecosystems (WallisDevries *et al.* 1998).

6.2.2 Nitrogen intake by free-ranging cattle and net N balance per habitat

We studied the role of free-ranging cattle in the process of nitrogen redistribution using field observations, measurements and literature data. We assessed seasonal nitrogen (N) intake by grazing and included body retention, excretion and volatilization to calculate a net N balance per habitat per hectare. We presented consecutive steps in Fig. 6.2. and explained them below (numbers between brackets correspond with numbers in Fig. 6.2).

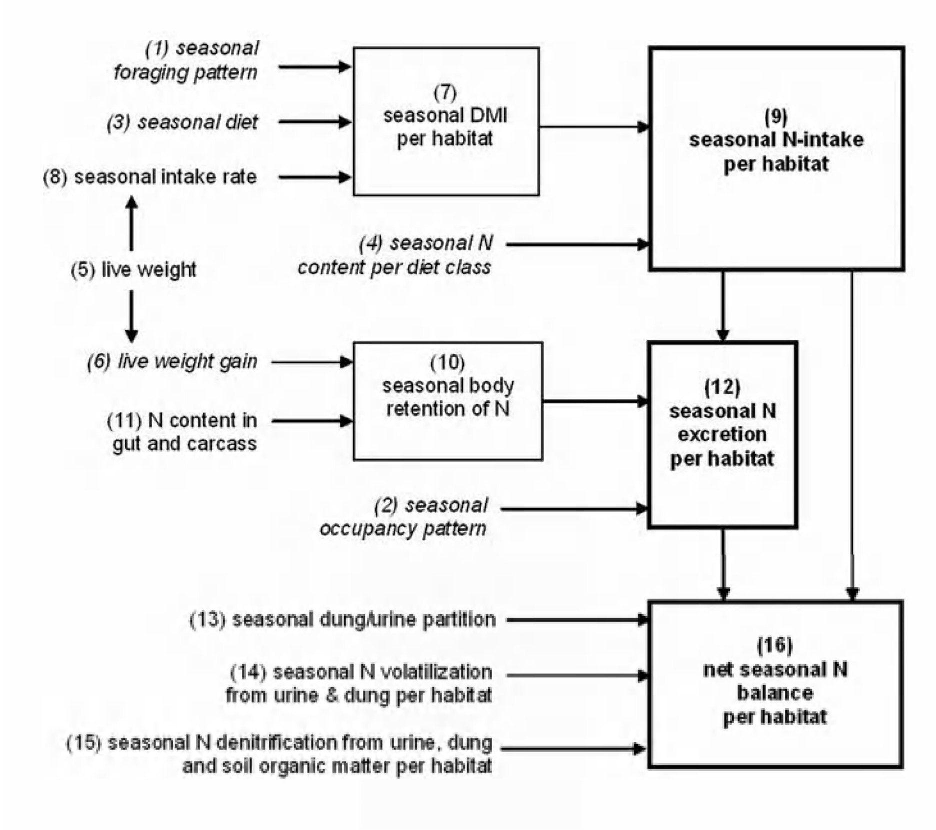

Figure 6.2: Nitrogen flows determining N intake by cattle and net N balance in a grazed mosaic landscape. Calculated results (in boxes, bold ones presented in figures) are based on field data of the study site (italics) and literature data (regular font).

6.2.2.1 Field observations: seasonal foraging (1) and occupancy pattern (2), seasonal diet (3), number of urinations and defecations

We assessed habitat use (foraging and occupancy) and diet using direct observations on time budget in the field. In 2005-2006, we conducted 36 observation sessions spread over one year: nine per season (spring=March-May; summer=June-August; autumn=September-November; winter=December-February). Observation sessions lasted five hours and were conducted in the morning (6-11h), afternoon (11-16h) or evening (16-21h). In each season, we conducted three morning, three afternoon and three evening sessions. We used an instantaneous sampling method (Lamoot *et al.* 2005). We recorded position, habitat, behavior, diet and drinking every 15 minutes for all animals of the herd. All individuals were used to the presence of people and could be approached easily (within a 2m range) without apparent impact on their behavior.

We distinguished four habitat types: forest, grassland, wooded pasture and "other habitat" (dust roads, paths and ponds situated in the three former habitats). We determined seasonal habitat preference using Jacobs' (1974) index of selection. We divided diet in four classes which were easily distinguishable during field observations: grasses, herbs, grasses-herbs mix and woody vegetation. Time budget (%) of habitat use, foraging and diet of all individuals was pooled and averaged per season.

6.2.2.2 Diet nitrogen content (4)

During one year, we took biomass samples each month in one permanent quadrate (PQ, 20m x 20m) per habitat type. Each time, we took four random samples of 0.25m² per PQ, avoiding re-sampling of previous sampled cells. We subdivided samples in monocotyledons (grasses, sedges, rushes), dicotyledons (herbs), mosses, woody vegetation and litter. For these classes, we determined nitrogen content monthly. We excluded mosses and litter for further calculations because we had no observations of cattle foraging on these classes in our study site. We determined crude protein concentration after a drying period of 48 hours (65 ±2°C), using the Kjeldahl-method. We calculated nitrogen content per season (i), habitat (j) and diet class (k) (=$\%N_{i,j,k}$) using Bradstreet's (1965) formula:

$$\%\mathbf{N}_{i,j,k} = \%\textbf{crude protein}_{i,j,k} * \mathbf{6.25}^{-1}\ (\%)$$

We pooled and averaged data per season. For the classes grasses, herbs, grasses-herbs mix and wood, we used the data on nitrogen content of monocotyledons, dicotyledons, average of both former classes and woody vegetation, respectively.

6.2.2.3 Live weight (5) and live weight gain (6)

We measured live weight of all animals of the herd in winter, before and after one year grazing. Live weight gain per season was estimated: spring: +35%; summer: +40%; autumn: +25%; winter: ± 0%. These estimates were based on growth curves for the breed (Butaye & De Baerdemaecker 1972). Body mass losses in winter are reported for cattle and wild herbivores in natural environments, but highly depend on site and severity of winter season (WallisdeVries 1992). For the nutrient-rich study site and the mild winter of 2005/2006, we assumed a stand still in live weight gain.

6.2.2.4. Dry matter intake (DMI) (7)

For cattle, Van Dyne *et al.* (1980) found a daily dry matter intake (dDMI) (8) of 0.021 kg kg^{-1} live weight (LW) (confirmed in WallisDeVries 1994). For the herd in Bos t' Ename, this meant an average dry matter intake of 4391 kg per animal per year (i.e. 48310 kg for the whole herd). We differentiated dDMI per season: 0.030 and 0.015 kg kg^{-1}LW for summer and winter respectively (Senft *et al.* 1987b; Bokdam 2003); intermediate values for spring and autumn: 0.023 and 0.017 kg kg^{-1}LW respectively, allowing us to calculate the seasonal DMI for the herd ($DMI_{herd,i}$).

$$\mathbf{DMI_{herd,i}}\ (kg{*}season_i^{-1}) = \mathbf{91.25}\ (day{*}season_i^{-1}) * \mathbf{dDMI_i}\ (kg{*}day^{-1}kg^{-1}LW^{-1}) * \mathbf{LW_{herd,i}}\ (kgLW)$$

We used foraging time (%) to calculate seasonal dry matter intake per habitat for the herd ($DMI_{herd,i,j}$)

$$\mathbf{DMI_{herd,i,j}}\ (kg{*}season_i^{-1}{*}habitat_j^{-1}) = \mathbf{DMI_{herd,i}}\ (kg{*}season_i^{-1}) * \textbf{foraging time}\ (\%{*}habitat_j^{-1})$$

We used seasonal foraging time (%) on different diet classes to calculate the seasonal DMI of the herd for each diet class per habitat ($\mathrm{DMI}_{herd,i,j,k}$). We assumed a constant intake rate for all diet classes. For woody vegetation, this is probably an overestimation.

$$\mathbf{DMI_{herd,i,j,k}}\ (\mathrm{kg}*\mathrm{season}_i^{-1}*\mathrm{habitat}_j^{-1}*\mathrm{dietclass}_k^{-1}) = \mathbf{DMI_{herd,i,j}}\ (\mathrm{kg}*\mathrm{season}_i^{-1}) * \mathbf{foraging\ time_k}\ (\%*\mathrm{dietclass}_k^{-1})$$

6.2.2.5 Nitrogen intake (9)

Using the measured seasonal N concentrations in the diet, we calculated N intake per season, habitat and diet class ($\mathrm{Nintake}_{i,j,k}$). We further calculated total seasonal nitrogen intake per habitat ($\mathrm{Nintake}_{i,j}$).

$$\mathbf{Nintake_{i,j,k}}\ (\mathrm{kg\ N}*\mathrm{season}_i^{-1}*\mathrm{habitat}_j^{-1}*\mathrm{diet\ class}_k^{-1}) = \mathbf{DMI_{herd,i,j,k}}\ (\mathrm{kg}*\mathrm{season}_i^{-1}*\mathrm{habitat}_j^{-1}*\mathrm{dietclass}_k^{-1}) * \mathbf{\%N_{i,j,k}}$$

$$\mathbf{Nintake_{i,j}}\ (\mathrm{kg\ N}*\mathrm{season}_i^{-1}*\mathrm{habitat}_j^{-1}) = \sum_{k=1}^{n} \mathbf{Nintake_{i,j,k}}\ (\mathrm{kg\ N}*\mathrm{season}_i^{-1}*\mathrm{habitat}_j^{-1}*\mathrm{dietclass}_k^{-1})$$

6.2.2.6 Body retention (10)

Body retention is the N incorporated in the yearly live weight gain. Live weight gain is the sum of carcass live weight increment (85%) and gut fill increment (15%, Agricultural Research Council 1980, in WallisDeVries *et al.* 1998). We assumed N concentrations (11) in the carcass and in the gut =2.56% and =0.52% respectively (Bokdam 2003). We used the seasonal live weight gain of the herd to calculate the seasonal body retention of nitrogen ($\mathrm{BR}_{herd,i}$).

$$\mathbf{BR_{herd,i} = 2.56\% * 85\% * LWgain_{herd,i} + 0.52\% * 15\% * LWgain_{herd,i}}$$

6.2.2.7 Nitrogen excretion (Nexcr_i) (12)

$$\mathbf{Nexcr_i}\ (\mathrm{kg\ N}*\mathrm{season}_i^{-1}) = \mathbf{Nintake_{i,}}\ (\mathrm{kg\ N}*\mathrm{season}_i^{-1}) - \mathbf{BR_{herd,i}}\ (\mathrm{kg\ N}*\mathrm{season}_i^{-1})$$

We further incorporated the seasonal occupancy time (Occuptime) (2) of cattle to calculate excretion distribution among different habitats ($Nexcr_{i,j}$).

$$\mathbf{Nexcr_{i,j}}\ (kg\ N*season_i^{-1}*habitat_j^{-1}) = \mathbf{Nexcr_i}\ (kg\ N*season_i^{-1}) * \mathbf{Occuptime_{i,j}}\ (\%*habitat_j^{-1})$$

6.2.2.8 Gaseous nitrogen losses from excreta and soil organic matter (13-14-15)

Ammonia volatilization from urine patches usually ranges from 10-40% of the total N in urine (Ruess & McNaughton 1988). From fine textured soils, losses are lower than from coarse textured soils (Schimel *et al.* 1986). Volatilization is positively related to soil humidity (Milchunas *et al.* 1988) and negatively related to soil cation-exchange capacity, plant biomass concentration. Volatilization is higher in summer than in winter (Ruess & McNaughton 1988; Frank & Zhang 1997). Given the fine textured soils with a comparable humidity in all habitats of the study site and the volatilization percentages in the above mentioned studies, we estimated seasonal nitrogen volatilization (14) from urine patches ($Nvolu_i$): 5-15 % (summer =15%; autumn and spring =10%; winter =5%). We incorporated the partition (13) of N in urine and dung respectively, to estimate N loss by volatilization per season and habitat ($Nlossvolu_{i,j}$). We assumed that the partition of total N in excreta was 50-70% in urine and 30-50 % in dung, varying among seasons (N percentage in urine (Nu_i): summer = 70%; autumn and spring = 60%; winter = 50%; Frank *et al.* 1994; Senft *et al.* 1987b; Bokdam 2003).

$$\mathbf{Nlossvolu_{i,j}}\ (kg\ N*season_i^{-1}*habitat_j^{-1}) = \mathbf{Nvol_i}\ (\%) * \mathbf{Nu_i}\ (\%) * \mathbf{Nexcr_{i,j}}\ (kg\ N*season_i^{-1}*habitat_j^{-1})$$

Ammonia volatilization from dung (14) is much lower than from urine, but may be of ecological importance (Saarijärvi *et al.* 2006). We estimated N volatilization from dung ($Nvold_i$) was 3% for all seasons (percentages in literature range from 0.5-8%, Sugimoto & Ball 1992a; Jørgensen & Jensen 1997; Saarijärvi *et al.* 2006). Seasonal N percentage in dung (Nd_i) = 100-Nu_i.

$$\mathbf{Nlossvold_{i,j}}\ (kg\ N*season_i^{-1}*habitat_j^{-1}) = \mathbf{Nvold_i}\ (\%) * \mathbf{Nd_i}\ (\%) * \mathbf{Nexcr_{i,j}}\ (kg\ N*season_i^{-1}*habitat_j^{-1})$$

Further, we incorporated N losses from urine, dung and soil organic matter due to denitrifcation (N_2O-emission) (15). Most literature describes intensively used pastures, but it is known that reduced exploitation (e.g. in grazed nature reserves) decreases denitrification (Loiseau *et al.* 2005). Therefore, our assumptions of N losses are rather conservative. The literature does not provide a consistent seasonal pattern for N_2O-emission. It seems rather dependent on soil moisture and rainfall (Groenigen *et al.* 2005a). Therefore, we used equal denitrification rates for all seasons. We assumed that the N_2O-emission from urine ($Nlossdenu_{i,j}$) was 0.5% and from dung 1.0% ($Nlossdend_{i,j}$) (ranges 0.05-0.9 and 0.33-4.0 respectively, Sugimoto & Ball 1992b; Wachendorf *et al.* 2008; Van Groenigen *et al.* 2005a).

$$\mathbf{Nlossdenu_{i,j}}\ (\text{kg N*season}_i^{-1}\text{*habitat}_j^{-1}) = 0.5\ (\%) * \mathbf{Nu_i}\ (\%) * \mathbf{Nexcr_{i,j}}\ (\text{kg N*season}_i^{-1}\text{*habitat}_j^{-1})$$

$$\mathbf{Nlossdend_{i,j}}\ (\text{kg N*season}_i^{-1}\text{*habitat}_j^{-1}) = 1.0\ (\%) * \mathbf{Nd_i}\ (\%) * \mathbf{Nexcr_{i,j}}\ (\text{kg N*season}_i^{-1}\text{*habitat}_j^{-1})$$

We assumed that background N_2O-emission from soil organic matter ($Nlossdens_j$) was 1.5 kg N_2O-N $ha^{-1}y^{-1}$ for wooded pasture and forest habitat, 2.5 kg N_2O-N $ha^{-1}y^{-1}$ for grassland and 5 kg N_2O-N $ha^{-1}y^{-1}$ for other habitats (mostly compacted soils of paths) (Van Groenigen *et al.* 2005b; Saggar *et al.* 2008).

Total seasonal gaseous nitrogen loss (Nlossg) from the system was than calculated as:

$$\mathbf{Nlossgas_{i,j}}\ (\text{kg N*season}_i^{-1}\text{*habitat}_j^{-1}) = \mathbf{Nlossvolu_{i,j}} + \mathbf{Nlossvold_{i,j}} + \mathbf{Nlossdenu_{i,j}} + \mathbf{Nlossdend_{i,j}} + \tfrac{1}{4}\ \mathbf{Nlossdens_j}$$

6.2.2.9 Net N balance (16)

We used the previous calculated seasonal intake, seasonal excretion and seasonal gaseous nitrogen losses per habitat to calculate the net N balance:

$$\mathbf{NetNbalance_{i,j}}\ (\text{kg N*season}_i^{-1}\text{*habitat}_j^{-1}) = \mathbf{Nintake_{i,j}} - \mathbf{Nexcr_{i,j}} + \mathbf{Nlossgas_{i,j}}\ (\text{kg N*season}_i^{-1}\text{*habitat}_j^{-1})$$

6.2.3 Habitat proportion scenarios and foraging strategy scenarios

In addition to the actual situation in Bos t' Ename, we assessed N flows in four extra scenarios with variable habitat proportions, i.e. with woodland-grassland-wooded pasture proportions of 33.3-33.3-33.3% (equal proportions), 60-20-20% (forest dominance), 20-60-20% (grassland dominance) and 20-20-60% (wooded pasture dominance) respectively. For each habitat proportion scenario, we calculated two subscenarios representing different foraging strategies (see introduction). In the first subscenario, we assumed that a non-cognitive foraging pattern will be related to habitat proportion. We changed time budget of foraging and occupancy relatively to the proportion of the habitats in accordance with the observed pattern in the real situation. In the second subscenario, the foraging pattern is independent on habitat proportion: time budget of foraging and occupancy in different habitat proportion scenarios remained the same as in the observed situation at the study site.

6.2.4 Excretion free areas

We counted urination and defecation events in six observation sessions. On average 12.0 (± 2.4) urinations and 11.8 (± 2.9) defecations cow^{-1} day^{-1} were counted. This corresponds with Afzal & Adams (1992), reporting 10.7 ±0.36 and 10.9 ±1.8 dung and urination patches respectively cow^{-1}day^{-1}. Affected areas of 0.04m^2 (Afzal & Adams 1992; own observations) for dung and 0.4m^2 for urine patches (averaging Afzal & Adams 1992 and Bokdam 2003) were assumed. We calculated total area of seasonally excretion free habitat at the study site and in all habitat proportion scenarios using affected area, occupancy time and habitat surface.

6.2.5 Integrated nitrogen balance (at ground level)

We included atmospheric N deposition to study the relative importance of nitrogen redistribution by free-ranging cattle. The OPS-model (van Jaarsveld 2004) provided modeled deposition data (resolution =1 km^2) for Flanders (N-Belgium). For Bos t' Ename, total N deposition was 30 kg N ha^{-1}y^{-1}, which is at present the average deposition level in Flanders and in two nearby and comparable deciduous forest sites (Flemish Level II sites of the European ICP-forests network, Genouw *et al.* 2005; EC-UN/ECE 1996). We corrected N deposition with an interception factor (IF) based on vegetation roughness (Fowler et al. 1999; Bokdam 2003): IF =1.5, 0.6 and 1.1 for forest, grassland and wooded pasture respectively. Further, for forest habitat, we corrected real throughfall at ground level with a factor 0.7 (data Level II sites).

6.3 Results

6.3.1 Nitrogen intake by free-ranging cattle

6.3.1.1 Foraging and occupancy pattern of free-ranging cattle

Except for winter, grassland habitat was the most important habitat for both occupancy (60.17%) and foraging (63.10%) and grassland and wooded pasture were strongly preferred and preferred forage habitats respectively (Fig. 6.3b). Forest is used in all seasons, but only in wintertime it is a substantial forage habitat (Fig. 6.3a). Throughout the year 1.62% of the time budget was spent on non-foraging sites (roads and paths used for resting: 87.40%; ponds for drinking: 12.60 %).

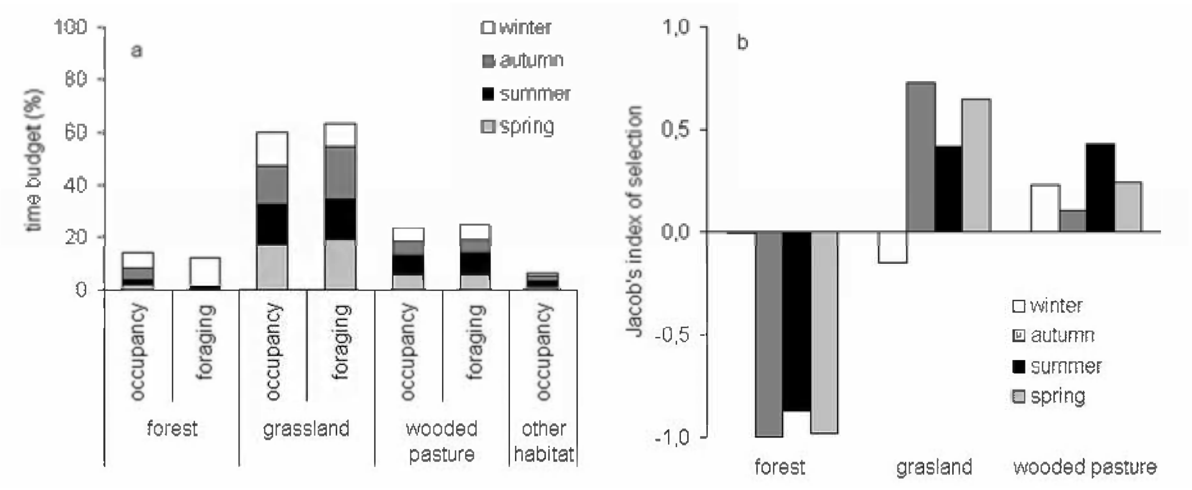

Figure 6.3. Habitat use (occupancy) and foraging pattern of free-ranging cattle in four habitat types in Bos t' Ename. a) seasonal variation in time budget (other habitat= roads, paths and ponds; b) seasonal variation in habitat preference for foraging, using Jacob's index of selection. no selection: -0.08<index<0.08; avoidance: -0.4<index<-0.08; strong avoidance: index<-0.4; preference: 0.08<index<0.4; strong preference: index>0.4

6.3.1.2 Diet and diet nitrogen content

Overall, grasses were the major food source. Woody vegetation became an important food source during winter (>20% of the foraging time, mostly *Rubus sp.*) and summer (mostly young tree shoots and leaves; Fig. 6.4b). N concentrations in forage range from 0.97 to 2.03% and were highest in spring for all diet classes (Fig. 6.4a). After spring, concentrations decreased gradually with the lowest concentrations in winter. Herbs had the highest nitrogen concentration; woody vegetation had the lowest concentration, showing a sharp decline in autumn and winter.

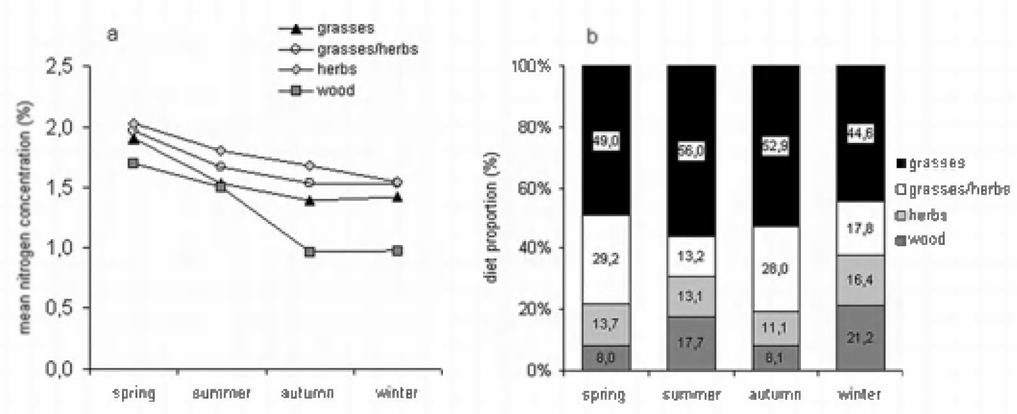

Figure 6.4. a: seasonal variation in nitrogen concentration in four diet classes; b: seasonal variation in diet of free-ranging cattle in Bos t' Ename.

6.3.1.3 Live weight, live weight gain and body retention

Initial individual live weight ranged from ±150 (calves) to ±700 kg (bull). Initial total live weight of the herd was 5675 kg. Total live weight gain for the whole herd was 1341 kg y^{-1} (mean per animal: 121.9 kg ± 135.9). Live weight gain in spring was +469 kg; in summer +536 kg; in autumn +335 kg; in winter 0 kg. Body retention comprised 30.20 kg N for the whole herd, i.e. 3.74% of N intake.

6.3.1.4 Nitrogen intake

Nitrogen intake in grassland and wooded pasture was comparable: -19.9 and -22.2 kg N ha^{-1}y^{-1} respectively and mainly realized in spring and summer. In forest, nitrogen intake is almost negligible (-2.3 kg N ha^{-1}y^{-1}) and almost completely confined to wintertime (Fig. 6.5).

6.3.1.5 Nitrogen excretion

On average, N-excretion was 92.5% of total N intake. The redistribution pattern was strongly related to the N intake pattern (Fig. 6.5), except for forest (excretion evenly spread throughout the year) and non foraging habitats, which received 24.7 kg N ha^{-1}y^{-1}.

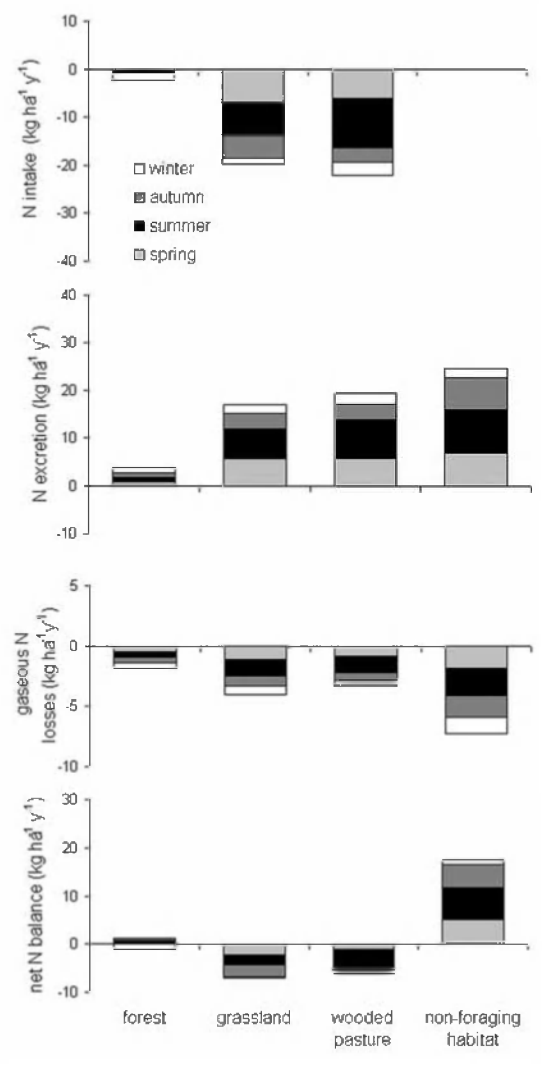

Figure 6.5: N intake, excretion, gaseous losses and net N balance as a result of cattle effects in three foraging habitats and in non-foraging (forest) habitats in Bos t' Ename (negative values indicate N losses from the habitat, positive values indicate N input in a habitat).

6.3.1.6 Gaseaous N losses

Volatilization from urine patches and denitrification from soil organic matter contribute the most to gaseous N losses. Gaseous losses are generally low (max. loss: -4.0 kg N ha^{-1}y^{-1} for all habitats, except for non foraging habitat: -7.3 kg N ha^{-1}y^{-1})(Fig. 6.5).

6.3.1.7 Net nitrogen balance

We found a negative N balance (i.e. N losses) per hectare in grassland and wooded pasture (-6.9 and -6.2 kg N ha^{-1}y^{-1}, respectively; Fig. 6.5). In grassland, all seasons, except for winter, evenly contributed to this negative balance. In wooded pasture, mainly summer contributed to N losses. In forest and non foraging habitats a net nitrogen input of 0.3 and 17.4 kg N $ha^{-1}y^{-1}$ respectively was calculated.

6.3.2 Habitat proportion and foraging strategy

N intake strongly depended on habitat type and habitat proportion (Fig. 6.6). In forest, N intake was small in all scenarios (maximum: -4.5 kg N $ha^{-1}y^{-1}$). In grassland, yearly N intake ranged from -14.5 to -43.4 kg N $ha^{-1}y^{-1}$. The highest intake was found when grassland habitat proportion was small (20%). In general, N intake in grassland was higher in scenarios with a foraging strategy that was independent on habitat proportion. In wooded pasture, N intake ranged from -5.7 to -27.3 kg N $ha^{-1}y^{-1}$. N intake was highest in the forest dominance scenario. For wooded pastures in general, we found a higher intake in scenarios with a foraging strategy that was dependent on habitat proportion.

Yearly differences among habitats and scenarios were much smaller for the net N balance (range: -12.2 – 1.9 kg N $ha^{-1}y^{-1}$). A small yearly net N input was found in forest habitat (< 2.0 kg N $ha^{-1}y^{-1}$). Nitrogen losses and inputs are most balanced in the grassland dominance scenario (range: -5.9 – 1.9 kg N $ha^{-1}y^{-1}$). The highest N losses were found in grassland (-12.2 kg N $ha^{-1}y^{-1}$), given its proportion was small and foraging strategy was independent on habitat proportion. N losses in wooded pasture were lower than or equal to those in grassland (-7.8 – -2.7 kg N $ha^{-1}y^{-1}$).

6.3.3 Excretion free areas

At the study site, we found high percentages of yearly excretion free area for all habitats: forest: 98,8%; grassland: 95.3%; wooded pasture: 94.7% and other habitat 93.1% (Table 6.1). For all habitats and in all proportion scenarios, yearly excretion free areas comprised more than 89.8 % of the total surface (Table 6.1). Percentages given are minimal values, assuming no overlap in excretion patches, which is a conservative assumption.

6.3.4 Integrated nitrogen balance

With an atmospheric N input of 30 kg N $ha^{-1}y^{-1}$, we found an average net N input for almost all habitat types and scenarios (Table 6.1). Only in grassland habitat, we found net N losses in excretion free areas: a small loss in the actual situation (-1.9 kg N $ha^{-1}y^{-1}$) and a considerable loss (-27.9 kg N $ha^{-1}y^{-1}$in habitat proportion scenarios with a relatively small grassland proportion (Table 6.1).

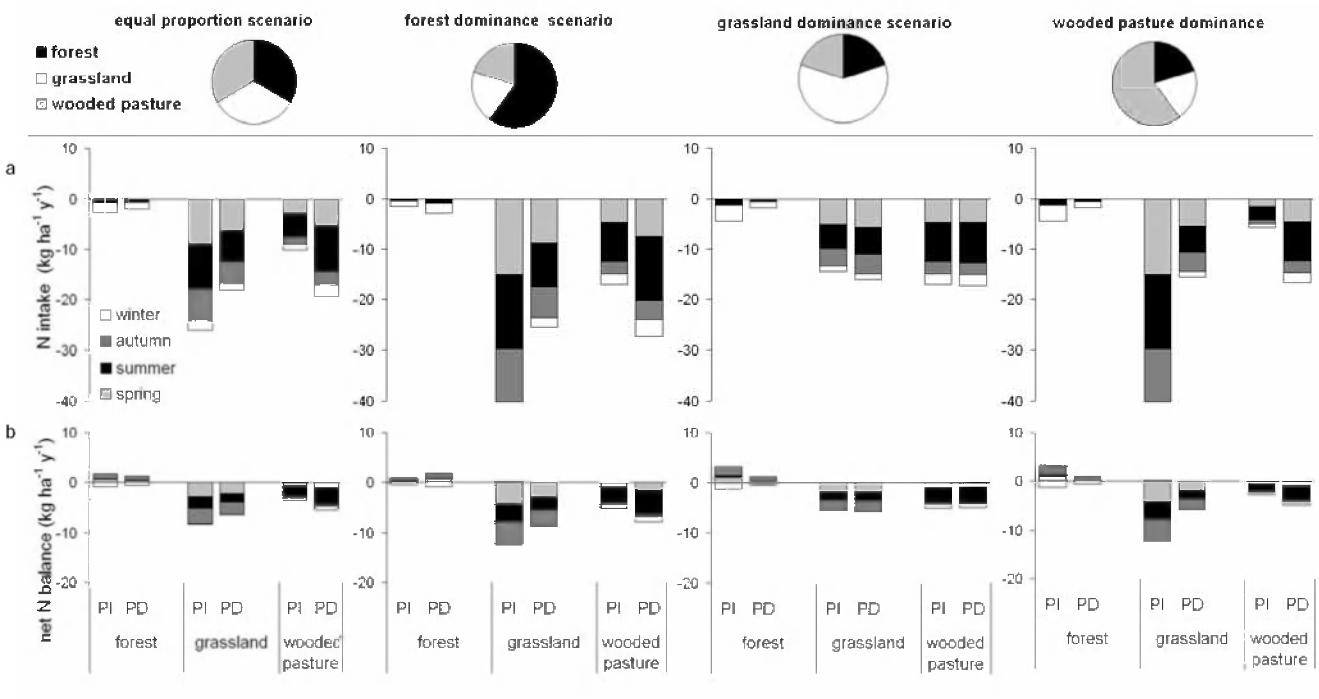

Figure 6.6. Seasonal and yearly N intake and net N balance in a mosaic landscape grazed by cattle in four habitat proportion and two foraging strategy scenarios. Proportion scenarios differ in the dominance of a particular habitat. Two contrasting foraging strategies (inside each diagram) were derived from the actual foraging pattern of the cattle herd at the study site Bos t' Ename. In a first strategy, foraging is based on cognitive mechanisms allowing habitat selection independent on habitat proportion (PI). In the second strategy, foraging is based on non-cognitive mechanisms, dependent on habitat proportion (PD). a: nitrogen intake by foraging cattle; b: net N balance (including body retention, return by excretion and gaseous N losses; positive values indicate a net N-input, negative values indicate N losses per habitat).

6.4 Discussion

Although our calculations are generally conservative, they clearly indicate a spatial N redistribution among different habitats. In general, free-ranging cattle cause N transport from grassland and wooded pasture to forest and non-foraging habitats as a result of seasonal differences in foraging, occupancy, diet and diet N content. However, calculations indicate that the magnitude of redistribution strongly depends on proportional habitat availability. Variances are expected at two levels. First, N intake will be influenced by both cognitive and non-cognitive foraging mechanisms. We expect intermediate values of N intake, i.e. between both extreme foraging strategies, to be more

Table 6.1: Integrated N balance with an atmospheric input of 30 kg N ha^{-1}y^{-1} and yearly proportion of excretion free areas for the actual situation in Bos t' Ename and for different habitat proportion and foraging strategy scenarios. Average values are given for the entire habitat and for excretion free areas. Negative values indicate a yearly net N loss; PI = proportion independent foraging strategy; PD = proportion dependent foraging strategy.

		integrated N balance entire habitat (kg N ha^{-1}y^{-1})		integrated N balance excretion free areas (kg N ha^{-1}y^{-1})		excretion free area (%y^{-1})	
actual situation	forest	32.3		29.2		98.7	
40-44-16 %	grassland	11.1		-1.9		95.3	
	wooded pasture	29.8		13.9		94.7	
proportion scenarios		**PI**	**PD**	**PI**	**PD**	**PI**	**PD**
equal proportion	forest	32.5	32.2	27.3	28.0	98.5	98.9
33-33-33 %	grassland	9.7	11.5	-10.5	-2.6	93.9	95.7
	wooded pasture	32.4	30.5	24.3	15.1	97.6	95.4
forest dominance	forest	31.9	32.5	28.5	27.0	99.2	98.5
60-20-20 %	grassland	5.8	9.3	-27.9	-10.0	89.8	94.1
	wooded pasture	30.9	28.2	17.5	7.2	95.9	93.6
grassland dominance	forest	33.4	32.1	26.7	27.2	97.6	99.0
20-60-20 %	grassland	12.3	12.1	1.0	-0.6	96.6	96.1
	wooded pasture	30.9	31.1	17.5	17.3	95.9	95.8
wooded pasture	forest	33.4	32.1	25.5	28.3	97.6	99.0
dominance	grassland	5.9	12.2	-27.8	-0.1	89.8	96.3
20-20-60 %	wooded pasture	33.3	31.1	28.8	17.8	98.7	96.0

realistic. Our results may indicate the range of uncertainty caused by cattle foraging. Second, we assumed a constant intake rate for all diet classes. However, it is known that intake rates differ according to food type and season (Pastor & Cohen 1997; van Langevelde *et al.* 2008). As a result, weight (gain) may decrease in the dormant season (WallisDevries *et al.* 1998). This may lead to an overestimation of N intake in autumn and winter and to an underestimation of intake in spring and summer. This may mask a more pronounced N flux to the forest. Further, our results show relative changes in the inorganic N pool of different habitats. Real nutrient availability for plants also depends on the amount and nature of litter (Wedin & Pastor 1993), N retention in above ground woody biomass (Magill *et al.* 2000), the functioning of the decomposer trophic level and feedback mechanisms of grazing on litter decomposition (Semmartin *et al.* 2008). All these factors influence the rate of N turnover in different seasons and habitats. They need further examination, but are beyond our current objectives.

We may expect N depletion of a habitat if the integrated balance shows yearly N losses. Different habitat proportion scenarios for the nutrient-rich study site show that depletion is possible in excretion free patches in grassland and wooded pasture (i.e. in patches where N intake determines the net N balance completely), given that the proportion of these habitats is relatively small. The pattern is more pronounced for the preferred grassland habitat and with a foraging pattern that corresponds to a cognitive foraging mechanism of habitat selection. This is in line with results of Moe & Wegge (2008) for deer, concentrating their grazing on small preferred patches, while depositing dung mostly elsewhere. Translocation of nutrients may be considerable when herbivores show so-called camping behavior (Güsewell *et al.* 2005) and it may be an efficient way to counteract soil nutrient enrichment on at least a part of the grassland area (Dahlin *et al.* 2005). Our own data showed that cattle often congregate in the forest, in non foraging habitats such as roads and paths to rest and to a lesser extent at drinking sites (all with a relatively small surface) and as a consequence defecations are congregated as well. The importance of habitat proportion is related to the functional diversity of habitats and to the functional differences among plant species in nutrient uptake rates and herbivore preference (Pastor & Cohen 1997). These functional differences will strongly influence nutrient return. In our study, a relatively small proportion of preferred habitat in a nutrient-rich mosaic landscape seems to be of major importance to induce shifts in N transport and potential depletion. It may lead to high intake and a negative net N balance with N losses >40 kg N $ha^{-1}y^{-1}$. Van Langevelde *et al.* (2008) showed that grassland lawns, developed this way, can be maintained over longer periods. This is due to the limited ability to process information about food quality at the moment of foraging, reducing the consumer's ability to make diet choice decisions. This phenomenon may speed up depletion. Grazing may also cause over-compensatory growth of palatable species at fertile sites, resulting in the dominance of grasses (Hawkes & Sullivan 2001). Brathen *et al.* (2007) could not confirm this, but although productive and palatable plants were suppressed (reindeer grazing) at fertile sites, there was no evidence for a persistent loss of productivity.

The critical load for boreal and temperate forest ground flora is estimated at 10-15 kg N $ha^{-1}y^{-1}$ (Achermann & Bobbink 2003). The same load is critical for forest soil processes and biodiversity indicators such as lichens, algae and mycorrhizae. Critical loads of 10-20(25) kg N $ha^{-1}y^{-1}$ were set for a broad range of species-rich grasslands and temperate heathlands (Achermann & Bobbink 2003). In our study, the relative influence of N transport by cattle on exceeding or declining beneath critical loads is largely scale dependent: i.e. a small impact on the landscape and habitat level and a possible large impact on excretion free areas. While integrated N balances at ground level, under a high deposition of 30 kg N $ha^{-1}y^{-1}$, on average show N surpluses, relatively high proportions of excretion free areas remained present in all habitats (± 90% y^{-1}). In these excretion free areas of nutrient-rich grassland and wooded pasture, N intake by free-ranging cattle reduced net N input to an extent that critical loads of 20-25 kg N $ha^{-1}y^{-1}$ (Achermann & Bobbink 2003) were not exceeded in almost all scenarios. For low productive ecosystems, Kooijman & Smit (2001) also found that grazing compensates for high atmospheric N loads. However, in the productive, grazed (with 0.55 cattle ha^{-1}) grasslands of the flooding pampa (Argentina), Chaneton *et al.* (1996) found N accumulation, even with very low atmospheric inputs (7.5 kg $ha^{-1}y^{-1}$). This might be the case for a broad range of nutrient-rich grassland types where leaching is restricted (Dahlin *et al.* 2005). In these studies however, no other habitats, nor mosaic landscapes were involved. N intake in excretion free forest and N transport to forest at the habitat level were negligible compared to atmospheric input, whether moderate or high. Severe effects on vulnerable forest ground vegetation is only to be expected under excretion patches. N in urine patches of cattle may be equivalent to 1000 kg N $ha^{-1}y^{-1}$ (Silva *et al.* 1999). In our study site however, a maximum of 2.5% of the total forest area was affected per year.

Our data suggest that N redistribution by free-ranging cattle strongly depends on the proportional availability of different habitats. It is mainly in the highly preferred grassland habitat that N balances can be altered and N poor conditions can be restored. Given that potential N depletion is restricted to excretion free grassland and wooded pasture patches when their habitat proportion is small (our results), attained dominance of grasses at fertile sites (Hawkes & Sullivan 2001) or persistence of productivity (Brathen *et al.* 2007), the restoration potential for oligotrophic grasslands is limited. Even so, N depletion by grazing, as a trigger for the establishment of woody species, is not likely to be of major importance in nutrient-rich mosaic landscapes (cf. the Resource mediated Successional Grazing Cycle theory, Bokdam 2003). Woody species establishment rather seems dependent on the availability of microsites, created by disturbances or ecosystem dynamics (de Villalobos *et al.* 2005; Jentsch 2007; Van Uytvanck *et al.* 2008a). N redistribution by cattle in relation to the potential exceeding of critical N loads on vulnerable ground vegetations is almost negligible compared to atmospheric deposition. In grassland and wooded pastures, cattle grazing can compensate for high N inputs, maintaining grassland types that support N loads of 20-25 kg N $ha^{-1}y^{-1}$.

Implications for practice

- In grazed, nutrient-rich mosaic landscapes, which support a relatively high grazing pressure (here ±9000 kg $km^{-2}y^{-1}$), proportional habitat availability has a considerable effect on N redistribution.
- The restoration potential for oligotrophic habitats, as a result of N transport and depletion by cattle, is limited. The proportion of grassland or wooded pasture habitat has to be small (< 40%), compared to other, less used habitat types such as forest. Moreover, nutrient depletion is only to be expected in excretion free areas under low atmospheric N input (less than ±20 kg N $ha^{-1}y^{-1}$). N depletion as a trigger for the regeneration of woody species is of minor importance in grazed nutrient-rich ecosystems.
- In the preferred grazing habitats of mosaic landscapes, grazing can compensate for high atmospheric N input and maintain grassland types that support N loads of 20-25 kg N $ha^{-1}y^{-1}$.
- N transport in low intensity grazed nutrient-rich mosaic landscapes does not lead to the eutrophication of vulnerable forest ground flora, given the proportion of forest habitat is not too small (min. 25-30%). On this condition, only small areas that receive excreta (< 3% y^{-1}), can be severely affected.

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Chapter 7. Large herbivores and forest plant diversity

Van Uytvanck J, Hoffmann M, 2009. Impact of grazing management with large herbivores on forest ground flora and bramble understorey. *Acta Oecologica* 35: 523–532

Abstract

We investigated whether grazing management with large herbivores is appropriate to reduce expanding bramble (*Rubus* sp.) in an ancient forest in Flanders (N. Belgium). We further studied interaction effects of four years (all year-round) grazing and *Rubus* cover on the presence, cover, and flowering of five forest ground flora species (unpalatable: *Anemone nemorosa* and *Primula elatior;* palatable*: Hyacinthoides non-scripta, Vinca minor* and *Hedera helix).* We collected data on eight transects and in 412 plots in adjacent grazed and ungrazed forest and used baseline datasets of 1996 and 2002 in the same area (i.e. before grazing). In a field experiment, we simulated grazing (by clipping) and trampling (by pressing a weight) in eight homogeneous *A. nemorosa* vegetation stands.

Large *Rubus* thickets had a clear negative impact on cover and flowering of *A. nemorosa* due to competition for light. Four years of cattle grazing reduced bramble cover by more than 50%, but then the limiting factor for *A. nemorosa* cover and flowering shifted to trampling damage. We also found lower cover and flowering of *H. non-scripta* in grazed plots, as a consequence of direct grazing. The evergreen species *V. minor* and *H. helix* totally disappeared from the grazed forest. Simulated once-only effects of grazing and trampling had a small and short term negative impact on cover of *A. nemorosa*, but flowering was strongly reduced. Grazing reduced biomass with 25-30 % in the following years.

Year-round grazing with large herbivores is an appropriate measure for bramble control in forests, but negative effects on ground flora are possible if grazing pressure is high. A low or moderate grazing pressure (< 0.25 animal units $ha^{-1}y^{-1}$) should be maintained in landscape mosaics with grassland and forest; or intermittent periods of non-grazing should be provided to maintain forest ground flora diversity.

Key words

Anemone nemorosa; associational resistance; bramble control; competition for light;
Hyacinthoides non-scripta; treading and trampling

7.1 Introduction

Woodland conservation and large herbivore grazing (or grazing management) are often reported as conflicting aims. On the one hand, livestock production and high numbers of wild herbivores are considered as a serious threat for biodiversity in native woodlands (e.g. Rackham 1980; Steinfeld *et al.* 2006; Dufour-Dror 2007; Tremblay *et al.* 2007). On the other hand, evidence is growing that large herbivore grazing is a key process in the dynamics of naturally grazed ecosystems, including woodland (Olff *et al.* 1999; Bokdam 2001; Bakker *et al.* 2004). In Europe, grazing by domestic herbivores in forests is mainly restricted to woodpasture remnants (Putman 1986; Mountford & Peterken 2003), although it is still a widespread land use form in Britain (Armstrong *et al.* 2003), mainly in small forests. In Flanders (N. Belgium), livestock has been gradually removed from woodland areas since early Medieval times (Tack *et al.* 1993). However, since the end of the twentieth century, there is growing interest in forest grazing with domestic large herbivores as part of conservation and restoration management practices (Rodwell & Patterson 1994; Bakker & Londo 1998; WallisDeVries *et al.* 1998).

Apart from the general conservation goals (concerning naturalness or increasing vegetation structure heterogeneity), grazing management in forests is sometimes used for specific reasons, in an attempt to counteract unfavourable processes (e.g. control of dominant species, Van Wieren & Bakker 1998). In the past decades, one of the most obvious changes in many forests in Flanders is the strong expansion of the bramble understorey (in most cases *Rubus fruticosus* agg., below mentioned as *Rubus*). *Rubus* also invades forests with high conservation interest, such as ancient forests. Ancient forests are characterised by a species-rich ground flora, mainly consisting of stress tolerant geophytes and hemicryptophytes with poor regeneration capacities (Hermy *et al.* 1999). McEvoy *et al.* (2006a) also found a trend of bramble expansion in woodlands in Northern Ireland. There is evidence that species richness declines as bramble cover increases or the species becomes dominant (Kirby 1990; Kirby & Woodell 1998).

There are three major factors described that may cause *Rubus* expansion in Flemish (and other European) forests. First, since the second half of the twentieth century, a conversion took place from coppice wood with standards to high wood in nearly all ancient forests. Not the conversion by itself, but rather the cessation of the accompanying measures, such as repeated cutting of thickets (including *Rubus*, often bundled in faggots for burning) and the removal of litter, benefited the development of *Rubus* (Kirby *et al.* 2005). Second, fragmentation of ancient forest led to increased forest edge length and area and promoted *Rubus* cover increase (Honnay *et al.* 2002). Finally, increased atmospheric nitrogen deposition, caused by intensive animal husbandry and increased traffic intensity, favoured nitrophilous vegetation (including bramble species), decreased vegetation diversity and changed community composition in forests (Landmann 1995; Bobbink *et al.* 1998; Diekmann & Falkengren-Grerup 2002; Skrindo & Okland 2002; Bernhardt-Römermann *et al.*, 2007).

Conservation practitioners are concerned about a possible shift towards a decreased species richness with dominance of plants with a competitive strategy (sensu Grime *et al.* 1988), such as *Rubus*. Grazing management in ancient forests may be a tool to affect competition between *Rubus* and forest ground flora (McEvoy *et al.* 2006b; Kirby & Thomas 2000). *Geum urbanum, Lysimachia nemorum, Potentilla sterilis, Primula vulgaris* and *Viola riviniana* were among forest species that increased in a cutting trial when cover of *Rubus fruticosus* was removed (Kirby & Woodell 1998). On the other hand, neither the effects of grazing nor trampling on ancient forest ground flora are studied comprehensively. It is known that herbivory can affect the physical structure, species composition and population structure of woodland communities (Jenkins *et al.* 2007). Trampling can alter soil processes (e.g. N-mineralisation) by increasing soil bulk density (Breland & Hansen 1996). Trampling effects on plants and vegetation include damage to the root system and a reduction of plant species diversity, cover, biomass and height (Cole & Bayfield 1993; Liddle 1997). Mesophilous forest communities are found to be susceptible to trampling (Roovers *et al.* 2004; Amrein *et al.* 2005). However, little is known about effects on forest specialists.

In this study, we investigated the effects of four years of year-round grazing on ground flora and *Rubus* cover in an ancient forest. We hypothesized that increasing *Rubus* cover has negative effects on cover and flowering of forest ground flora species. We further hypothesized that large herbivore browsing reduces *Rubus* cover. We hypothesised that the reduction of *Rubus* cover, as a consequence of grazing management, has a positive influence on presence, cover, flower abundance and growth of forest ground species. However, we expect interaction with the palatability of these species: for palatable species such as *Hyacinthoides non-scripta, Vinca minor* and *Hedera helix,* positive effects of reduced cover might be counteracted by negative effects due to grazing damage.

For unpalatable species such as *Anemone nemorosa* and *Primula elatior*, we expect a positive effect of reduced *Rubus* cover through grazing.

To get an insight into the direct short term effects of grazing and trampling, we set up a field experiment with *A. nemorosa*, in which total grazing and trampling were simulated as a once-only and extreme event. We hypothesized that direct trampling and grazing have a negative effect on cover, flower abundance and biomass of this species and explored recovery in two consecutive years.

We discuss the relative importance of possible positive effects (as a result of bramble control) and possible negative effects of grazing management (as a result of direct damage) on forest groundflora in a context of altered competition, associational resistance and palatability of plant species.

7.2 Methods

7.2.1 Study sites and studied species

The study was carried out in two nature reserves on moist, nutrient rich soils: Bos t' Ename (50°86' N, 3°65E; ± 100 ha; sandy loam) and Hayesbos (50°78N, 3°77E; ± 50 ha; loam) in Flanders (N. Belgium). Forests in these two reserves mainly consist of *Carpinion* (most abundant trees: *Fagus sylvatica*, *Quercus robur* and *Populus* x *canadensis*) and smaller parts of *Alno-Padion* vegetation (most abundant trees: *Fraxinus excelsior*, *Alnus glutinosa*, *Ulmus minor*). Large areas with species-rich ground flora, flowering in early spring, are present (most abundant are *Anemone nemorosa* and *Hyacinthoides non-scripta* in the *Carpinion* forest and *Primula elatior, A. nemorosa,* and *Vinca minor* in the *Alno-Padion*). Both study areas are among the most species-rich forests in a survey of 234 randomly chosen deciduous forest patches (0.5-5216 ha) in western Belgium and the most northern part of France (Honnay *et al.* 1999a). Bos t' Ename has been the subject of both intensive historical and ecological research (Tack *et al.* 1993; Honnay *et al.* 1999b).

Since the end of the 20th century, Bos t' Ename and Hayesbos are managed as nature reserves. In Bos t' Ename, forest (25.1 ha) is grazed together with adjacent grassland (25.5 ha) and former arable land (10.7 ha) since 2004 (Fig. 7.1). A small herd of the local and hardy cattle breed "white and red of East-Flanders" (10 -14 animals, including cows, heifers, calves and one bull) and two Konik horses were introduced in the area for year-round grazing (i.e. ±0.25 animal units (AU) ha^{-1}y^{-1}). This grazing pressure is rather high (≥ 125 kg herbivore biomass ha^{-1}y^{-1}, cf. WallisDeVries *et al.* 1998) and fairly constant. Both cattle and horses were introduced to trigger and guide the development of more natural vegetation patterns on formerly intensively used grassland (summer grazing) and arable land.

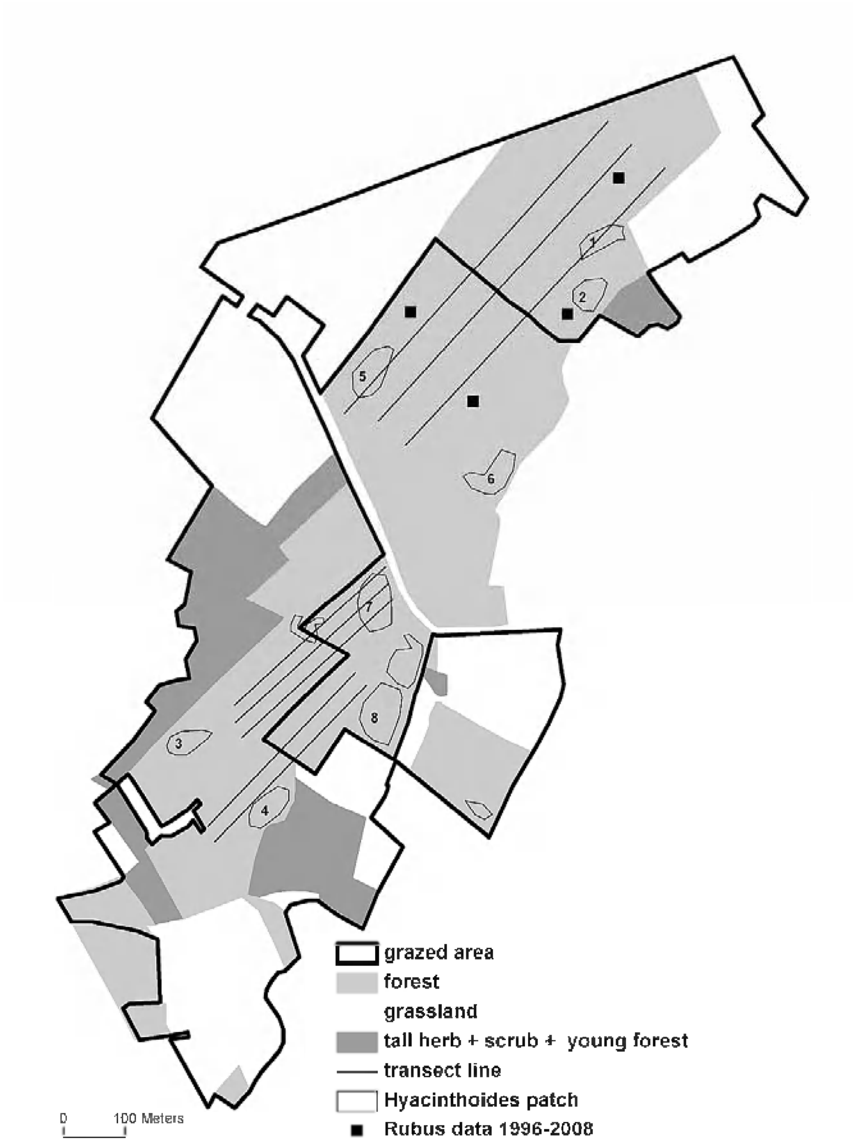

Figure 7.1: Study site Bos t' Ename: location of 8 transects with 412 plots (2008), 8 sampled *Hyacinthoides* patches (2008, with number) and 4 permanent plots (each divided in an exclosure and an open subplot, 1996-2008) in grazed (since 2004) and permanently ungrazed forest. One transect in the NW of the forest was set out in *Alno-Padion,* the other transects were set out in *Carpinion* forest.

Forest was included in the grazing block to join former agricultural land, but in particular, the managers wanted to amass knowledge concerning the effects of large herbivores on forest development (e.g. natural regeneration, bramble control, effects on ground flora). Part of the forest, adjacent to grazed parts (20.2ha) and some isolated forest patches remained ungrazed. For grazing, browsing and resting, the forest was almost exclusively used by the cattle herd. During one year, the cattle's time budget for foraging was 12.3% in the forest, 63.1% in grassland and 24.6% on former arable land. 88% of the grazing time in the forest occurred during winter, mainly late winter (Van Uytvanck, unpublished data of 36 observation sessions of five hours, evenly spread over seasons and daily period). Horses did not use the forest for foraging since their introduction in the area. In Hayesbos, only spontaneous development occurs (no grazing, no other management measures). The latter study area was used for the grazing and trampling simulation experiment. Deer were absent in both study sites. An introduction trial of roe deer (*Capreolus capreolus*) in Bos t' Ename in 1997 (six individuals) failed. All roe deer disappeared from the area less than 2 months after introduction.

We studied six plant species (the forbs are typical ancient forest species): *Rubus fruticosus* group (representing >95 % of all *Rubus* species), *Anemone nemorosa*, *Primula elatior*, *Vinca minor*, *Hedera helix* and *Hyacinthoides non-scripta*. The former five species are spread throughout the forest, with *A. nemorosa* forming large stands, massively flowering in spring. *H. non-scripta* mainly occurs in almost pure, spatially distinct stands (Fig. 7.1). *H. non-scripta*, *H. helix* and *V. minor* are known as palatable plants. The presence of irritant and antibiotic protoanemonine and ranunculine in *A. nemorosa* and the acrid tasting triterpene-saponins in *P. elatior* makes both species unpalatable for livestock (van Genderen *et al.* 1997; Grime *et al.* 1988; De Cleene & Lejeune 1999; Okrslar *et al.* 2007).

7.2.2 Data collection

Four different datasets were used to study grazing management effects: (1) four permanent plots (1996-2008; *Rubus*) and (2) 42 relevés (2002; *Rubus*, ground flora) were used as baseline for (3) a fence line study with 412 plots (2008; *Rubus*, ground flora). A simulation study (4) with eight plots was used to underpin the mechanistic effects of defoliation and treading (2006-2008; ground flora). The sampling procedure and datasets used for the different aims of this study are summarised in Table 7.1.

7.2.2.1 Rubus cover before and after grazing management (datasets 1996-2008 and 2002)

To assess cover percentages of *Rubus* before and after grazing management, we acquired a dataset of 1996. This dataset (Cornelis 1996) contains 32 relevés (decimal scale of Londo 1984), made in 4 permanent plots, marked in the field (Fig. 7.1). Each plot was divided into two subplots (one roe deer exclosure and one open subplot) of 20m x 20m. In all subplots, 4 relevés (10m x 10m) were made, resulting in a total of 32 relevés. In 2004, two of the four plots were included in the grazing block. One exclosure subplot in the grazing block (near to *Hyacinthoides* patch 2, Fig. 7.1) was destroyed before cattle and horses were introduced in 2004, resulting in one more grazed subplot. From this dataset, we used *Rubus* cover percentages. In April 2008, we revisited all subplots and assessed cover percentage of *Rubus* again.

To assess the starting conditions of the study area before grazing, we used a set of 42 relevés (2m x 2m; decimal scale of Londo 1984) made in the study area in August 2002, i.e. two years before the start of year-round grazing in Bos t' Ename (Buckens 2003). These relevés were located in the area where transects were established (Fig. 7.1): 19 in the forest block grazed since 2004, 23 in the permanently ungrazed forest block. From this dataset, we used cover data of *Rubus* (Table 7.1).

7.2.2.2 Frequency of forest ground flora before (dataset 2002) and after grazing (dataset 2008)

We used the 42 relevés (dataset Buckens 2003) to obtain presence/absence data from three frequently occurring forest ground flora species: *H. helix, P. elatior* and *V. minor*. We used presence/absence because cover was always low (< 10%). Because relevés were made in August (student's thesis), *A. nemorosa* and *H. non-scripta* could not be accurately recorded. In the same area, we recorded presence/absence of the same species in 412 plots in April 2008 (see 2.2.3; Table 7.1).

Table 7.1: Overview of sampling procedure and research aims at Bos t' Ename. G = grazed/grazing blocks; U = ungrazed blocks; BG = before grazing; comp. = comparison (including statistical testing); perm. = permanent; non-perm. = non-permanent; meas. = measurement.

Subject/ variable	research aim	plot type	plot set up	plot no.	relevé/meas. type	data source
Rubus						
- cover	1) comp. G-U 1996-2008	perm. (40x20m)	random	4, each with U & G subplot (20x20m)	Londo, 4 per subplot i.e. 32 relevés (10x10m)	Cornelis 1996; this study
	2) comp. blocks BG 2002	non-perm. (2x2m)	random	42 (19 U, 23 before G)	Londo	Buckens 2003
	3) comp. G-U 2008	non-perm. (2x2m)	8 transects	412 (206 G, 206 U)	Londo	this study
- height	4) comp. G-U 2008	non-perm. (2x2m)	8 transects	64 (32 G, 32 U)	320 meas., 5 per plot	this study
Forest ground flora						
- frequencies of *V. minor, H. helix, P. elatior*	5) comp. blocks BG 2002	non-perm. (2x2m)	random	42 (23 BG, 19 U)	presence/ absence	Buckens 2003
	6) comp. G-U 2008	non-perm. (2x2m)	8 transects	412 (206 G, 206 U)	presence/ absence	this study
	7) comp. BG-G-U 2002-2008	non-perm. (2x2m)	random+ transects	454 (23 BG, 206 G, 225 U)	presence/absence	Buckens 2003; this study
- *A. nemorosa*						
* cover	8) comp. G-U 2008	non-perm. (2x2m)	8 transects	412 (206 G, 206 U)	Londo	this study
* no. flowers	9) comp. G-U 2008	non-perm. (2x2m)	8 transects	412 (206 G, 206 U)	counts	this study
- *H. non-scripta*						
* cover	10) comp. G-U 2008	non-perm. (3x3m)	8 grids (27x27m)	72 (36 G, 36 U)	Londo	this study
* no. flowers	11) comp. G-U 2008	non-perm. (cross point)	8 grids (27x27m)	800 (400 G, 400 U)	counts	this study
* stem	12) comp. G-U 2008	non-perm. (cross point)	8 grids (27x27m)	800 (400 G, 400 U)	presence/absence	this study
* stem height	13) comp. G-U 2008	non-perm. (cross point)	8 grids (27x27m)	800 (400 G, 400 U)	meas.	this study
* G damage	14) comp. G-U 2008	non-perm. (cross point)	8 grids (27x27m)	800 (400 G, 400 U)	presence/absence	this study

7.2.2.3 Comparing Rubus and forest ground flora in grazed and ungrazed forest (dataset 2008)

We applied two different strategies for the fence line study of forest ground flora and *Rubus* in Bos t' Ename, according to different species distribution: clumped (*H. non-scripta*) vs. scattered (all other species).

For the species, occurring scattered throughout the forest, we set out eight transects, each of them crossing adjacent grazed and ungrazed forest parcels (Fig. 7.1). Individual transects were parallel to the topographic contours of the main slope in the study site and were 10-30 m apart. Transect length ranged from 315-630 meters. In each transect an equal number of plots (2m x 2m) in ungrazed and grazed forest was established. Distance between plots was 7.5-15 m. One transect with 56 plots was situated in *Alno-Padion* forest; seven transects with 356 plots were situated in *Carpinion* forest. Measurements on transects were conducted in the second half of April 2008. We recorded absence or presence of *H. helix, P. elatior* and *V. minor.* Apart from *A. nemorosa,* these were the most frequent species (present in more than five % of all plots, but cover % was always lower than 10%). We further estimated *Rubus* and *A. nemorosa* cover with a decimal scale (Londo 1984) and counted the number of *A. nemorosa* flowers in each plot. We used *A. nemorosa* as a model species for unpalatable ground flora in general, because it was present throughout the forest in more than 90 % of all plots. On a cloudless day, we measured *Rubus* canopy height and photosynthetic photon flux density (µmol photons $s^{-1}m^{-2}$) in 64 plots (eight per transect) at four fixed points per plot. Light measurements were made at 20 cm (four fixed points) and 150 cm (one point without interfering vegetation) above ground level with a photometer (Skye Instruments LTD, SKP 200). Light penetration in the vegetation was determined as the ratio between paired measurements at 20 and 150cm.

For *H. non-scripta,* mainly occurring clumped in distinct stands, we established eight grids, four in grazed forest and four in ungrazed forest. These grids (27m x 27m, with horizontal and vertical grid lines 3 m apart) were located in the centre of eight *H. non-scripta* stands and resulted in 100 cross points per stand. Measurements in grids were made in April 2008. We estimated *H. non-scripta* (as a model species for palatable ground flora) and *Rubus* cover in 9 squares (9m x 9m) within each grid. On the 100 cross points, we recorded presence/absence of the stem, presence/absence of grazing traces to leaves, stem height and number of flowers of the nearest individual *H. non-scripta* plant (Table 7.1).

7.2.2.4 Effects of simulated grazing and trampling on A. nemorosa (dataset 2006-2008)

At Hayesbos, we established eight plots (3m x 1m) in a *Carpinion* forest with large patches of *A. nemorosa* dominated vegetation (cover per patch: 80-100%; distance between plots: 20-250m). Each plot was divided in three adjacent subplots of $1m^2$ (appendix 7.1). Randomly, we assigned control, simulated grazing and simulated trampling treatment to the subplots. In this experiment, we tried to measure a pure, mechanical grazing and trampling effect. For this, we assumed that large herbivores forage on *A. nemorosa*, without selecting more palatable or nutritious food types. Before treatment, cover percentage was estimated with a decimal scale (Londo 1984) and the number of flowers was counted (second half of April 2006). We then cut off all aboveground biomass in the simulated grazing treatment. Collected biomass was dried at 70°C during 48 hours and weighed. Trampling was simulated with a wooden cattle paw dummy (basal "hoof" surface = $49cm^2$): in each trampling-subplot we made 40 imprints by pressing the dummy on the vegetation with a weight of ± 100kg (i.e. ± $2kg/cm^2$, a comparable pressure of an adult cow). This resulted in complete trampling of the vegetation and disturbance in the upper 5 (±2) cm of the soil in each trampling-subplot. We repeated all measurements in the second half of April 2007 and April 2008 and collected biomass again in the simulated grazing subplots. Simulated trampling was not repeated in 2007 to assess recovery after a once only trampling event. Therefore, differences between trampling and grazing effects can only be compared with the 2007 data (one grazing event vs. one trampling event), not with the 2008 data (one trampling event vs. two grazing events).

7.2.3 Data analysis

7.2.3.1 Rubus cover before and after grazing management (datasets 1996-2008 and 2002)

A comparison between *Rubus* cover percentages in the permanent plots of 1996 and 2008 was made using a Repeated Measurements ANOVA with mixed effects (S-plus 8.0). Because in each plot, adjacent subplots (ungrazed exclosure and open grazed) contain juxtaposed relevés, observations inside plots are not independent. For this, we entered plot as a random factor in the analysis, with relevé nested in plot. We decided not to sum the values of individual relevés per subplot to include variance within plots in the analysis. Response data were square root transformed. We entered treatment (grazed or ungrazed) and year (1996 and 2008) and the interaction between treatment and year as fixed factors. We defined a spatial correlation structure with plot as group variable. A significant impact of grazing was to be expected if the interaction term treatment*year was significant: no effect in 1996 (measurements before treatment), a possible effect in 2008. Multiple comparisons using Tukey tests, were made between years in grazed and ungrazed plots.

A comparison between *Rubus* cover percentages in the two blocks (one before grazing, one permanently ungrazed; dataset 2002) was made using generalised linear models (S-plus 8.0). Response variable *Rubus* cover was square root transformed; independent factor = treatment.

7.2.3.2 Presence/absence of forest ground flora before (dataset 2002) and after grazing (dataset 2008)

Comparison of presence/absence frequencies of *P. elatior*, *V. minor* and *H. helix* between the two blocks before grazing (dataset 2002) and between the same blocks (one permanently ungrazed and one grazed since 2004; dataset 2008) were analysed using Yates corrected χ^2 tests. Further comparisons between 2002 and 2008 were made in the same way.

7.2.3.3. Comparing Rubus and forest ground flora in grazed and ungrazed forest (dataset 2008)

We used generalised linear mixed models (S-plus 8.0) to analyse cover percentages of *Rubus*, *H. non-scripta* and *A. nemorosa,* number of *A. nemorosa* and *H. non-scripta* flowers, presence of flowering stems and grazing damage to *H. non-scripta*. We used general linear models to analyse stem height of *H. non-scripta*. A logit and square root transformation (giving the best results to meet conditions of normality) was performed for percentages or presence/absence data and counts, respectively. We defined a spatial correlation structure, using xy-coordinates for all plots or cross points on transects and grids, respectively.

Rubus cover percentages were modelled with treatment (grazed vs. ungrazed) and forest type as independent variables. The same was done for *H. non-scripta* and *A. nemorosa* analyses, but also *Rubus* cover was included as an independent factor. Before entering other independent variables in these models, we tested for correlations (Pearson's correlation coefficients) between light penetration and *Rubus* height ($r = -0.44$, $P<0.001$), *Rubus* cover and light penetration ($r= -0.42$, $P=0.03$) and between *Rubus* cover and *Rubus* height ($r= 0.38$, $P=0.008$). Given the significant correlations, we only included *Rubus* cover (more observations than light penetration and *Rubus* height), treatment and forest type as fixed factors in the models. We compared light penetration (%)

through *Rubus* understorey and *Rubus* canopy height in ungrazed and grazed plots (2x32) using generalised and general linear models respectively.

Plots where *A. nemorosa* was absent (8%) were removed from the analysis to exclude bias (e.g. absence as a consequence of historical forest use). We controlled for stochastic variation in ungrazed vs. grazed plots. Initial models include all variables and all interactions. Non-significant factors and interactions were gradually eliminated, using a backward procedure. AIC values for goodness of model fit were used to control, whether the model was improved or not after elimination.

7.2.3.4 Effects of simulated grazing and trampling on A. nemorosa (dataset 2006-2008).

Simulated grazing and trampling effects on *A. nemorosa* cover, flower abundance and biomass were analysed using Repeated Measurements ANOVA (S-plus 8.0). We entered treatment (grazing, trampling or control), year (2006, 2007 and 2008) and the interaction between treatment and year as fixed factors. A significant impact of grazing or trampling was to be expected if the interaction term treatment*year was significant: no effect in 2006 (measurements before treatment), a possible effect in 2007 and 2008. Biomass (data for grazed subplots only) was analysed using a Friedman Anova.

7.3 Results

7.3.1 Rubus cover before and after grazing management (datasets 1996-2008 and 2002)

We found a significant interaction year*treatment (Repeated Measurements ANOVA: F=57.64; P<0.001): *Rubus* cover decreased significantly (-29.6% ±19.6; Tukey test P= 0.009) in plots grazed since 2004; *Rubus* cover significantly increased (+19.5 ±17.9%; Tukey test P= 0.014) in permanently ungrazed plots (since 1996).

Glm analyses of the 2002 dataset revealed no significant differences in *Rubus* cover percentages between the block before grazing (34.7% ± 24.9) and the permanently ungrazed block (42.1 ±25.6) in the study area (den. d.f. = 40; F= 0.89; P=0.35).

7.3.2 Presence/absence of forest ground flora before (dataset 2002) and after grazing (dataset 2008)

Species frequencies of *H. helix*, *V. minor* and *P. elatior* are presented in Table 7.2. In 2002, we found no significant differences in presence frequencies of *H. helix*, *P. elatior* and *V. minor* between the block before grazing and the permanently ungrazed block. Frequency analysis in the same blocks in 2008 revealed a significant difference between grazed and ungrazed blocks for *H. helix* and *V. minor*. Both species had disappeared completely from the grazed block. Frequency analyses between years revealed significant differences between the "before grazing block" and the block grazed since 2004 for *H. helix* and *V. minor*. Frequencies of *P. elatior* did not differ between blocks before or after grazing, nor between years (Table 7.2).

Table 7.2: Frequencies (% of plots) of three forest ground flora species before grazing (2002), after 4 years grazing 2008) and in permanently ungrazed forest (2002 and 2008) in Bos t' Ename. Different letters between brackets indicate significant differences in frequency for each species separately (Yates corrected χ^2 test, P<0.01). UG = permanently ungrazed; G = grazed; BG = before grazing.

	2002		**2008**	
	UG	**BG**	**UG**	**G**
Species	(N=19)	(N=23)	(N=206)	(N=206)
H. helix	26.32 (a)	30.43 (a)	23.83 (a)	0.00 (b)
V. minor	5.26 (a)	8.70 (a)	6.64 (a)	0.00 (b)
P. elatior	15.79 (a)	13.04 (a)	12.89 (a)	10.55 (a)

7.3.3 Comparing Rubus and forest ground flora in grazed and ungrazed forest (dataset 2008)

In grazed plots, *Rubus* cover was significantly lower than in ungrazed plots (grazed: 22.4% ±25.7 vs. ungrazed: 43.6% ± 27.7; F=64.1; P< 0.001). Also canopy height of *Rubus* was significantly lower in grazed plots (grazed: 43.7cm ±33.2 vs. ungrazed: 78.6cm ± 56.8; F=8.2; P=0.006). Light penetration was significantly higher in grazed plots (grazed: 22.0% ±15.9 vs. ungrazed: 12.1% ±9.2; F=8.9; P=0.004).

Cover percentage and number of flowers of *A. nemorosa* were significantly lower in grazed plots (Fig. 7.2). Overall, *Rubus* cover had a negative impact on *A. nemorosa* cover and flowering (Table 7.3), but it was more clearly expressed in ungrazed plots (significant interaction between treatment and *Rubus* cover, Fig. 7.3).

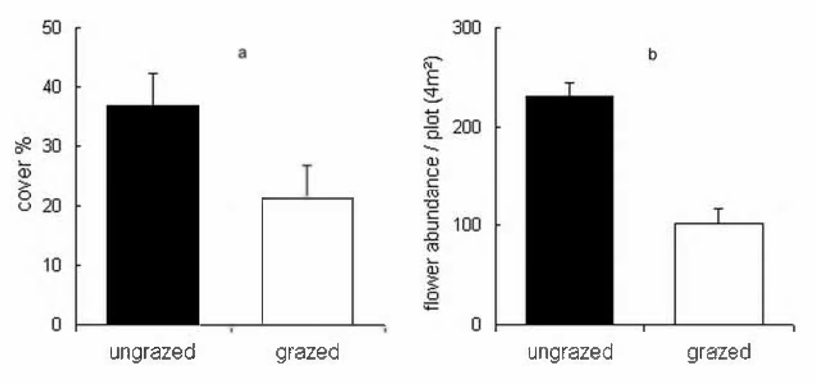

Figure 7.2: Characteristics of *Anemone nemorosa* vegetation in grazed (since 2004) and permanently ungrazed forest (Bos t' Ename); all histograms with standard error.

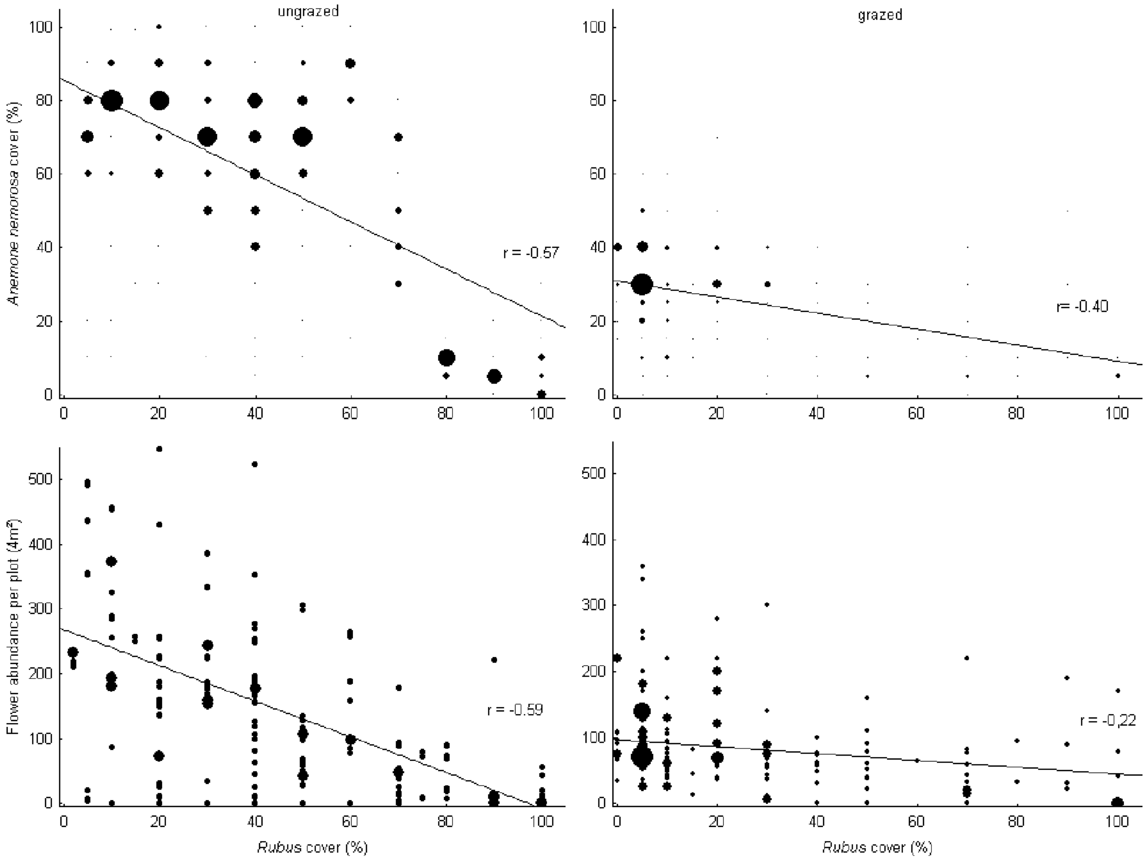

Figure 7.3: *Anemone nemorosa* cover percentages and flower abundance in relation to *Rubus* cover in grazed (since 2004) and permanently ungrazed forest (Bos t' Ename). Larger dots represent more relevés with the same *Rubus/Anemone* cover ratio than smaller dots (5 frequency classes with a linear relationship)

Table 7.3: Results of the GLM-analyses with *A. nemorosa* cover and flowering, *H. non-scripta* cover, flowering, stem height and number of fertile plants as response variables; treatment (grazing or not), and *Rubus* cover as independent variables.

response	independent	nom. d.f.	den. d.f.	F value	Pr>F
A. nemorosa					
cover	treatment	1	408	302.16	<0.001
	Rubus cover	1	408	39.13	<0.001
	treatment**Rubus* cover	1	408	33.88	<0.001
flowering	treatment	1	408	52.47	<0.001
	Rubus cover	1	408	67.26	<0.001
	treatment**Rubus* cover	1	408	41.28	<0.001
H. non-scripta					
cover	treatment	1	68	7.10	0.009
	Rubus cover	1	68	0.07	0.957
	treatment**Rubus* cover	1	68	0.01	0.802
no. of fertile ind.	treatment	1	796	54.50	<0.001
	Rubus cover	1	796	0.071	0.203
	treatment**Rubus* cover	1	796	0.012	0.269
stem height	treatment	1	650	409.25	<0.001
	Rubus cover	1	650	3.96	0.102
	treatment**Rubus* cover	1	650	0.012	0.854
flowering	treatment	1	650	171.93	<0.001
	Rubus cover	1	650	2.97	0.085
	treatment**Rubus* cover	1	650	2.56	0.110

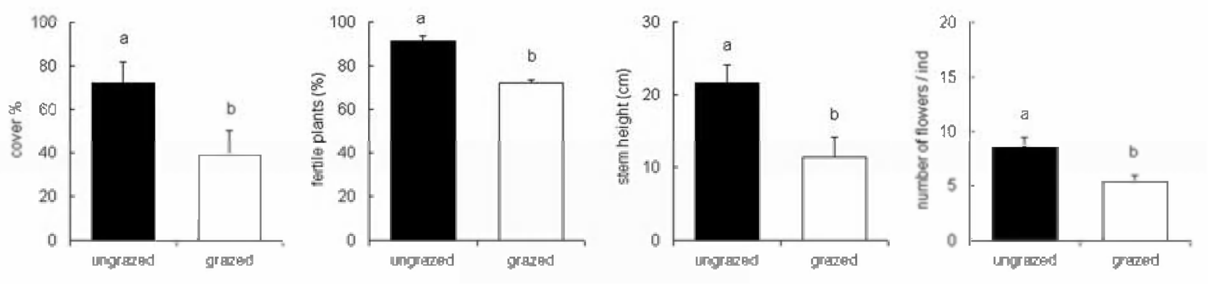

Figure 7.4: Characteristics of *Hyacinthoides non-scripta* stands in grazed (since 2004) and permanently ungrazed forest (Bos t' Ename); all histograms with standard error. Different letters indicate significant differences between values (Tukey test P<0.05).

For *H. non-scripta*, cover percentage, number of flowers per individual, number of fertile plants and stem height were significantly lower in grazed plots (Fig. 7.4). 71% of *H. non-scripta* individual plants showed grazing traces to leaves or stems. No significant effect of *Rubus* cover and no interaction with grazing was found (Table 7.3). In none of the analyses, did forest type (*Carpinion* vs. *Alno-Padion*) have a significant effect.

7.3.4 Effects of simulated grazing and trampling on A. nemorosa (dataset 2006-2008).

Cover percentages of *A. nemorosa* were significantly lower after simulated grazing and trampling (treatment*year: F= 8.069; P = 0.003). However, the reduction in cover percentages was small (less than 10%, Table 7.4) for both grazing and trampling. Trampled plots recovered in the second year after disturbance. Grazed plots, that were subject to two treatments (2006 and 2007) did not recover by 2008. Flower abundance (F=8.247; P=0.032) was lower in trampled and grazed plots, but the reduction was only significant after 2 years. Biomass decreased significantly after simulated grazing, on average with 25% after one year, with ± 30% after two years (Friedman Anova χ^2 (N=8; df= 2) = 14.000; P=<0.001, Table 7.4; no biomass data for the other treatment types available).

Table 7.4. Mean (± st. dev.) cover percentage, flower abundance and biomass of *A. nemorosa* in 2006 (before treatment), 2007 and 2008 (one and two years after treatment, respectively) in control and simulated grazing and trampling plots. Different letters between brackets indicate significant differences (Tukey test P<0.05) between years for each treatment.

	cover (%)			**flower abundance/m^2**			**biomass (g)**		
treatment	**2006**	**2007**	**2008**	**2006**	**2007**	**2008**	**2006**	**2007**	**2008**
control	91 ±6(a)	93 ±7(a)	92 ±6(a)	128 ±26(a)	122 ±16(a)	115 ±25(a)	-	-	-
grazing	96 ±5(a)	89 ±4(b)	85 ±5(b)	133 ±23(a)	96 ±20(ab)	66 ±20(b)	120 ±21(a)	82 ±15(b)	78±14(b)
trampling	91 ±8(a)	87 ±8(b)	93 ±9(a)	108 ±21(a)	87 ±20(ab)	80 ±14(b)	-	-	-

7.4 Discussion

Large herbivore grazing (± 0.25 AUha^{-1}y^{-1}) reduced cover and height of *Rubus* and cover and presence of forest ground species in the forest. *Hyacinthoides non-scripta* cover and flowering decreased and stem height and number of flowers per individual plant was reduced. The effects of large herbivores on cover and flower abundance of the dominant vernal, unpalatable ground flora species *A. nemorosa* was comparable to effects on *H. non-scripta*, but it was likely that the effects on *A. nemorosa* were mainly due to trampling. The evergreen *Vinca minor* and *Hedera helix* disappeared from grazed forest parts.

7.4.1 Rubus control

Analyses of different datasets showed that grazing reduced *Rubus* cover significantly (±30-50%) as compared to ungrazed control plots. In a survey of 105 woodland areas in Northern Ireland overall cover percentages of *Rubus* were much lower, but in grazed woods cover percentages were significantly lower than in ungrazed woods: 3.9 % and 9.6 % respectively (McEvoy *et al.* 2006b). Given the generally high regeneration capacity of *Rubus* spp., with even over-compensation of lost tissues (Focardi & Tinelli 2005), grazing pressure needs to be high enough to reduce *Rubus* cover considerably. McInnes *et al.* (1992) suggest that moderate levels of shrub browsing by moose (*Alces alces*) may even increase production efficiency of the shrubs involved. In our study site, cattle grazing pressure in the forest increased in late winter - early spring. In this period of the year, the cattle herd spent more than 28% of its foraging time in woodland habitat, foraging (65% of the time) on the wintergreen leaves and branches of *Rubus* (Van Uytvanck, unpublished data). Putman (1986) and Maublanc *et al.* (1991) report a similar seasonal dependent forage behaviour for browsers.

Other factors that might affect *Rubus* cover and forest ground flora are related to the tree and shrub layer canopy. De Keersmaeker *et al.* (2004) showed the importance of a well developed coppice or woody shrub layer to enable suppression of *Rubus* in favour of ground flora. The permanent plots in our study site showed an increase in *Rubus* cover in ungrazed forest in the period 1996-2008. However, given the relatively short time between the observations and the non-intervention management during the past decades, important changes in shrub and tree canopy structure were not to be expected at the study site.

For conservation management, it is important to assess the importance of *Rubus* in the diet of large herbivores. The forage quality of *Rubus* is characterised as mid-low (González-Hernández & Silva-Pando 1999), mainly because the digestibility is low. In dune ecosystems, *Rubus ceasius* is a desired food source for horses (Cosyns *et al.* 2001), donkeys (Lamoot *et al.* 2005a), ponies and cattle (Lamoot *et al.* 2005b). Crude protein concentrations in *Rubus* are relatively high: 8.8 ±3.8% (Heens, unpublished measurements of *Rubus* in the study site). These concentrations meet the minimal nitrogen requirements of cattle (= 7.2 %, Agricultural Research Council 1980, in WallisDeVries *et al.* 1998). However, the best forage quality for *Rubus* is reached in late spring. At this time of the year, *Rubus* is not a substantial part of the cattle's diet. It is clear that *Rubus* will only be eaten if food supply in other habitats and of other, less grazer defensive plant species is too low. This may have important consequences when grazing is applied for *Rubus* control in forests.

7.4.2 Forest ground flora

High *Rubus* cover percentage had a clear negative effect on the flowering and cover percentage of *A. nemorosa.* Although we expected a positive effect of large herbivore grazing on both cover and flowering due to reduced competition, our data did not confirm this hypothesis. Occasional grazing of *A. nemorosa* is noted (personal observations; observations P. Blondé), but it is not yet clear, whether the species is a valuable food source for large herbivores. For roe deer, *A. nemorosa* is a constituent of the summer diet (Costa Pérez 1992, in González-Hernández & Silva-Pando 1999). Among a list of 33 species of trees, shrubs, forbs, grasses and ferns, *A. nemorosa* had the highest digestibility (69.1%) and a very high crude protein concentration (20.8%, González-Hernández & Silva-Pando 1999), but the presence of protoanemonine makes it unpalatable for livestock (Grime *et al.* 1988; De Cleene & Lejeune 1999).

Therefore, it is likely that mainly trampling by cattle inhibits growth and flowering of *A. nemorosa*. Results of the grazing-trampling experiment clearly indicate that trampling can decrease cover and flowering, though simulation effects were relatively weak if compared with the transect study. Possible explanations for this are the once-only treatment in the experiment, the combined effect of grazing and trampling in the field and differences in treatment period. Once-only grazing or trampling might allow a better recovery than repeated disturbance in the field (Cole 1995; Godefroid *et al.* 2003) and although *A. nemorosa* is an unpalatable species, the combined effect of grazing and trampling may be larger than grazing or trampling alone, as was simulated in the experiment. Moreover, large herbivore trampling of *A. nemorosa* in the field occurs mainly in late winter-early spring, when shoots are small and possibly more vulnerable than in April. Generally, soils are wetter in late winter than in April, resulting in a higher susceptibility to trampling (damaging also the root system of plants) and compaction (Kuss 1986). Possibly, *A. nemorosa* uses the energy reserves in the rhizomes to compensate trampling damage to below and above ground plant parts. These reserves are largely responsible for above ground growth and flowering and therefore, a reduction in biomass and flowering potential is likely to happen. It could take many seasons of additional irradiance to build up these reserves. In mesophilous forests communities, Roovers *et al.* (2004) found high recovery percentages, but mainly herbaceous species, with a relatively high resilience after trampling were involved. In our trampling experiment, we also found a rather fast recovery of *A. nemorosa* leaves (conversely to grazing treatment), but flowering remained low in the following years.

For the palatable *H. non-scripta*, we found no effects of *Rubus* cover, though it was much higher in ungrazed forest. Probably, *H. non-scripta* is more shade tolerant than *A. nemorosa.* The emergence and flowering period of *H. non-scripta,* which are 2-3 weeks later than those of *A. nemorosa,* when trees already have leaves and light penetration is restricted, strengthen this idea. Grazing had a clear negative impact on both individual plants and stands. McEvoy *et al.* (2006b) showed significant differences in cover of *H. non-scripta* in grazed (2.5%) and ungrazed (10.1%) woods. However, together with *A. nemorosa*, it is not considered as a preferential food species for either grazed or ungrazed woods. In our study, we found very high percentages of grazed *H. non-scripta* individuals, resulting in reduced cover (mainly due to increased damage to leaves) and stem height. Cooke (1997) found comparable results: shorter stems and leaves due to grazing of muntjac (*Muntiacus reevesi*). The most profound impact may be on the ability to produce seeds (Littlemore & Barker 2001). Our results are in line with these findings, showing a significant reduction in the number of fertile plants and the number of flowers per individual.

From the total disappearance after four years of grazing, we can deduce that *H. helix* and *V. minor* were highly preferred forest species. Experiences with a comparable forest in the east of Flanders (Altenbroek - Voeren, 43 ha) are similar: in wintertime, cattle are attracted to species with wintergreen leaves and forage on it till the food source is depleted (Van Uytvanck, unpubl. data). For *H. helix*, González-Hernández & Silva-Pando (1999) showed excellent forage quality and, together with *Rubus*, it was the primary forage for roe deer in autumn and winter (Jackson 1980). The complete removal through grazing of *H. helix*, which often dominates ground flora in drier parts of deciduous forests, may trigger important changes in community composition by providing a release in competition for less vigorous forbs (McEvoy & McAdam 2002). This could be of interest in N-

American forests, where *H. helix* is a non-native, invasive species, having a substantial negative impact on forest ground flora (Biggerstaff & Beck 2007).

In our study, *Primula elatior* was not eaten by cattle. Kirby (2001) reports grazing of this species by deer: muntjac usually eats flowers, fallow deer (*Dama dama*) additionally eats leaves. This appears to weaken plants such that they were less able to withstand the effects of dry summers. Kirby (2001) also found evidence of grazing-sensitivity for a number of forest species such as *Vaccinium myrtillus, Luzula sylvatica, Mercurialis perennis, Dryopteris dilatata* and *Lonicera periclymenum* in British woods.

Other factors that might have influenced forest ground flora cover and flowering in the study site are changes in soil, hydrology, litter quality and quantity (Hérault *et al.* 2005). However, these changes may rather affect forest ground flora condition on the long term (Honnay *et al.* 1998) than in a time span of four years.

7.4.3 Rubus cover and grazing interaction

The interaction between grazing and *Rubus* cover suggests that in grazed plots, the limiting factor for *A. nemorosa* shifts from light deficiency under large, tall and dark *Rubus* thickets to grazing and trampling damage in plots with low *Rubus* cover. The active mechanism here, is probably related to associational resistance (Callaway *et al.* 2005; Bossuyt *et al.* 2005; Van Uytvanck *et al.* 2008b). Although *Rubus* is considered as a palatable species and sometimes as a preferred food source for large herbivores (Garin *et al.* 2000), it has spiny stems and large thickets offer, to a certain extent, protection to ground flora such as *A. nemorosa*. Gómez *et al.* (2001) showed that shrub thickets have to be large enough to function as an effective barrier for grazers. For *Rubus*, this means that large patches, offer more protection to grazing and trampling than small ones. Large patches are also higher and darker due to the growth form of *Rubus fruticosus* agg., which has outgrowing suckers that support each other. Small *Rubus* patches are more easily eaten and entered by cattle and are probably less able to protect *A. nemorosa* from trampling. Larger patches are eaten as well, but they hinder movement of animals through it (Kirby & Woodell 1998). For early growing and flowering woodland specialists like *A. nemorosa* (March-April), increased light penetration, due to the reduction of the shading canopy of the evergreen *Rubus fruticosus* agg. (dominantly present at the study site), may enhance vitality (Barkham 1992). In our study site, this advantage probably was counteracted by severe trampling damage. The increase in light may also allow non-woodland species to establish (Kirby 2001).

Our data concerning large herbivore effects, only cover four years. Given the fact that cattle were able to reduce *Rubus* cover considerably, it is possible that the forest will gradually lose its role as winter food supply habitat. Therefore, the real grazing and trampling pressure on forest ground flora may decrease in time. In contrast with *H. non-scripta*, rhizomatous species such as *A. nemorosa* may benefit from moderate disturbance (Grime *et al.* 1988; Van Staalduinen *et al.* 2007). Shoots of this species may be produced from very small broken rhizome fragments remaining in the upper surface of the soil after disturbance (Barkham 1992).

7.4.4 Conclusions and practical applications

Year-round grazing with cattle is an effective and quick-working measure to control *Rubus* vegetation in small forests. However, in the short term, mainly negative effects on forest ground flora were found. Trampling by cattle, mainly foraging on *Rubus* in late winter – early spring, had negative effects on cover and flowering of the unpalatable *A. nemorosa.* Grazing clearly has a negative impact on the palatable *H. non-scripta* and evergreen ground flora species. The latter may disappear quickly from grazed forests. Extrapolation to long-term effects is uncertain, but these may include indirect effects on the whole system as well (e.g. soil compaction). Managers should try to find a balance between positive and negative effects of large herbivore grazing in ancient forests. We advise to maintain a low or moderate grazing pressure, i.e. below 0.25 AU $ha^{-1}y^{-1}$ in a mosaic where also a substantial part of grassland is included. This grazing pressure however, may be too high for less productive ecosystems or other vegetation types and needs evaluation in the field. Providing temporal time gaps in grazing may prevent excessive grazing and trampling damage to vulnerable forest ground flora (e.g. in spring) and may give opportunities for recovery (e.g. a 1-2 years exclosure period).

Both management measures are in line with guidelines provided by Mountford & Peterken (2003) for the New Forest (0.3 cows or 0.15 ponies ha^{-1}; short periodic reductions and increases in herbivore numbers). Seasonal grazing may prevent the loss of wintergreen species. Insight into the effects of different grazing regimes clearly needs further research. Managers should be aware that a massive flowering vernal forest most often reflects a high and long lasting cultural impact and a rather intensive forest management. Certainly, some of these massive flowering forests need preservation and an appropriate management. In large woodland areas, a more heterogeneous, patchy vegetation pattern as a result of the implementation of modern, less intensive and more natural grazing systems may be a valuable conservation alternative (Hodder *et al.* 2005), but it may imply a reduction in the presence, cover or flowering of forest ground flora species that often dominate the ungrazed forest.

Acknowledgements

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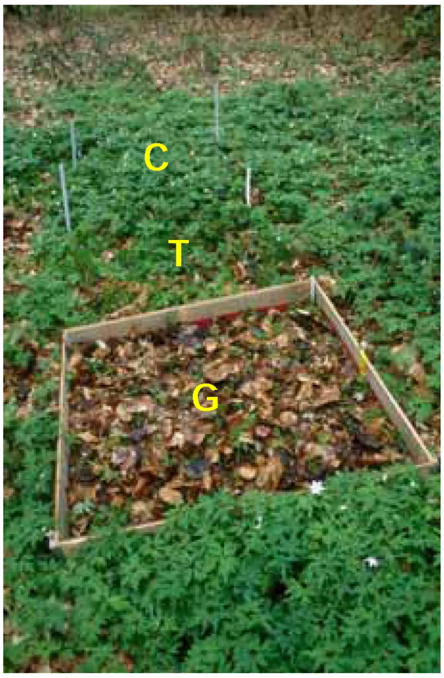
At the left: ungrazed forest with abundant bramble (Rubus sp.) understorey;
At the right: grazed forest with decreased bramble cover and flowering, but trampled, Anemone nemorosa stands.

Appendix 7.1:
Experimental set up grazing and trampling experiment on forest ground flora

Plot design

All plots are situated in large homogeneous *Anemone nemorosa* stands in the ungrazed study site Hayesbos

plot before treatments



plot after treatments

plots consist of 3 adjacent subplots (1m x 1m):

C = control subplot; no treatments

T = trampling subplot; complete trampling of plant cover and upper soil

G = grazing subplot; all above ground biomass removed

}

X 8

subplots randomised

Chapter 8. General discussion and conclusions

8.1 Introduction

In the previous chapters, we presented woodland regeneration patterns on grazed former agricultural land in a broad geomorphologic range (floodplain to plateau). We presented mechanisms and processes that determine and affect these patterns in different life stages of woody species (from seed to established juvenile tree) and in different successional stages of a vegetation patch (**chapter 2-5**). As the younger life stages of trees are more susceptible to herbivory than well-established trees, studies that focus on the earliest demographic stages have the greatest potential to reveal underlying mechanisms controlling community structure. In this view, biotic factors that affect the establishment of trees in successional habitats are of great importance, as there is the potential to affect the long term structure and composition of regenerating forest (Meiners & Handel 2000). Further, we assessed the role of different foraging strategies and habitat use of large herbivores in the redistribution of nitrogen among the habitats of mosaic landscapes. We related this process to the potential depletion of nitrogen as a trigger for woody species establishment in grassland and to the potential eutrophication of vulnerable forest vegetation (**chapter 6**). We studied the direct impact of large herbivore activities on forest vegetation in **chapter 7**, and discussed actual management questions concerning bramble control and the conservation of the vernal flora of ancient forests.

In this concluding chapter I will address some main synthesizing items concerning patterns, mechanisms and processes in woodland regeneration on nutrient rich soils on (sandy) loam and clay and go back to the main questions raised in the introduction (**chapter 1**) of this thesis. To conclude, I will discuss implications for conservation management and perspectives for future research.

8.2 Regeneration patterns

8.2.1 Vegetation structure

Herbivores interact with their environment, which can be viewed as a mosaic of spatial units at different scales. The spatial units, encountered by large herbivores while foraging can be ordered in an ecological hierarchy consisting of the regional system, the landscape system, the plant community (or large patch, or habitat) and the small patch (or feeding station) (Senft *et al.* 1987). In this thesis, we studied large herbivores at the three lower scale classes. We found evidence that tree regeneration patterns are mainly determined by structural differences among small patches, when grazing is the dominant, but fine-scale disturbance factor (cf. tall herb and scrub patches in grassland, **chapter 3**). If large-scale disturbances such as ploughing and flooding are part of the system, the large patch or habitat scale determines regeneration patterns (e.g. regeneration patterns on former arable land, **chapter 4)**.

The regeneration of woody species in grazed environments occurs in a non-random pattern, related to abiotic and spatial heterogeneity. The important role of vegetation structure (s.l.) is addressed in **chapters 2-5**. A mosaic pattern of tall stands (tussocks, tall herb mats, scrub patches) and short stands (lawns) is a common feature of grazed environments. The development of such a spatial heterogeneous vegetation structure may be a result of permanent grazing interacting with differences in plant palatability and abiotic heterogeneity such as light, soil and litter features (Quilchano *et al.* 2008). It may also be a result of changing human land use, e.g. decreasing herbivore pressure on former agricultural land. Conversely, vegetation structure is also found to be important with regard to the availability and quality of food resources for large herbivores (e.g. low quality of wood or tall herb stands vs. low grass or herb stands), and therefore it affects the foraging strategy and/or habitat use of large herbivores (**chapter 6**). Model simulations of grazer-plant interactions generated patterns of short and tall stands that were highly constant over time, given the productivity was high enough and the area was large enough. In this way, grazers do not shorten the entire vegetation. Moreover, a preference for previously grazed patches (because of better nutritional content), results in the permanent presence of tall stands in the area (Mouissie *et al.* 2004). Consequently, it is to be expected, that in these tall stands opportunities for the establishment of woody species arise (Olff *et al.* 1999; Bokdam 2003, **chapter 3 & 5**).

Throughout this thesis, we found interaction effects of vegetation structure and large herbivore activities on woodland regeneration. These effects differ among life stages and functional traits of woody species. They result in a series of bottlenecks for regeneration (presented chronologically in the tree life cycle in Table 8.1). We did not treat seed stages in this thesis, except for patterns in the relation between frequencies of established woody species and the distance to potential seed trees (**chapter 4** & 5; next paragraph). The importance of endozoochorous and epizoochorous seed dispersal by large herbivores was shown recently by Cosyns (2004) and Mouissie (2004). However, the role of large herbivores in dispersing seeds of woody species and their indirect effects on small rodents as dispersers and predators remain unclear. The bottlenecks discussed in this thesis are related to direct effects of browsing and grazing by large herbivores and to the interactions between vegetation structure, diet and habitat selection and (other) natural disturbances (**chapter 2, 3, 4, 5, 6**).

Table 8.1: Bottlenecks in woody species regeneration: indirect and direct negative (⊖) and positive (⊕) effects of large herbivores and vegetation structure.

life stage	bottleneck	potential large herbivore effect	potential vegetation structure effect	chapter/reference
seed	production	⊖ foraging on flowers, immature fruits	-	Or & Ward (2003); Danell *et al.* 2003
	viability	-	-	
	dispersal	⊕ epi-/endozoochory .	⊕ hides for seeds	Kollmann & Schill (1996); Gomez (2003)
		⊖ predation	⊖ increased predation ⊖ decreased reach of soil substrate	Smit *et al.* (2006)
	germination	⊕/⊖ affecting light conditions	⊕/⊖ determining light conditions	Rousset & Lepart (2000)
		⊕/⊖ dung micro-environment (in the case of endozoochory)		Myers *et al.* (2004); Brodie *et al.* (2009)
seedling	emergence	⊕ pre-emergence grazing/trampling (release of competition + change in abiotic conditions)	⊕/⊖ affecting abiotic conditions	ch. 2
		⊕/⊖ nutrient redistribution	⊕/⊖ nutrient accumulation/ depletion	ch. 6
	survival	⊖ predation	⊕/⊖ facilitation/competition	ch. 3, 4, 5
	growth	⊖ grazing and browsing	⊕/⊖ facilitation/competition	ch. 3, 4, 5
		⊕ browsing induced shoot growth & stem branching		ch. 4, 5
sapling/ juvenile tree	survival	⊖ predation	⊕/⊖ facilitation/competition	ch. 4, 5
	growth	⊖ grazing and browsing	⊕/⊖ facilitation/competition	ch. 4, 5
		⊕ suppression of competitors		
adult tree	survival	⊖ browsing, bark-stripping		McInnes *et al.* 1992; Kuiters *et al.* 2006

Successive bottlenecks for survival and growth during different life stages of woody species interact with vegetation structure type: our results show that in grazed ecosystems, there is a discordant relationship between seedling emergence and seedling survival-growth in the process of tree recruitment. Survival and growth of a seedling is highly restricted in vegetation types that favour emergence. Survival and growth is restricted in unprotected, short grazed vegetation types, while emergence is enhanced due to reduced competition from neighbouring vegetation (e.g. short grazed grassland) (**chapter 2**). In contrast, protective vegetation types such as scrub, and to a lesser extent tall herbs, favour survival and growth due to associational resistance (**chapter 3**), but hamper emergence due to strong competition. A conceptual model of successive bottlenecks in woodland regeneration is given in Fig. 8.1. Counteractive effects of grazing on different stages increase the complexity of the recruitment process and the uncertainty about the amount of individuals reaching the adult stage (Pérez-Camacho & Rebollo 2009).

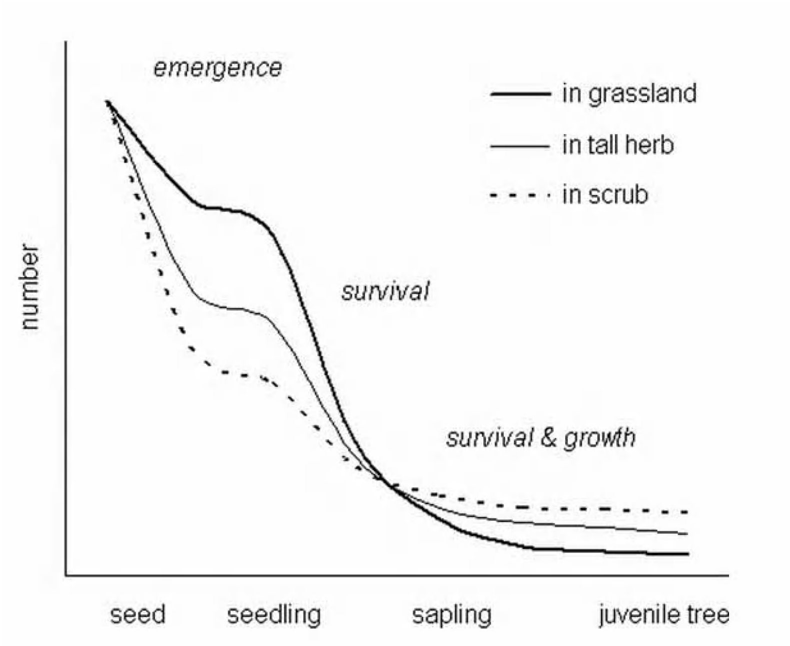


Figure 8.1: conceptual model for postdispersal shifts in numbers of individuals passing different life stages during the establishment process of a palatable tree species in three structurally different vegetation types in a grazed environment.

8.2.2 The impact of land use history: former pasture versus former arable land

The role of vegetation structure is emphasized in the contrasts between the two major points of departure for woodland regeneration in practice, i.e. former pasture grassland and former arable land. Former intensively used and structurally uniform pastures gradually change in patchy vegetation with tall and short stands when grazing intensity is lowered. The pattern of woody species colonisation is a rather slow process that interacts with developing vegetation structure and is determined by associational resistance (**chapter 5**). The regeneration pattern is patchy or scattered. In contrast, the colonisation process on former arable land is a fast process that rather depends on the resistance traits (related to herbivory) of pioneer trees, i.e. tolerance and defence (**chapter 4**). The initial regeneration pattern is massive and dense, but grazers increase horizontal and vertical heterogeneity by browsing seedlings and saplings. On former arable land, defensive tree species (e.g. *Betula pendula*) gradually take over the role of grazing tolerant species (e.g. *Salix caprea*) and are able to grow beyond the browse line. These species will dominate the tree layer in the early forest stages and can be present for decades (Falinski 1998; Dostalova 2009). In contrast, it are mainly non-resistant species (e.g. *Fraxinus excelsior* and *Quercus robur*) that will determine the tree layer on former pastures, although the defensive *Alnus glutinosa* may locally be important when regenerating in tall herb patches (**chapter 5**). Also the defensive *Populus canescens* may invade grasslands, but it highly depends on clonal growth of nearby trees (own observations). Our results show that on former arable land, initially the role of associational resistance is of minor importance compared to resistance traits of establishing trees.

8.2.3 The impact of the surrounding landscape: distance to seed source

In grazed mosaic landscapes, we expected that the presence/absence of potential seed trees and distance to potential seed trees would affect establishment patterns. We found some contrasting results for the role of distance to seed trees between former arable land and pastures. For the six most abundant woody species, among which predominantly wind- (*S. caprea, S. alba and B. pendula)* as well as predominantly animal-dispersed species (*A. glutinosa*, *Q. robur* and *C.monogyna*), frequencies per plot were not correlated with distance to seed source in nine former arable land study sites (**chapter 4**). The same was true for the less abundant *F. excelsior* (new analysis) (Fig. 8.2). At the 6 pasture study sites, distance to the nearest potential seed source did have an effect: distance was negatively correlated with sapling frequency per plot for *F. excelsior, Q. robur* and *C. monogyna*; no significant correlation was found for *A. glutinosa, S. alba* (**chapter 5**) and *S. caprea* (new analysis) (Table 8.2; Fig. 8.2).

Table 8.2: Correlation between established woody species frequencies and distance to the nearest potential seed sources (R=Spearman rank order correlation coefficient).

	former pasture			former arable land		
Species	R	p	analysed range (m)	R	p	analysed range (m)
Fraxinus excelsior	-0.78	<0.01	0-200	-0.54	0.211	0-140
Quercus robur	-0.90	<0.01	0-160	-0.23	0.420	0-160
Alnus glutinosa	-0.62	0.144	0-180	-0.73	0.053	0-120
Salix alba	0.19	0.655	0-460	0.28	0.880	0-140
Salix caprea	0.68	0.102	0-500	-0.15	0.980	0-200
Betula pendula		no data		-0.49	0.220	0-200
Crataegus monogyna	-0.95	<0.01	0-200	-0.69	0.123	0-200

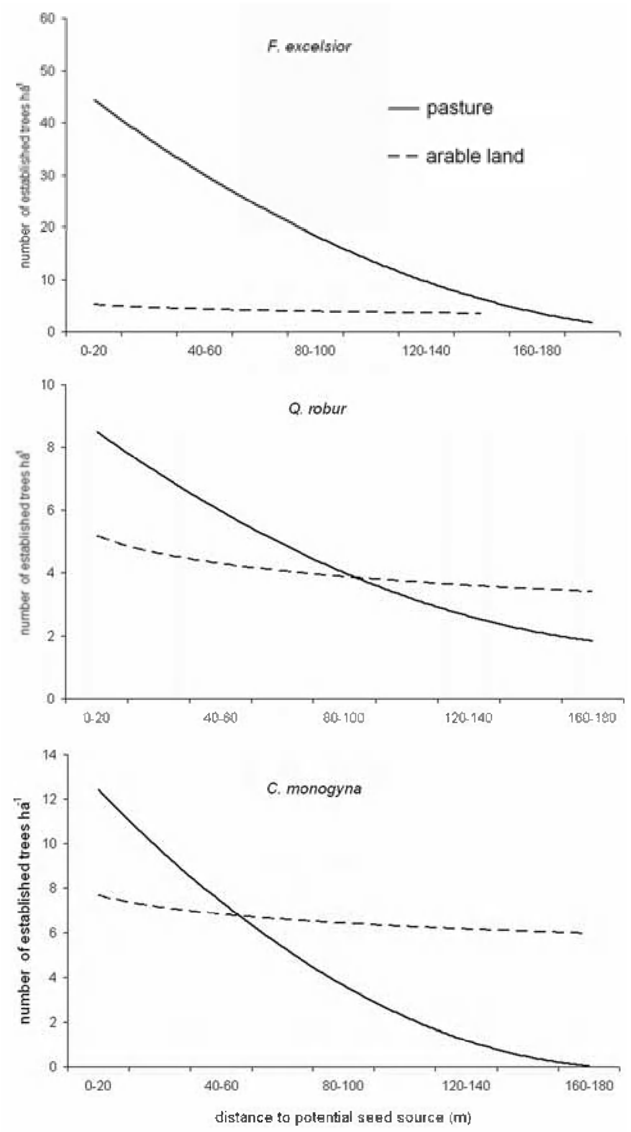


Figure 8.2: Contrasting results in pastures and former arable land regarding the correlation between distance to seed source and frequencies of established woody species (trend lines given).

Our data show that in small-scaled mosaic landscapes, the close vicinity of forest patches with seed sources enhances and accelerates tree establishment. Gardescu & Marks (2004) also found that the location of an old field (former arable land) in a forested matrix results in rapid arrival of tree species. At the scale of a particular old field however, distance to seed source had little impact on sapling frequencies due to excellent colonisation capacities of the mainly wind dispersed pioneer species. For *Salix* species and *Betula pendula*, the examined ranges (max. 200m) are relatively small (Myking 2002), but relevant for old fields in mosaic landscapes with sufficient seed supply. In such conditions, early successional trajectories are not primarily dictated by seed dispersal as is usually the case in old field succession (Martineau & Saugier 2007). The contrasting results between former arable land and pastures for animal dispersed species (*Q. robur, C. monogyna*) with larger and heavier seeds are probably due to the greater homogeneity of soil conditions and bare ground cover of former arable land. Probably, the establishment of species dispersed by granivorous animals (small rodents, birds) rather occurs by coincidence because for these animals (birds, small rodents) bare ground is inhospitable to live and not suitable to hide seeds. On the other hand, seeds on bare soil will experience less predation (Kelt *et al.* 2004). In contrast, structurally heterogeneous pastures offer good conditions to live and hide seeds for such animals. Acorns (*Q. robur*) are mainly dispersed by small rodents (Takahashi *et al.* 2006), which may explain the distance to seed source pattern. Berries of *C. monogyna* may be transported over long distances by birds, but frequencies significantly decreased with distance to seed source on a smaller scale (0-180m), suggesting that the majority of seeds may also be dispersed by small animals. In contrast, for *Fraxinus excelsior*, the significant negative correlation between distance to seed source may be explained by its limited long distance wind dispersal capacity (Wardle 1961). In general, our results suggest that the presence and number of suitable sites better explain establishment patterns than distance to seed source.

In Box 8.1, I give an example of how distance to seed source, colonising capacity, episodic disturbance and grazing interact and determine the establishment pattern of a tree species.

Box 8.1

In Boelaremeersen, one of the study sites in the floodplain of the river Dender, the establishment frequencies of *Salix caprea* showed a surprising peaky pattern in relation to distance to potential seed source (Fig. 8.3a). All trees were even aged (15 y), wide growing and heavily branched at ground level. Peaks in establishment numbers coincided with the riparian zone of overgrown side-ditches of the old drainage system of the area (Fig. 8.3b). A possible explanation is that long term inundation caused die back of the vegetation. As the pattern was not related to vegetation type (which reflects soil drainage class and ground level height), this was not a satisfying explanation.

Finally, I found out that single deposition of ditch slurry as a result of maintenance works during former use of the pasture caused the creation of an ideal seed bed for the pioneer tree species *S. caprea*. This species is highly tolerant for grazing in an early life stage, which resulted in the high percentages of branched stems at ground level.

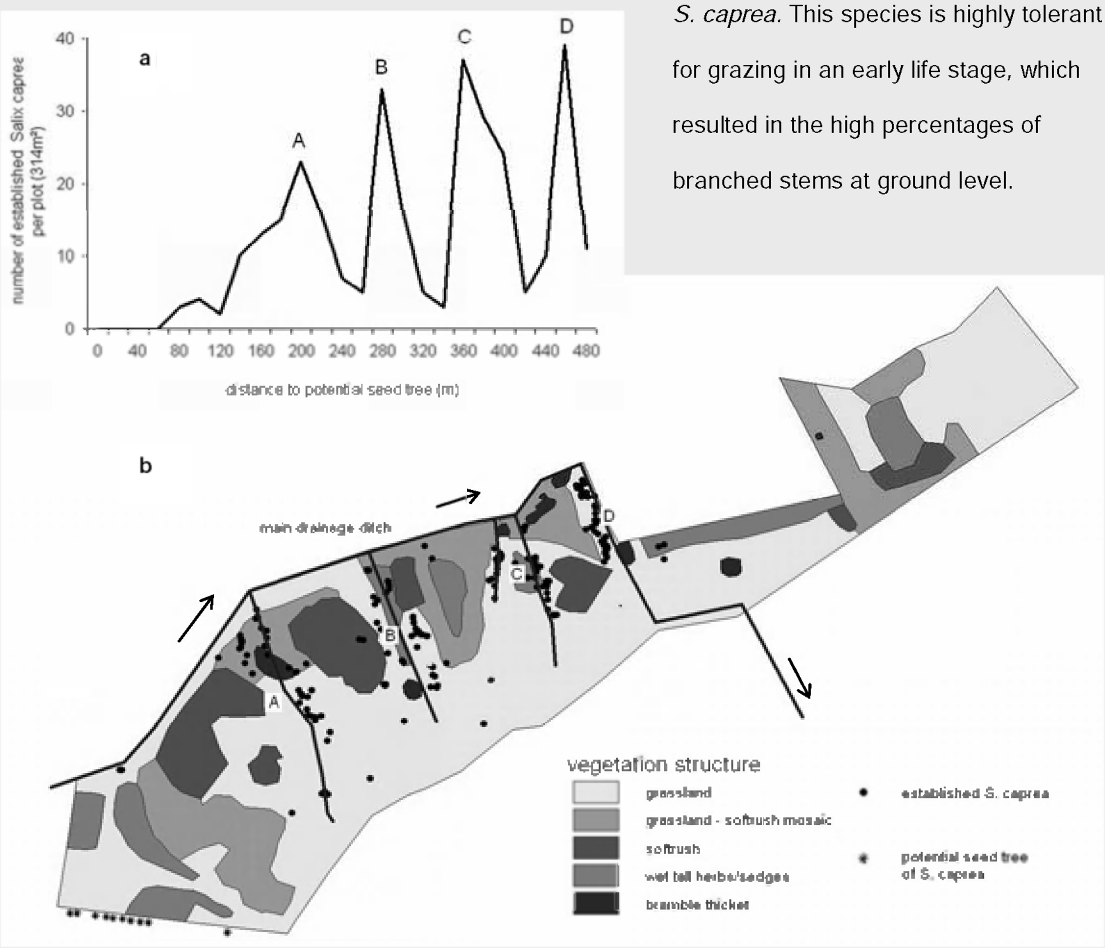


Figure 8.3: Establishment pattern of the pioneer tree species *Salix caprea* in floodplain grassland. a: tree number in relation to distance to potential seed trees with peaky pattern (A→D); b: vegetation map and old drainage system of the area, with the coinciding pattern of established trees, side-ditch riparian zones and peaks in distance pattern (A→D).

8.3 Regeneration mechanisms

In the previous paragraphs, I showed that vegetation structure and large herbivore activities interact and affect the regeneration pattern of woodland regeneration. But what are the underlying mechanisms?

Interaction mechanisms include both positive and negative effects on woody species regeneration (Fig. 8.4). In a palatable vegetation patch, an establishing woody species may be promoted by grazing or trampling due to reduced competition if it escapes from grazing: cf. emergence and early growth (**chapter 2**) and establishment in floodplain grassland (**chapter 5**). Associated palatability negatively affects establishment if seedlings or saplings are eaten together with the surrounding palatable vegetation by non-selective foraging large herbivores (**chapter 3, 4 & 5**). In an unpalatable/resistant vegetation patch, establishing woody species may be favoured through associational resistance (**chapter 3 & 5**), but avoided patches may also experience increased competition, neighbour contrast susceptibility and prohibition of emergence and growth (**chapter 2 & 3**) (Fig. 8.4).

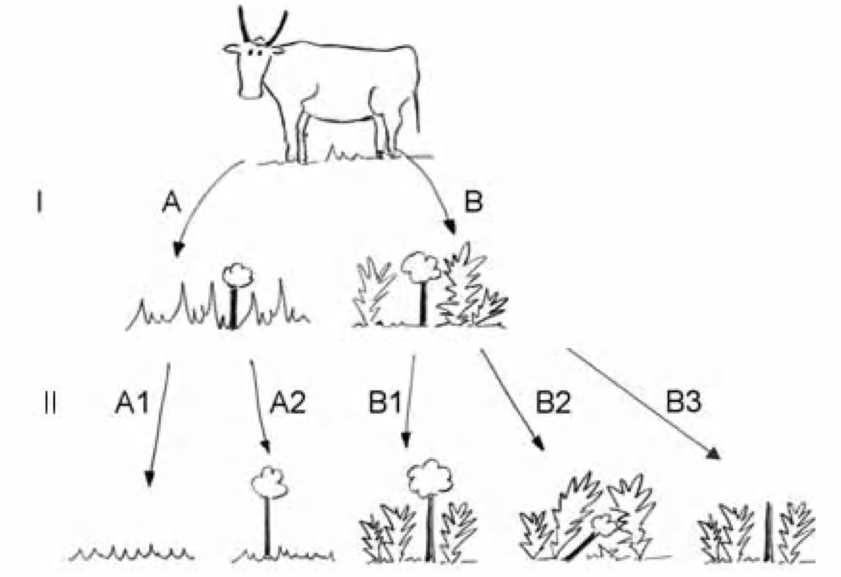


Figure 8.4: grazing and vegetation structure interaction: positive and negative effects on woody species establishment. I. herbivore effects on vegetation patches: A: large herbivores forage in palatable vegetation patch; B: large herbivores avoid unpalatable vegetation patch. II: indirect effects on establishing trees: A1: associational palatability; A2: reduced competition; B1: associational resistance; B2: increased competition; B3: neighbour contrast susceptibility.

8.3.1 Facilitation and safe sites

Facilitation mechanisms between different plant species is thought to play a key role in tree establishment in grazed ecosystems (Olff *et al.*, 1999). The mechanism is based on the protective capacities of nurse plants that are unpalatable or spiny and provide safe sites against herbivores. Spatial associations between young trees and nurse plants are described for different grazed ecosystems (Kuiters & Slim 2003; Rousset & Lepart 2000, **chapter 5**). In the present study, we tested the potential facilitating capacities of different vegetation types in successional seres on former agricultural land, i.e. unpalatable or spiny ruderal vegetation (on former arable land), unpalatable tall herbs (soft rush, tall sedge vegetation) and spiny scrub (bramble, sloe, hawthorn, roses). Besides associational resistance, we tested if alternative or interacting mechanisms played a role for woody species establishment.

We found empirical evidence for tree seedling and sapling survival and growth as a result of associational resistance (**chapter 3, 4 & 5**). *Rubus* (bramble) scrub, frequently occurring on former agricultural land, was found to facilitate establishment and growth on both former arable land and intensively used pastures. Vegetation types with a higher light penetration than scrub, such as tall herbs, facilitated survival, but subsequent growth was hampered. The present study provides some new insights into the regeneration process in tall herb patches, which is an important element (successional stage) in low intensity grazed mosaics. In such patches, the process of associational resistance is enhanced or interacts with other processes:

(1) Grazing by large herbivores creates gaps in the vegetation. Whereas, in ungrazed conditions, seedling emergence is highest in low swards or sparse vegetation types, grazing also enhances emergence (**chapter 2**) in tall herbs by locally changing the light regime.

(2) Trampling by large herbivores also creates gaps in vegetation as well as in soils. Especially on soils that are susceptible to trampling, such as floodplains, this offers opportunities for establishment. Our results show that trampling enhances emergence on flooded soils (**chapter 2**). Subsequently, established trees (such as *Fraxinus excelsior*) are protected in wet tall herb vegetation, at least temporarily, because this vegetation type is less intensively used for foraging **(chapter 5).**

(3) Time gaps in grazing particularly promote growth in tall herb patches, because already established but browsed trees can survive for years and then grow out quickly when grazing

temporarily ceases (**chapter 3**). Most pioneer tree species can grow beyond the browse line within three years (**chapter 4**).

Not all potential nurse vegetation types will effectively promote woody species establishment, e.g. nurse vegetation types may hamper germination and emergence or may compete for light an nutrients with seedlings. Seed removal may be higher inside safe sites than outside. Smit *et al.* (2006) found that aggregated predation by different granivorous animals (insects, rodents, birds) was of great importance under nurse structures, that did not prevent but rather enhanced seed predation. Further, some pioneer shrub species only promote their own replacement and hamper further succession and woodland regeneration will finally depend on the frequency of facilitative and competitive interactions between nurse plants and woody seedlings (Siles *et al.* 2008). In our study, we found that competition for light may be a factor that retards succession by reducing emergence (e.g. *Rubus* scrub, **chapter 2**).

8.3.2 Release of competition, associational palatability and neighbour contrast susceptibility

Competition for light, nutrients and water are stress factors found to be highly important to cause death of woody seedlings (Frost & Rydin 1997; Pépin *et al.* 2006). Moreover, a dense and high surrounding vegetation drastically reduces the availability of favourable microsites for germination and emergence and subsequent growth (above and below ground) and survival rate of seedlings (Eriksson and Ehrlén 1992; Davis *et al.* 1999; Mazia *et al.* 2001; Harmer and Robertson 2003; Setterfield 2002). The intensity of competitive effects on seedling growth increases with increasing site quality (productivity). Thus, competition and physical stress trade off along a site-quality gradient, with the result that tree seedling growth generally is slow (Berkowitz *et al.* 1995). In contrast to the results for growth, the survival of tree seedlings may be facilitated by the presence of intact vegetation (protection against drought) (Vandenberghe *et al.* 2006).

In the nutrient rich sites of this study, we expected competition to be important in the regeneration process. It is likely that large herbivores affect competition through selective foraging on high quality grasses, but that they fail to affect competition in scrub. In scrub, there were no significant differences in emergence, survival and growth (**chapter 2 & 3**) between grazed and ungrazed plots.

Compared to other vegetation types, we found the highest mortality of seedlings in bramble thickets when large herbivores were absent. Clonal expansion by above ground suckers form dense and dark thickets, which grow out in a horizontal direction. Low seedling emergence and high mortality was also found in ungrazed and rather dark, tall herb vegetations, confirming that in the absence of large herbivores, light is the limiting factor for tree regeneration (Kelly 2002). Emergence, survival and growth were enhanced in these vegetation types by grazing (**chapter 2 &3**). Our results suggest a shift from light deficiency to large herbivore grazing damage as the limiting factor for survival of seedlings in grazed mosaic vegetations with grassland and tall herbs (**chapter 3**). The underlying mechanism is a release of competition: grazing and trampling by large herbivores open tall herb swards, increase favourable microsite availability for emergence and establishment (**chapter 5**) and decrease competition for light (**chapter 2**). This mechanism was earlier described by Posada *et al.* (2000), de Villalobos *et al.* (2005) and Vandenberghe *et al.* (2006), but was now confirmed for nutrient rich former agricultural land.

However, large grazers such as cattle and horses, which have a preference for short vegetation types with sufficient nutrient rich leaves (WallisDeVries *et al.* 1999), caused a very low survival rate of tree seedlings in grassland vegetation through the mechanism of associational palatability (**chapter 3**). In palatable vegetation types such as grasslands, it is likely that grazing pressure and/or selective capacities of grazers may tip the balance to either dominating associational palatability or release of competition. High grazing pressure and low selective capacities (mainly herbivores with large mouths) will promote associational palatability, while low grazing pressure and high selective capacities (mainly herbivores with small or pointed mouths) will promote release of competition. Pollock *et al.* (2005) found that browsing intensity, of selective foraging sheep, on saplings was negatively related to height and good quality biomass available of adjacent vegetation, pointing at release of competition. Conversely, the mechanism of neighbour contrast susceptibility (Bergvall & Leimar, 2005) may attract herbivores to saplings. In such a case, palatable saplings, growing out above the canopy of unpalatable neighbouring vegetation, catch the eye of herbivores and experience increased browsing. In such a case, selective browsing by cattle in an unpalatable patch may explain the damage pattern of palatable saplings and their apparent inability to grow out beyond the browse line, which was an observed pattern of saplings growing in tall sedge vegetation (Pelster *et al.* 2004) and in tall herb vegetation that was grazed after a time gap (**chapter 3**).

8.3.3 Resistance traits of establishing trees

Besides the mechanisms that function through positive or negative interaction with surrounding vegetation, resistance traits of establishing woody species affect the rate and nature of woodland regeneration. Avoidance (a reduction of the amount of damage) and tolerance represent two general strategies of plant resistance against herbivores. Since resources available for allocation to defence are limited and resistance and tolerance are likely to serve the same functions for plants, there is a trade off between these two strategies (Leimu & Koricheva 2006). In this study, both strategies are at stake in the regeneration process of woody species. In **chapter 4**, we found that on grazed former arable land, resistance traits - tolerance and defence - were more important than the mechanism of associational resistance.

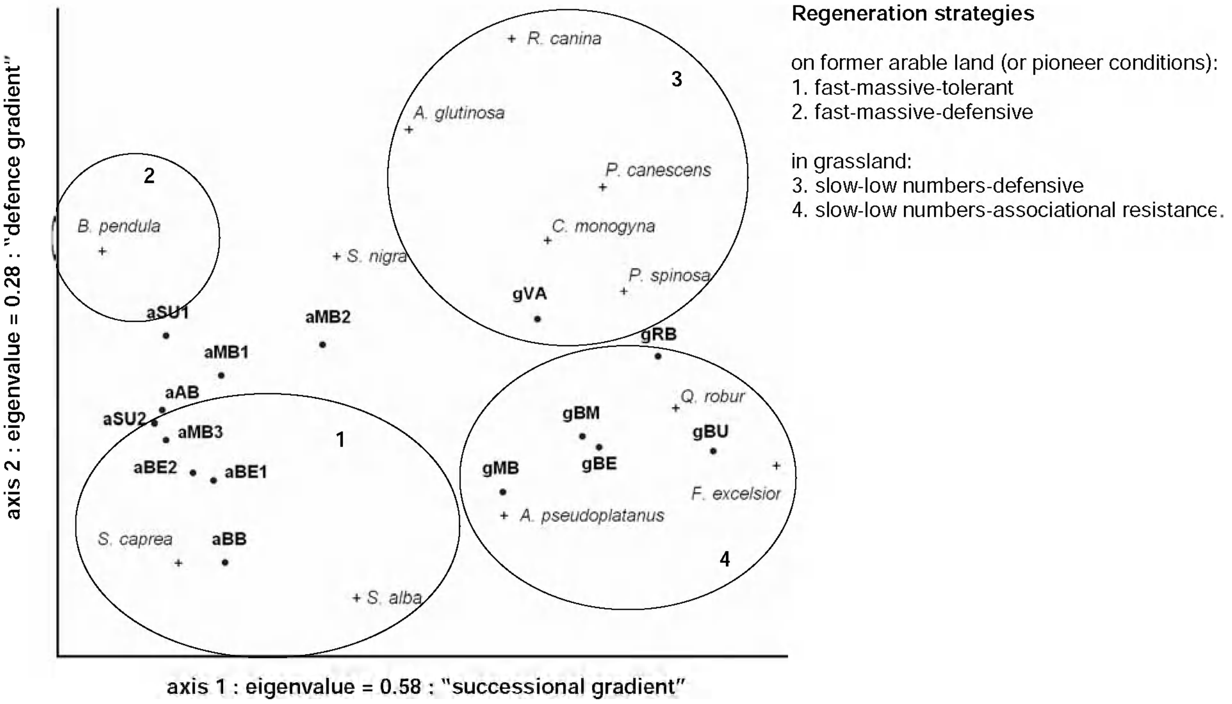


Figure 8.5: Ordination diagram of the DCA analyses with 15 study sites (•; prefix a = former arable land; prefix g = former pasture grassland) and 12 frequent occurring woody species (+) with different resistance traits (non-resistant: *Q. robur, F. excelsior* & *A. pseudoplatanus*; tolerant: *S. caprea* & *S. alba*; defensive (unpalatable): *A. glutinosa*, *P. canescens, S. nigra* & *B. pendula*; defensive (spiny): *C. monogyna*, *P. spinosa* & *R. canina*). Ellipses (1-4) represent 4 regeneration strategies.

To explore whether there is a general pattern in plant strategy or in mechanism for regeneration in either grassland or former arable land, I analysed the presence of tree species in the six grassland study sites of **chapter 5** and the nine grazed former arable land study sites of **chapter 4** together. For all analysed species, occurrence per site was expressed as the percentage of plots in which the species was present. These percentages were analysed with a DCA. The ordination diagram (Fig. 8.5) shows that the first axis probably corresponds with a successional gradient from bare soil with pioneer tree species (left) to grassland with mid and late successional species (right). The first axis explains the variation much more than the second (relatively high eigenvalue = 0.58; R^2 = 0.68). The interpretation of the second axis is more difficult (eigenvalue = 0.28; R^2 = 0.19). Resistant species, whether with morphological or chemical defence are in the upper half of the diagram; non-resistant and tolerant species are in the lower half. The results of the explorative analysis show that for the species pool in this study, species composition, plant strategies and regeneration mechanisms are different for former arable land and pastures. On former arable land, woody species with a massive seed production, combined with high dispersal capacities and grazing tolerant or defensive (unpalatable) determine the early and fast regeneration process. In grasslands, the slow regeneration process is determined by trees with a combination of rather low dispersal capacities and seed production and defence (spiny) or low dispersal capacities and seed production and associational resistance. These combinations represent four regeneration strategies in grazed systems (Fig. 8.5). Essentially, these different regeneration mechanisms are likely to be the result of trade off mechanisms between allocation of resources to:

- massive seed production and morphological defence (spines)
- high seed dispersal capacities and morphological defence.
- tolerance and defence (spiny or unpalatable)

In general, defensive and tolerant woody species are favoured when herbivores are present (Anderson & Briske 1995). Both strategies should succeed equally well in grazed ecosystems (Vandermeijden *et al.* 1988; Mauricio *et al.* 1997). In **chapter 4**, it was shown that defensive and tolerant woody species determined the early successional regeneration pattern, but that there were interaction effects with the mechanisms of associational resistance and competition. The presence of ruderal ground vegetation with unpalatable and spiny species and *Rubus* scrub, commonly occurring and soon (after 2-3 years) developing on former arable land, enables also the gradual establishment of non-resistant and morphological defenced woody species. It was also shown that selective grazing affects competition by suppressing the tolerant, but highly productive and palatable species such as *S.*

caprea (cf. Bilyeu *et al.* 2007). In this way, defensive and already established species (e.g. *B. pendula*) gradually take over. *Sambucus nigra* was found almost exclusively on former arable land, but it regenerates in low numbers. *A. glutinosa* and *S. alba* had no clear preference for former arable land, nor for grassland.

A gradual establishment process of defensive species was seen in pastures by clonal growth from adjacent forest or linear sideway stands. In particular *P. canescens* (unpalatable) and *P. spinosa* (spiny) regenerate in this way. *R. canina* and *C. monogyna* regenerate in a more scattered way in short grassland vegetation (**chapter 5**, personal observations). All those species are able to expand if grazing pressures is lowered during a number of successive growing seasons or when grazing abruptly ceases (Weeda *et al.* 1987-1994), i.e. when time gaps in grazing occur.

8.3.4 Time gaps in grazing

Research on the effects of short term exclusion of large herbivores is scarce, although it might be an alternative to the mechanism of associational resistance in native woodland regeneration, in particular in small areas. In **chapter 3**, it was shown that the seedling's first year was important for definitive establishment. Therefore, a time gap before grazing had a large positive impact on survival and growth of palatable tree saplings, but this result was highly dependent on vegetation structure. Time gaps of two years were too short to enhance seedling and sapling survival in grasslands. In tall herbs and ruderal vegetation, survival was enhanced but definite outgrowth was hampered, confirming results of Clarke (2002) for sheep grazing. Our results indicate that longer periods are needed for successful regeneration in grassland ecosystems (**chapter 3 & 5**), but that this is not the case on former arable land (**chapter 4**), where mainly tolerant and defensive trees establish. The duration of time gaps also depends on regeneration capacities of tree species, in particular the time that is needed to grow above the browse line. Since we used planted seedlings from a commercial nursery in our experiments, the period to grow out was shorter than in a real situation, where also the germination and emergence stage may be infuenced by herbivores. Therefore, for most hardwood species, two years will be to short to grow above the browe sline but already established *F. excelsior* saplings for example are able to regenerate and grow fast after periods of browsing (**chapter 3**). *Q. robur* is less able to survive a long period of repeated browsing and regrowth is much slower.

Probably, there are more woody species that would take advantage of time gaps, e.g. *Fagus sylvatica* (Mountford & Peterken 2003), *Carpinus betulus* and *Prunus avium* (own observations).

In this study however, we were particularly interested in the interaction effects of short time gaps and different vegetation structure types. There is a strong lack of knowledge about the role of time gaps in grazing on woodland regeneration. In particular the role of short time gaps, that are important in the conservation of woodpasture systems with high conservation value in the herb layer, is poorly studied and results are difficult to compare. In general, temporal absence of large herbivores can create a window of opportunity for woody species to establish. Ramírez & Díaz (2008) propose a rotational set a side system to enhance recruitment of *Quercus ilex* for the dehesa system in southern Europe, but they found that optimal time gaps should be at least 20 years. Prolonged exclusion of livestock allowed abundant regeneration of trees and shrubs in Denny Wood (New Forest, UK), whereas recruitment was completely restricted in adjacent, permanently grazed woodland (but resulted in the development of species rich grasslands; Mountford & Peterken 2003). Putman *et al.* (1989) mention that continuous and high grazing pressure also prohibited the establishment of potentially protective shrubs in which trees can regenerate (see mechanism above). In the majority of woodpastures of the New Forest (one of the largest remnant woodpasture landscapes in temperate Europe), the local dominants *Quercus robur* and *Fagus sylvaticus* fail to recruit for several decades already (Tubbs 2001). Kuiters and Slim (2002) argue that browse-sensitive woody species such as *Quercus* species will successfully regenerate, only if temporal and spatial variation in browsing pressure can occur. If not, woodland regeneration on abandoned land may take several decades (Smit & Olff, 1998; Harmer *et al.*, 2001). Augustine & McNaughton (1998) found that high levels of nutrient inputs or recycling and an intermittent temporal pattern of herbivory (often due to migration) are key factors increasing the regrowth capacity of palatable species and hence maintaining their dominance in plant communities supporting abundant herbivores. However, some authors argue that on the long term and at large scales even with a continuous presence of large herbivores, regeneration will always be possible, i.e. that opportunities for recruitment will occur patchily and intermittently due to habitat heterogeneity and fluctuations in herbivore populations (Morgan 1987a; Morgan 1987b). Also Vera (2000) opposes the idea of recruitment failures in continuously grazed systems, assigning for instance *Quercus* sp. to the browse-tolerant trees, but depending simultaneously on protective vegetation types.

8.4 Regeneration processes

8.4.1 Disturbance processes

Herbivory by wild and domestic ungulates is a chronic disturbance that can have dramatic effects on vegetation dynamics. Herbivory effects, however, are not easily predicted under different combinations of other episodic disturbances such as floods, fires & storm (Wisdom *et al.* 2006). One of the central ideas in ecology is that disturbance plays an important role in natural communities, influencing population persistence, community composition and maintenance of diversity (Pickett & White 1985). The relevant disturbance parameters to describe drivers of mosaic cycles are spatial extent, frequency, and magnitude (biomass loss). The importance of spatial, temporal and magnitude aspects were illustrated in **chapter 7**, in which disturbance effects of trampling forest ground flora were described in a grazed mosaic vegetation. It was shown that the interaction of large herbivore activities and shrub cover(*Rubus* sp.) affects forest ground vegetation. Cattle are able to affect the balance between the protective and competitive capacities of shrubs in relation to forest ground flora. Positive effects of *Rubus* control on cover of *Anemone nemorosa* shift to trampling damage when cover of *Rubus* becomes low. This is the case in late winter, when forest is a substantially used habitat by large herbivores (**chapter 6**) and when soils are wet and more susceptible to disturbance.

Individual disturbances may be seen as "ecological reset" mechanisms, creating a link to the concept of succession. In this context, grazing may be seen as fine-scale disturbance, affecting the re-vegetation dynamics after disturbances at a larger scale (e.g. fire, floods, storms, insect plagues; Vandvik *et al.* 2005). In semi-natural and cultural landscapes, disturbances that "reset" succession are often caused by human intervention, (e.g. fire in heathland systems; Webb 1998). Disturbances by man (land use) are usually regular and deterministic in space and time, in contrast to year-round grazing by free-ranging large herbivores (Kleyer et al 2007). E.g., in **chapter 4**, soil disturbance on former arable land (resulting in bare ground) was the dominant disturbance factor, strongly determining community structure of the establishing wind dispersed pioneer species. Large herbivore grazing mainly affected vegetation structure.

Trimble & Mendel (1995) showed that large herbivores themselves (such as cattle) are important agents of geomorphological change. On the uplands, heavy grazing compacts the soil, reduces infiltration, increases runoff, and increases erosion and sediment yield. In riparian zones, grazing decreases resistance against erosion by reducing vegetation and exposing more vulnerable substrate. Trampling directly erodes banks, thus increasing turbulence and consequent erosion. It is clear that heavy disturbance by grazing and trampling will prevent woodland regeneration.

An important item in this thesis was the interaction of temporary flooding (large scale disturbance) and grazing/trampling activities of large herbivores. Winter floods in valleys and floodplains may cause die back of vegetation and retard growth (Deiller *et al.* 2003; Kühne & Bartsch 2007), resulting in a spatially diverse vegetation pattern that corresponds with differences in micro-relief. In this study, winter flood disturbance interacted with large herbivore activities and enhanced seedling emergence (**chapter 2**) and establishment (**chapter 5**) via the creation of trampling holes in soil and gaps in vegetation. Recruitment of woody species in grassland ecosystems that depend on disturbance were described for dry ecosystems (e.g. Putman 1986; Clarke 2002). In this thesis, the regeneration pattern of woody species in floodplains reflected wet soils that were susceptible to soil trampling combined with protective tall herb vegetation patches on flooded soils (e.g. *Carex* vegetation).

Interaction of other biotic disturbances (insect plagues) and grazing may also trigger cyclic succession: e.g. defoliation by beetles and trampling by cattle killed *Calluna* shrubs and favoured grass invasion, grazing and gap creation by cattle in *Deschampsia* vegetation favoured the establishment and recovery of *Calluna*. However, the underlying mechanism is related to differential light and nutrient levels in *Calluna* and *Deschampsia* gaps and nutrient redistribution by larger large herbivores (Bokdam 2001).

8.4.2 Nutrient redistribution

Large herbivores influence the nitrogen (N) cycle of ecosystems by changing litter quality, affecting conditions for N mineralization, and by adding readily available N to upper levels of the soil in urine and faeces. Natural heterogeneity in the spatial distribution of N within landscapes is amplified by herbivore selection of habitats and patches (Hobbs 1996). Bokdam (2003) found partial evidence for his "resource-mediated successional grazing cycle" theory, in the fact that free-ranging large grazers can act as driving force for successional woodland-grassland cycles by causing nutrient depletion in grassland lawns and induce the invasion of dwarf shrubs (*Calluna vulgaris*) and trees (*Pinus sylvestris* and *Betula* spp.). Wood invasion and abandonment by the cattle induced replenishment of the soil nutrient pool. Further, the results showed that cattle induced the development of grassland lawns in gaps in the heathland and woodland. They suppressed woody and tall herbaceous species in the woodland undergrowth and introduced grass seeds by dung deposition. The results showed the important role of the interaction between herbivory and plant resources in cyclic plant succession.

In this study, the first aim was to examine if an underlying process of spatial redistribution of nitrogen among habitats in nutrient-rich ecosystems on heavy soils, could restore nutrient-poor conditions in preferred foraging habitats, and consequently induce the mechanism of shrub and tree establishment in avoided vegetation patches (Olff *et al.* 1999; Bokdam 2003; **chapter 6**). Additionally, we examined whether such translocation conversely could lead to a risk of extreme eutrophication in preferred resting habitats, such as ancient forest (cf. **chapter 7**). Concerning the latter question, we found that N redistribution by cattle in relation to the potential exceeding of critical N loads on vulnerable ground vegetations was almost negligible compared to present-day atmospheric deposition. In grasslands and wooded pastures, cattle grazing can compensate for high N inputs, maintaining grassland types that support N loads of 20-25 kg N $ha^{-1}y^{-1}$, but the restoration potential for oligotrophic grasslands is limited (**chapter 6**).

We found that N was transported from grasslands and wooded pastures to forest habitat. Our data suggest that N redistribution by free-ranging cattle strongly depends on the proportional availability of different habitats. It is mainly in the highly preferred grassland habitat that N balances can be altered and N poor conditions can be restored. Different habitat proportion scenarios for the nutrient-rich study site showed that N depletion is possible in excretion free patches in grassland and wooded pasture (i.e. in patches where N intake determines the net N balance completely), given that the proportion of these habitats is relatively small (i.e. ± 20%, **chapter 6**). The pattern is more pronounced for the preferred grassland habitat and with a foraging pattern that corresponds to a cognitive foraging mechanism of habitat selection. It may lead to high intake and a negative net N balance with N losses >40 kg N $ha^{-1}y^{-1}$ and it may even compensate for high atmospheric N input. So, there is a potential role of N depletion in triggering the establishment of woody species in nutrient-rich ecosystems. However up till now, data on effective establishment of woody species in depleted patches in nutrient-rich areas are lacking. This may be due to some counteracting processes. First, grassland lawns may be maintained over longer periods due to the limited ability of herbivores to process information about food quality at the moment of foraging (van Langevelde *et al.* 2008). Second, grazing may also cause over-compensatory growth of palatable species at fertile sites, resulting in the dominance of grasses (Hawkes & Sullivan 2001) that favours the mechanism of associated palatability and prohibits tree establishment. Third, Brathen *et al.* (2007) found that in spite of suppression of productive and palatable plants at fertile sites, there was no evidence for a persistent loss of productivity. These phenomena may speed up soil depletion, but in the same time inhibit shrub or tree establishment. Further, grazing lawns contain plants of lower stature with higher bulk densities (i.e., g/m3) of leaves, and higher leaf N concentrations (McNaughton 1984). As a result, the amount of N per unit volume in the patch is several fold higher than in an un-grazed patch. Because also other nutrients are concentrated in grazing lawns, foraging large herbivores may enhance the conditions for future feeding as plants regrow. When this regrowth is rapid (as is the case in nutrient-rich conditions), there is a positive feedback to future grazing. These feedbacks have important implications for landscape structure. Positive feedback increases landscape heterogeneity by heightening the contrast between areas that are grazed and those that are not. Negative feedback achieves the opposite: landscape heterogeneity is reduced or fails to change because grazing intensity tends to become uniform (Hobbs 1996). Based on the data of **chapter 3** and **chapter 5**, I think that such a positive feedback mechanism, resulting in high structural diversity and episodic disturbance processes are more important than nutrient depletion for the regeneration of woody species in nutrient-rich ecosystems.

8.5 A conceptual model for woody species establishment under moderate grazing in low and high competitive environments

Based on the obtained insights in patterns, mechanisms and processes of woodland regeneration under grazing, I present a conceptual model for establishment success in high competitive (e.g. grassland) and in low competitive environments (e.g. former arable land) (Fig. 8.6). We found that that with moderate grazing (<0.3 AU ha^{-1} or <0.5 AU ha^{-1}, see following paragraph – "Implications for conservation management and ecological restoration"), a heterogeneous vegetation structure develops in both grassland (former intensively used pastures) and former arable land.

Establishment success of woody species is related to episodic disturbance processes (one event in Fig. 8.6), grazing or not and facilitating mechanisms (associational resistance). Time gaps may have similar effects on establishment success as a single disturbance event.

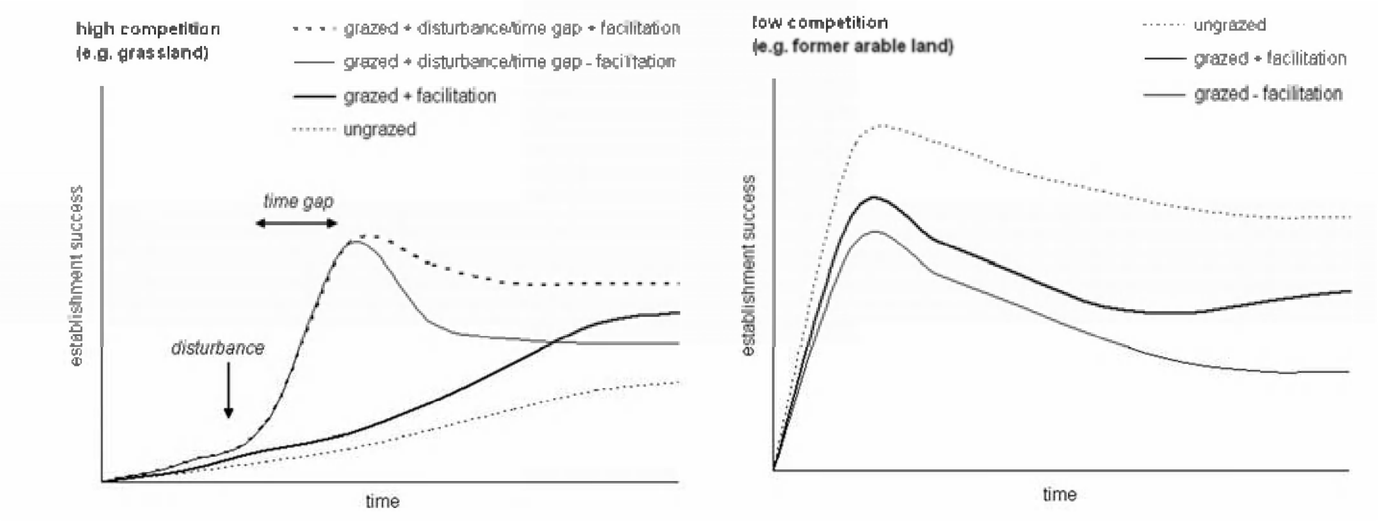


Figure 8.6: conceptual model for woody species establishment success in grazed high and low competitive environments

In grassland systems, slow establishment is accelerated by disturbance events (successional reset) and grazing (development of a heterogeneous vegetation structure). Facilitation (associational resistance) enhances establishment success. On former arable land, bare ground enables fast establishment of woody pioneers with resistance traits against herbivory (tolerance, unpalatability). Grazing reduces regeneration success by decreasing the number of seedlings (being vulnerable for grazing). Facilitation by invading spiny forbs and scrub becomes more important over time and enables establishment of palatable woody species. A conceptual model as described above may be useful to steer conservation management decisions.

To conclude, I summarize the main factors that are active or explain the differences between grazed former arable land and former pastures in the early woodland regeneration stages (Table 8.3).

Table 8.3: General differences between grazed former arable land and former pastures in the early woodland regeneration stages in a nutrient rich environment on fine textured soils.

	former pasture	**former arable land**
distance to seed source	important, establishment frequencies decrease with increasing distance	less important
seed dispersal	mainly animals	mainly wind
microsites for establishment	no. restricted, but diverse & positively influenced by large herbivores activities	numerous, but less diverse & no additional effect of large herbivores
colonizing woody species	mainly mid-successional species	pioneer species
colonization rate	slow	fast
initial establishment mechanisms	mainly associational resistance	mainly grazing avoidance & tolerance
subsequent establishment mechanisms	resistance (morphological defense)	resistance (morphological defence) & associational resistance
establishment pattern	patchy, strong match with vegetation structure	massive, often uniform
outgrowth above browse line	mainly in protective vegetation types	mainly resistant species

8.6 Implications for conservation management and ecological restoration

At the European level, there is widespread interest in the ecology, impact and use of large herbivores in woodland ecosystems (e.g. Kuiters *et al.* 1996; Humphrey *et al.*, 1998; Fuller and Gill, 2001), driven partly by the recent increase in ungulates (deer, fallow deer, roe deer, wild boar) in many European forests. Grazing has also been recognized as an important management tool in achieving biodiversity conservation aims, particularly in nature reserves, where it has become a major issue (Mountford & Peterken 2003).

The integrated results of the different studies presented in this thesis include suitable information for conservation management and ecological restoration. Understanding mechanisms and processes that determine and/or affect regeneration of native woodland (s.l.) in grazed mosaic vegetations on former agricultural land, was our departure point (**chapter 2-6**). It revealed also insights into achievable reference views for grazed nature reserves. Insights into these matters may also be useful for other conservation aims, such as the maintenance or development of open vegetation types, the restoration of nutrient poor grassland (**chapter 6**), control of shrub encroachment (**chapter 3 & 5**), the conservation of species rich forest ground flora (**chapter 7**) and the restoration of natural processes (**chapter 2 & 5**). Insight into the process of woodland development by shrub encroachment is useful to counteract negative effects on grassland biodiversity. When individual shrubs aggregate, a decrease in number of vascular plants may be expected when thresholds in scrub cover are passed. E.g. for the species rich V*eronica spicata–Avenula pratensis* community in a woodpasture system in Öland (Sweden) a dramatic decrease in species richness coincides with shrub cover exceeding the threshold of 70–80% (Rejmanék & Rosén 1992). Probably, comparable thresholds count for a large variety of species rich grassland communities throughout Europe. In most cases, conservation managers are able to interfere and steer grazing management and woodland development by controlling grazing pressure, choosing grazing regime and allowing time gaps in grazing. These items will be addressed in the following paragraphs.

8.6.1 Grazing pressure

From a nature conservation point of view, both undergrazing and overgrazing can cause problems in semi-natural landscapes such as woodpastures and semi-natural grasslands and forests (Rackham 2003; Green 1990; Gibson 1997). There is a remarkable duality in the appreciation of large herbivore grazing as a conservation tool. In North and South America, as well as in Australia, where livestock grazing has a much shorter history than in Europe, it is regarded by many conservationists as more of a threat to native flora and fauna than as a factor contributing to the management of biodiversity, cultural heritage or naturalness (Hecht 1993; Fleischner 1994). In Scandinavia, and in mountainous regions on the European continent, undergrazing and abandonment are the predominant problems, whereas elsewhere in Europe there is a tendency for overgrazing former semi-natural landscapes in the lowlands (Kumm 2003). However, all over the world there is a predominant vigilance and even fear for overgrazing of forest ecosystems. Goldberg *et al.* (2007) mention that the main threat to woodland is less often the catastrophic loss of whole woods, but the more insidious, creeping attrition, for example, from the prevention of tree regeneration by over-grazing, and the slow death of an aging population of trees. For ancient forests in particular, Rackham (2003) comments: "the ancient woods will remain on the map. A very few will be strenuously protected. More will escape through isolation or small size. The rest will have their guts eaten out of them by deer and sheep".

Here, I focus on grazing pressure that is related to woodland (s.l.) regeneration on former agricultural land. Combining the datasets of different studies and study sites, I tried to detect thresholds for woody species establishment. I used the 228 plots, located in 9 study sites and 15 sampling areas (**chapter 4 & 5**) on former arable land (87 plots) and former pastures (141 plots). All plots were subject to grazing for more than five years. Plots were divided into the three dominant vegetation structure types present at all sites, i.e. short grazed grassland, tall herb and scrub plots (cover dominance per plot > 80 %). I used the known stocking rate to estimate the real grazing pressure per structural vegetation type (in grazing days ha^{-1}) using a simplified key that was based on diet observations in Bos t' Ename (**chapter 6**) and data on browse patterns in Boelaremeersen, Steenbergbos, Bos t' Ename and Rietbeemd (**chapter 3**, also see next paragraph). I assumed that grazing pressure on grassland (grazing time = 60%) was twice the grazing pressure on tall herb (grazing time = 30%) and six fold the grazing pressure on scrub plots (grazing time = 10%). For all plots, established sapling density (saplings > 1 year, i.e. height > 0.5 m or height > 30 cm + diameter > 1 cm) was calculated per ha and related to estimated grazing pressure and structural vegetation type.

Fig. 8.7 gives a general insight into the role of grazing pressure on woodland regeneration in mosaic vegetations that develop on former pastures and former arable land. A threshold for regeneration on former pastures was found around 130 grazing days ha^{-1} y^{-1} (= ±0.35 AU ha^{-1} y^{-1}); on former arable land around 180 grazing days ha^{-1} y^{-1} (= ±0.50 AU ha^{-1} y^{-1}). For the main regeneration phases in the New Forest, Mountford & Peterken (2003) give comparable maximum thresholds for cattle (0.3 AU ha^{-1} y^{-1}). For ponies and deer 0.15 and 0.45 AU ha^{-1} y^{-1}, are given respectively. For woodpasture grazing, Chatters & Sanderson (1994) recommend 0.1-0.2 AU ha^{-1} y^{-1}. Kuiters & Slim (2003) found that Iceland ponies, grazing on former arable (0.08-0.1 AU ha^{-1} y^{-1}) restricted successful regeneration to patches with bramble shrub, but no thresholds are given for regeneration outside protective vegetation structures. Using grazing pressure thresholds for conservation should take into account the presence and densities of rabbits which may strongly affect woodland regeneration (chapter 3).

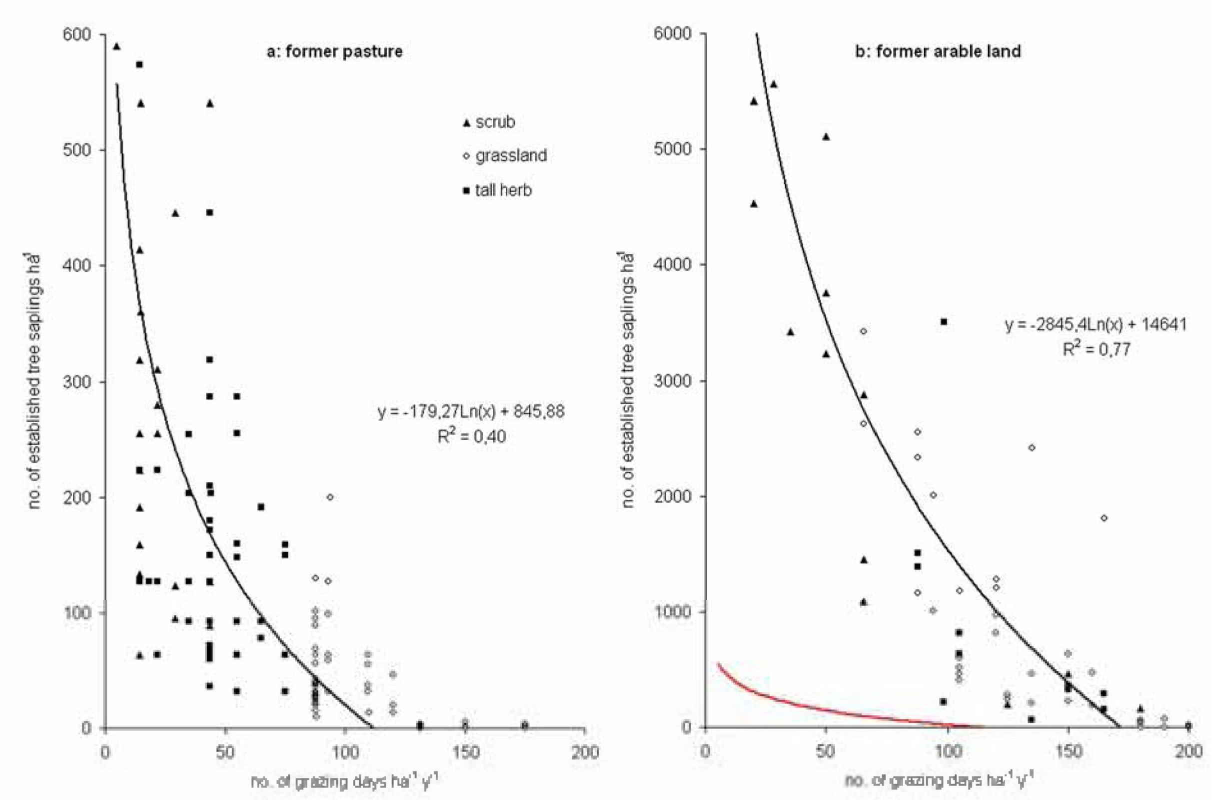


Figure 8.7: number of established trees related to grazing pressure on former pasture grassland (a) and former arable land (b). Y-axis scales differ with a factor 10: the red line in 8.7b is the trendline of 8.7a (former pasture) to make easy comparison.

A grazing pressure that is below the thresholds given here, allows woodland regeneration in a developing mosaic vegetation during the first 5-10 years after the cessation of agricultural use. Up till now, it is not clear whether such grazing pressures will be appropriate to either promote or reverse further woodland development and the possibility to grow beyond the browse line, but the results of this study strongly indicate that facilitation processes will dominate over grazing pressure (if it remains below the thresholds given) in the following successional stages (**chapter 4 & 5**). Studies that have controlled herbivore densities and observed ecosystem level response have documented striking, non-linear relations (Hobbs *et al.* 1996); the same may be true for the establishment process of trees on former agricultural land.

The role of vegetation structure type is pronounced strongest on former pastures, where a lower real grazing pressure in scrub and tall herbs results in higher establishment numbers. On former arable land, reduced real grazing pressure and higher establishment numbers were found in scrub (Fig. 8.7). There was no clear difference between the impact of tall herb and grassland plots, which is probably due to the dominance of tree species with resistance traits that establish on former arable land (**chapter 4**).

8.6.2 Grazing regime

In large areas where nature conservation is the principal objective, "natural grazing" by mixed, free-ranging feral herbivores grazing year-round with populations limited by late winter conditions, is preferred to seasonal grazing limited by summer fodder (Mountford & Peterken 2003). The former requires lower stocking rates and should produce greater habitat diversity and allow trees to regenerate in open areas (Helmer 2002). However, literature on the effects of different grazing regimes and woodland regeneration is very scarce.

Hester *et al.* (1996) compared summer and winter grazing regimes and three different grazing intensities (low: 0.6-1.2, medium: 1.2-2 and high: 2.1-3.8 sheep ha^{-1}). Seedling recruitment (*Betula pubescens*, *Fraxinus excelsior* and *Sorbus aucuparia*) was highest in the high intensity grazed plots. Growth and survival to the sapling stage (>0.3 m) was negatively correlated with grazing intensity, but was much greater in winter-grazed than summer-grazed plots. Although, browsing was highest in winter - probably due to increased visibility of saplings and scarcity of alternative food - sapling density was highest in winter-grazed plots, suggesting that browsing in wintertime is less detrimental for survival and growth. In this study, I could only compare summer-grazed (May-October) and year-round grazed sites. Survival and growth of *F. excelsior* and *Q. robur* did not differ between these grazing regimes (**chapter 3**). The browsing pattern of both species in two year-round grazed sites (Bos t' Ename and Rietbeemd) showed that browsing intensity on saplings was much greater in spring and summer than in winter (Fig. 8.8) for saplings in grassland and tall herb plots. Autumn browsing on both species was important in ruderal pioneer vegetations. Observations on cattle diet and habitat use in Bos t' Ename (**chapter 6**) showed that browsing of *S. caprea* saplings and juvenile trees on former arable land occurred mainly in late spring and early summer when saplings had nutritious buds and green leaves. Comparable seasonal patterns have been observed for roe deer, browsing on *Picea sitchensis* (Welch *et al.* 1988) and probably also on *Pinus sylvestris* (Palmer & Truscott 2003).

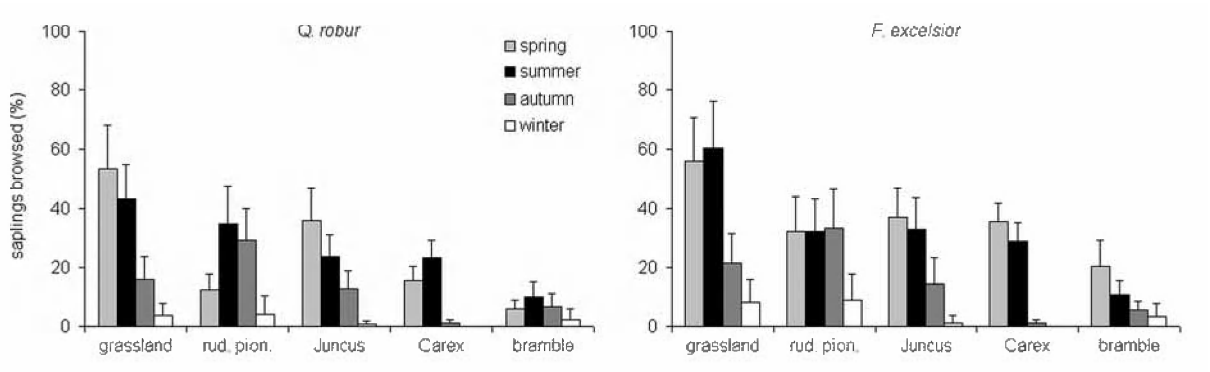


Figure 8.8: percentages of *Q. robur* and *F. excelsior* saplings browsed in two year-round grazed vegetation mosaics (Bos t' Ename and Rietbeemd).

In conservation management, there is often a general restraint concerning winter-grazing. Various authors report higher browsing levels on tree saplings (and trees in general) in winter for both wild and domestic herbivores (Hester *et al.* 1996; Palmer *et al.* 2004 (*Q. robur*, *Corylus avellana*, *Betula* sp.)). Our results and those of Hester *et al.* (1996) indicate that winter-grazing or year-round grazing by domestic herbivores (cattle, sheep) does not prevent woodland regeneration, nor decreases sapling densities compared to summer-grazing. However, there may be differences in browsing response according to species, plant size at the moment of browsing, local site conditions, frequency of browsing, amount and type of tissue eaten and competition with ground vegetation (cf. review by Hester *et al.* 2006).

8.6.3 Discontinuous management measures: time gaps in grazing and disturbances

The results of this study show that besides grazing pressure and grazing regime, also discontinuous measures (or events) should be incorporated or accepted in management plans. Short time gaps (a few years) before grazing often occur after cessation of agricultural use and before the start of conservation management. This may be the result of practical regulations or a purposeful measure to trigger succession and tree regeneration. Our data indicate that on former intensively used grassland, time gaps before grazing and intermittent time gaps in grazing can trigger and promote woody species regeneration (**chapter 3 & 5**). Time gaps before grazing should be longer than 2 years. Intermittent time gaps may be shorter, because regeneration can be accelerated due to already established saplings or juvenile trees that fail to grow out when grazing continues (e.g. *Fraxinus excelsior* in soft rush (*Juncus*)-grassland vegetation mosaics. Extending time gaps before grazing may be an option, but in species rich grasslands it carries the risk of biodiversity loss due to the strong expansion of tall herbs (ruderalisation; Bakker *et al.* 2002). Time gaps before grazing on former arable land should only be taken in consideration when there is a risk of insufficient seed dispersion, i.e. when sites are isolated, and therefore depend on wind dispersed species such as *Salix* and *Betula* species, that have to be dispersed over long distances (several hundreds of meters to kilometres; **chapter 4**). When there is sufficient seed available, time gaps before grazing will result in a fast and massive regeneration (within three years) with pioneer tree species. Therefore delayed grazing will only have a minor impact on woodland regeneration. Moreover, in **chapter 4**, it was shown that grazing increases spatial heterogeneity in woody pioneer assemblages, which is an important management objective for ecological restoration on former, intensively used land (e.g. the restoration of wooded pastures, Mitlacher *et al.* 2002). Intermittent time gaps in grazing may play a similar role as described for former intensively used grasslands.

Time gaps in grazing may also be of great importance in forest grazing. In **chapter 7**, it was concluded that temporal time gaps in grazing may prevent excessive grazing and trampling damage to vulnerable forest ground flora and may give opportunities for recovery. E.g., a time gap prohibiting trampling may be needed to restore energy reserves in the rhizomes to compensate trampling damage to below and above ground plant parts (e.g. *Anemone nemorosa*). It could take many seasons of additional irradiance to build up these reserves. As grazing and trampling in forest coincide with grazing on bramble understorey in winter and early spring, time gaps in grazing may occur spontaneously when this food supply is depleted. Consequently, the real grazing and trampling pressure on forest ground flora may decrease in time. However, for such shifts in diet and habitat use, large herbivores need alternatives. Therefore, large areas that meet the habitat requirements of large herbivores should be used to develop the design of nature reserves (WallisDevries 1994). If this is not possible, time gaps in grazing, seasonal grazing, short periodic reductions and increases in herbivore numbers, and permanent or temporal exclosures may be needed in conservation management planning. Insight into the effects of such measures clearly needs further research.

Natural and episodic disturbance dynamics and interaction effects with large herbivore grazing may strongly affect woodland regeneration (**chapter 2 & 5**). However, herbivory effects are not easily predicted under different combinations of episodic disturbance such as fire, timber harvest, floods, drought and insect defoliation. The lack of predictability poses a substantial obstacle to effective management with large herbivores (Wisdom *et al.* 2006). Nevertheless, conservation managers should primarily focus on the restoration of natural disturbances or processes in a given area and accept some uncertainties in community development when grazing management with large herbivores is applied (Cosyns & Hoffmann 2004).

8.6.4 Recruitment probabilities at different stages and expected canopy covers on grazed former agricultural land

In this paragraph, I will use the information on seedling emergence success (**chapter 2**), survival and growth of seedlings (**chapter 3**) and existing models (**this chapter**, García 2001) to give some quantitative recruitment probabilities for the model species of this thesis *Q. robur* and *F. excelsior* in a three year's time span. Secondly, I will use the observed establishment frequencies (**chapter 4 & 5**) related to grazing pressure (see 8.6.1), recruitment probability at the stage of outgrowth (above the browse line) and some basic demographic models (de Turckheim & Bruciamacchi 2005) to estimate the degree of canopy cover in developing grazed woodlands.

8.6.4.1. Recruitment probabilities

Table 8.4 shows the stage specific and stagewise cumulative recruitment probabilities obtained from our experiments on *Q. robur* and *F. excelsior*, respectively. Stage-specific recruitment probabilities assess the proportion of seeds or seedlings passing through the given recruitment limitation. Within a species, they show the relative importance of a certain stage. Stagewise cumulative recruitment probabilities represent the accumulated product of successive probabilities of recruitment. I had no data of seed removal & post-dispersal seed predation of *F. excelsior,* nor data of seed viability of both species. Therefore, both species should not be compared. Nevertheless, our data suggest that for both species the germination-emergence and growth stages are the most limiting for recruitment.

Because the strong effect of vegetation type that was found throughout this thesis, I refined probabilities for recruitment according to these types (Fig. 8.9). In all vegetation types, the germination-emergence stage and growth stage are important. Probably recruitment in the seedling survival/sapling survival stage is overestimated because in our experiments seedlings from a commercial nursery were used. Also our own field observations suggest a higher mortality.

Table 8.4 Summary of stages leading to recruitment of *Q. robur* and *F. excelsior.*

	stage	stage specific probability	stagewise, cumulative probability	data
Q. robur	post-dispersal seed predation	0.453	0.453	chapter 2
	germination-emergence*	0.149	0.068	chapter 2
	survival (after 3 years)*	0.450	0.031	chapter 3
	growth above browse line*	0.080	0.002	chapter 3
F. excelsior	post-dispersal seed predation	no data	no data	chapter 2
	germination-emergence*	0.134	0.134	chapter 2
	survival (after 3 years)*	0.439	0.059	chapter 3
	growth above browse line*	0.148	0.009	chapter 3

* average of different vegetation types

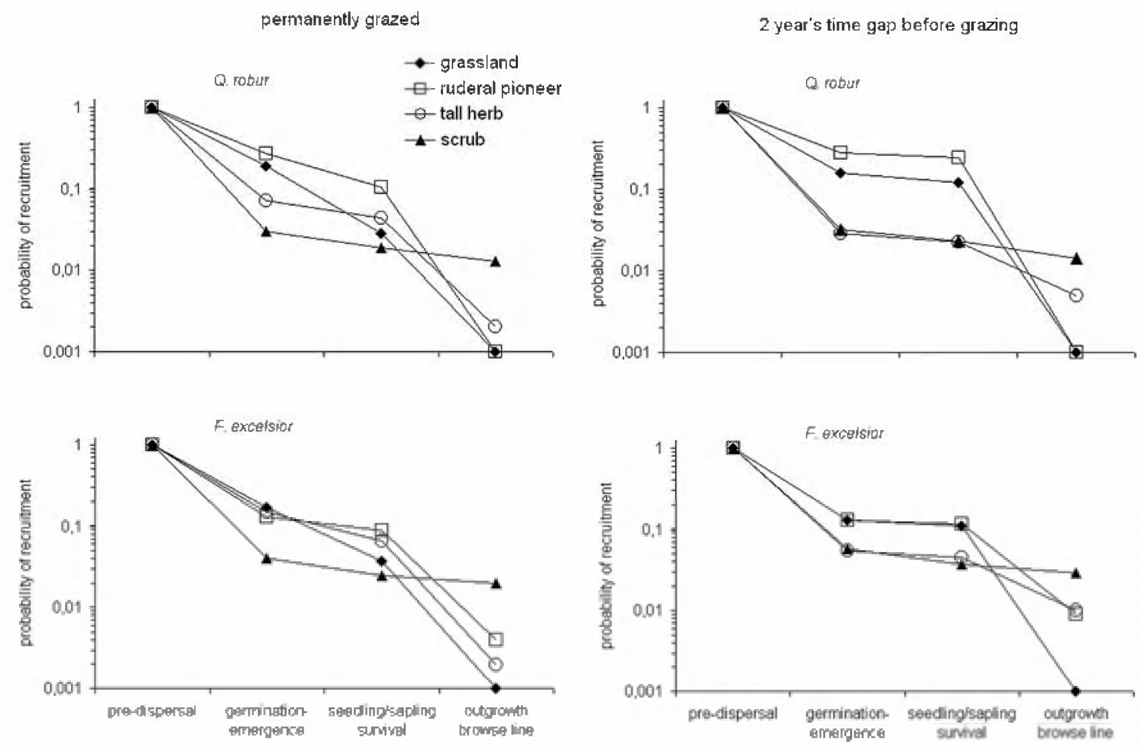


Figure 8.9: Probability of recruitment through the end of each stage (accumulated product of successive probabilities of recruitment, cf. García 2001). Predispersal expectancies have been arbitrarily set at 1. Recruitment probability at the germination-emergence stage also includes the effect of post dispersal seed predation (field data did not allow to make distinction between both effects). No possible effects of disturbances (germination-emergence stage) are included.

Nevertheless, there is strong selection in the following growth stage. Time gaps have a substantial effect on survival of seedlings/saplings and an even stronger effect in te following growth stage. This effect is most clearly expressed in tall herb and ruderal pioneer vegetation. Our data suggest that longer time gaps could increase recruitment in this stage considerably. Considering our experimental results of **chapter 2**, effects of trampling and flooding disturbance could also increase recruitment in the emergence stage with 50-150%, which would also have a positive effect of recruitment in the following stage.

Finally, very low percentages of seeds of *F. excelsior* and *Q. robur* produced by mother plants were able to produce a juvenile tree that was able to grow beyond the browse line. Our data suggest that finally appropriate microsites for germination-emergence and a protecting vegetation type are needed in permanently grazed sites. It is likely that species with resistance traits will have greater recruitment probabilities. Given these insights in recruitment probabilities, the next question is what kind of woodland will develop on grazed former agricultural land?

8.6.4.2. Expected canopy cover in developing woodland

I calculated the expected canopy cover in developing grazed woodlands using the establishment pattern in grazed former pastures and former arable land (**chapters 4 & 5, Fig. 8.7**) as a starting point. We made following assumptions to predict canopy cover:

(1) assumptions based on own data:

- on former pastures, established tree frequency after 25 years is 2.5 times the observed frequency (± 10 years), assuming new recruitment probabilities to remain stable.
- on former arable land, recruitment rate decreases after 5-7 years and equals the rate on former pastures from that moment on, assuming new recruitment probabilities to have decreased because the initial pioneer environment has disappeared.
- the above mentioned recruitment (establishment) rates remain equal for a certain grazing pressure in the following years (calculated for 25, 50, 75 & 100 years) on both former pastures and arable land.
- 0.08% of all established individuals is able to grow beyond the browse line (cf. the stage specific expectancy of *Q. robur*, see 8.6.4.1). This probability remains the same in the following years.

(2) assumptions based on the model of de Turckheim & Bruciamacchi (2005).

- 10% of trees that have grown out above the browse line in the first age class die.
- canopy diameter of a tree is ±20 times the stem diameter at 1.3m, except for the first age class (25 times).
- yearly diameter increment is 8 or 6 mm (two scenarios calculated).

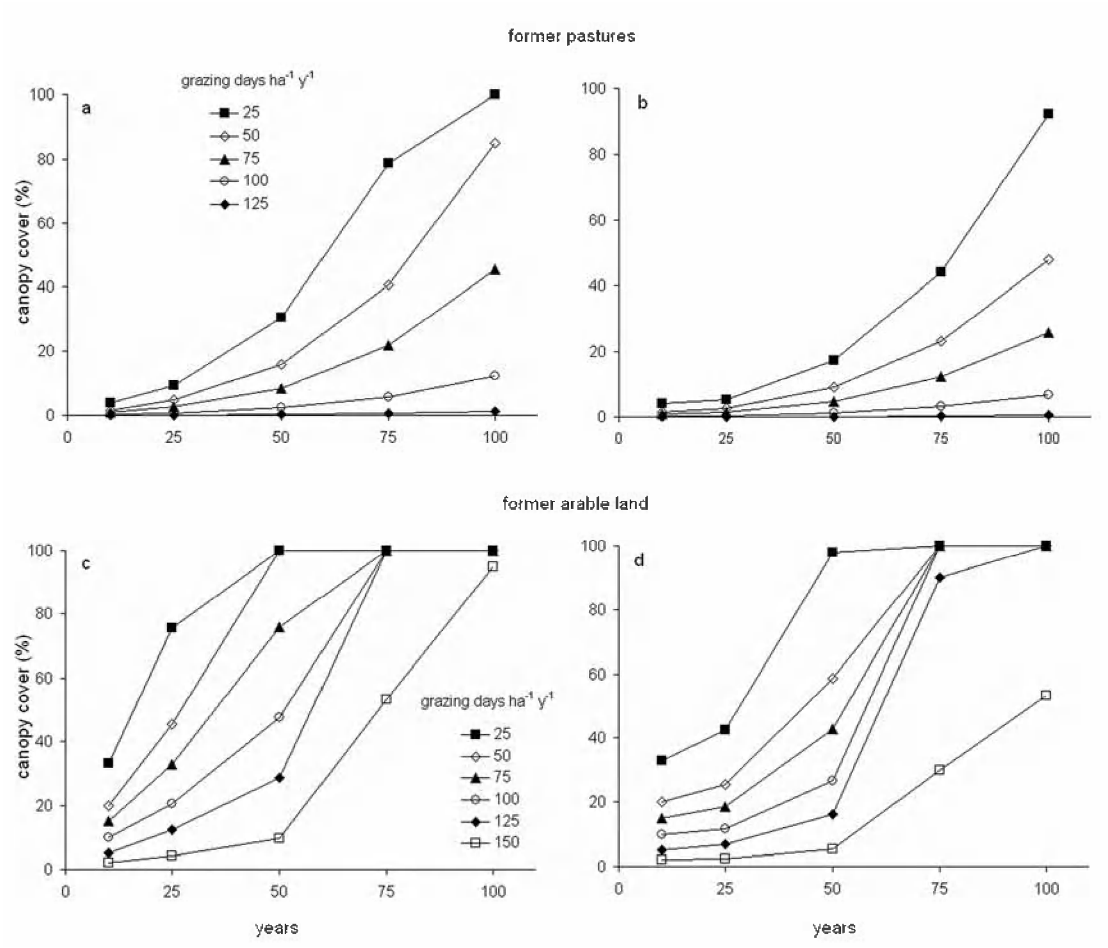


Figure 8.10: Predicted canopy cover in developing woodlands on former pasture and former arable land for different grazing pressures (animal unit grazing days ha^{-1} y^{-1}). a & c: assumed yearly diameter increment = 8mm; b & d: yearly diameter increment = 6mm.

Figure 8.10 gives predictions for canopy cover in a period of 100 years based on a the demographic model of de Turckheim and Bruciamacchi (2005). Probably scenario b and d are most appropriate because under grazing, diameter increment is lower than in ungrazed situations. On former pastures, a grazing pressure between 50 and 100 grazing days $ha^{-1}y^{-1}$ will result in an open woodland type in the next 75 years. Lower grazing pressure leads to a closed forest type. Higher grazing pressure almost prohibits woodland regeneration. On former arable land, even a moderate grazing pressure of 125 grazing days will result in a closed canopy in 50-75 years time span. For prediction at a longer time scale (>100 years), more detailed data (e.g. tree mortality) should be included. We also expect a decreasing recruitment rate once the canopy has closed. Therefore, we should test more scenarios (by changing recruitment probabilities, initial establishment frequencies…). This was beyond the aims of this study.

Nevertheless, we explored the sensitivity of the model by changing model parameters in two ways. First, we decreased the probability for newly established trees to grow beyond the browse line over time (0.08 first 25 years; 0.06 after 50 years, 0.04 after 75 years & 0.02 after 100 years), assuming an increasing trend in competition of neighbouring tree canopies. Second, we changed the probability for newly established trees to grow beyond the browse line corresponding to grazing pressure (0.08 for 25, 0.06 for 50, 0.04 for 75, 0.02 for 100 and 0.01 for >100 grazing days $ha^{-1}y^{-1}$), assuming that increasing grazing pressure negatively affects growth above the browse line. Both changes in probabilities had a rather low impact on the developing canopy covers over time. The first scenario reduced the rate of canopy cover development over time, but differences for a certain time and grazing pressure were always less than 7% for former pastures and less than 21% for former arable land. The second scenario had a similar effect but differences were less pronounced (5 and 17% resp.). These finding suggest that the initial establishment stages are very important for further development.

Though our results might give an idea of possible woodland views over time, caution is needed for further interpretation. First, the developing structure of ground and low shrub vegetation is highly unpredictable and strongly affects establishment (see previous chapters). Here, we used average establishment frequencies corresponding with a certain grazing pressure as starting points. Second, grazing pressure might fluctuate over time, creating windows of opportunity that result in higher probabilities for growth beyond the browse line. Third, there might be a strong site and species effect. Species with resistance traits related to grazing will have higher recruitment probabilities for growth beyond the browse line. Site effects may have a strong effect on initial recruitment rate and, as a consequence, on further development of tree canopy cover (**chapter 4 & 5**). Fourth, no mortality rates were included in the model (see above).

Most model scenarios point at an increase of canopy cover in a period of 100 years. These results contrast with results on forest development under nutrient poor conditions. Jorritsma *et al.*

(1997) developed the FORGRA model to predict the effects of ungulates (roe deer, deer, konik horse and highland cattle) in nutrient poor woodland ecosystems. Model results predict that Scotts pine (*Pinus sylvestris*) forest on land dunes soils and beech (*Fagus sylvatica*) forest on former heathland soils will dissapear within 10 years, even with a very low grazing pressure (0.01-0.08 cattle ha^{-1} y^{-1}). In general, the model predicts that ungulates will exert a strong influence on tree recruitment in nutrient poor forests. For cattle, grazing will result in the total disappearance of new recruitment in seven forest types (e.g. pine and oak forests) on nutrient poor soils. A second general trend was that grazing results in the development of deciduous pioneer forest types when cattle and horses are used. Although the concepts of the FORGRA-model (changes in existing forest) and the basic demographic model in this study (regeneration outside forest) strongly differ, the direction in which woodlands develop in a time span of 100 years is totally different for nutrient poor and nutrient rich environments, respectively. On nutrient poor soils, development of pioneer forest types is induced, while on nutrient rich soils, an increasing canopy cover over time is predicted. Caution for further interpretation is needed because mortality and natural degeneration processes are not included in the demographic model on nutrient rich sites. These processes will gain impact in later stages of woodland regeneration on nutrient agricultural land and they will only start after 50-60 years (Falinski 1998). Further, van Wieren & Kuiters (1997) conclude that (based on the model of Jorritsma et al. 1997) open landscape components of heathland-woodland complexes will gradually change in more closed woodlands through establishment of *Pinus sylvestris.* These authors state that the effects of heterogeneous habitat use are difficult to predict. The larger the ratio forest/heathland, the less effects on natural regeneration inside forest and the greater the effects on natural regeneration in heathland. The model also predicts that cattle en horses will be able to prevent woodland regeneration in heathlands for a longer period than deer will do.

8.6.5 A conceptual and management oriented state-and-transition model for grazed woodlands on former agricultural land

Conservation management has traditionally been based on equilibrium paradigms. The Range Model (Walker 1993) for example is largely a univariate approach, that emphasizes grazing as the primary driver of vegetation dynamics: grazing intensity proportionally counteracts secondary succession, in a continuous, directional manner, to modify species composition. Recently, studies from a wide range of habitats managed for conservation purposes have found that temporal and spatial heterogeneity (i.e. features of non-equilibrium systems, Table 8.5) may be of considerable importance for the diversity and community structure of these systems (Briske *et al.* 2003; Fuhlendorf & Engle 2004). For example: spatially and temporarily heterogeneous land-use practices, such as cutting, burning, turf-cutting and grazing, have resulted in complex mosaic landscapes that are of high priority for conservation in Europe. Contemporary land-use (and conservation) subjects these systems to management regimes that are generally less diverse, in terms of disturbances and fine-scale temporal and spatial variability. Grazing management increases variability among habitats and adds complexity to successional dynamics (Vandvik *et al.* 2005), given grazing regime and pressure are variable as well.

Table 8.5: Attributes of equilibrium and non-equilibrium systems are based on varying degrees of internal regulation and the corresponding response of system behaviour to external disturbances. (Briske *et al.* 2003)

	equilibrium systems	**non-equilibrium systems**
abiotic patterns	relatively constant	stochastic/variable
plant–herbivore interactions	tight coupling	weak coupling
	biotic regulation	abiotic drivers
population patterns	density dependence	density independence
	populations track carrying capacity	dynamic carrying capacity limits population tracking
community/ecosystem characteristics	competitive structuring of communities	competition not expressed
	internal regulation	external drivers

To conclude this chapter, I develop a conceptual, management oriented state-and-transition model for grazed woodland development on nutrient-rich former agricultural land (Fig. 8.11). It is based on results of this thesis and existing ecological theory and accommodates both equilibrium (i.e. continuous) and non-equilibrium (i.e. event-driven) vegetation dynamics. A state-and-transition model requires knowledge of (i) potential alternative vegetation states on a site, (ii) potential transitions between states and (iii) opportunities to achieve favourable transitions between vegetation states or avoid unfavourable transitions (Briske *et al.* 2003).

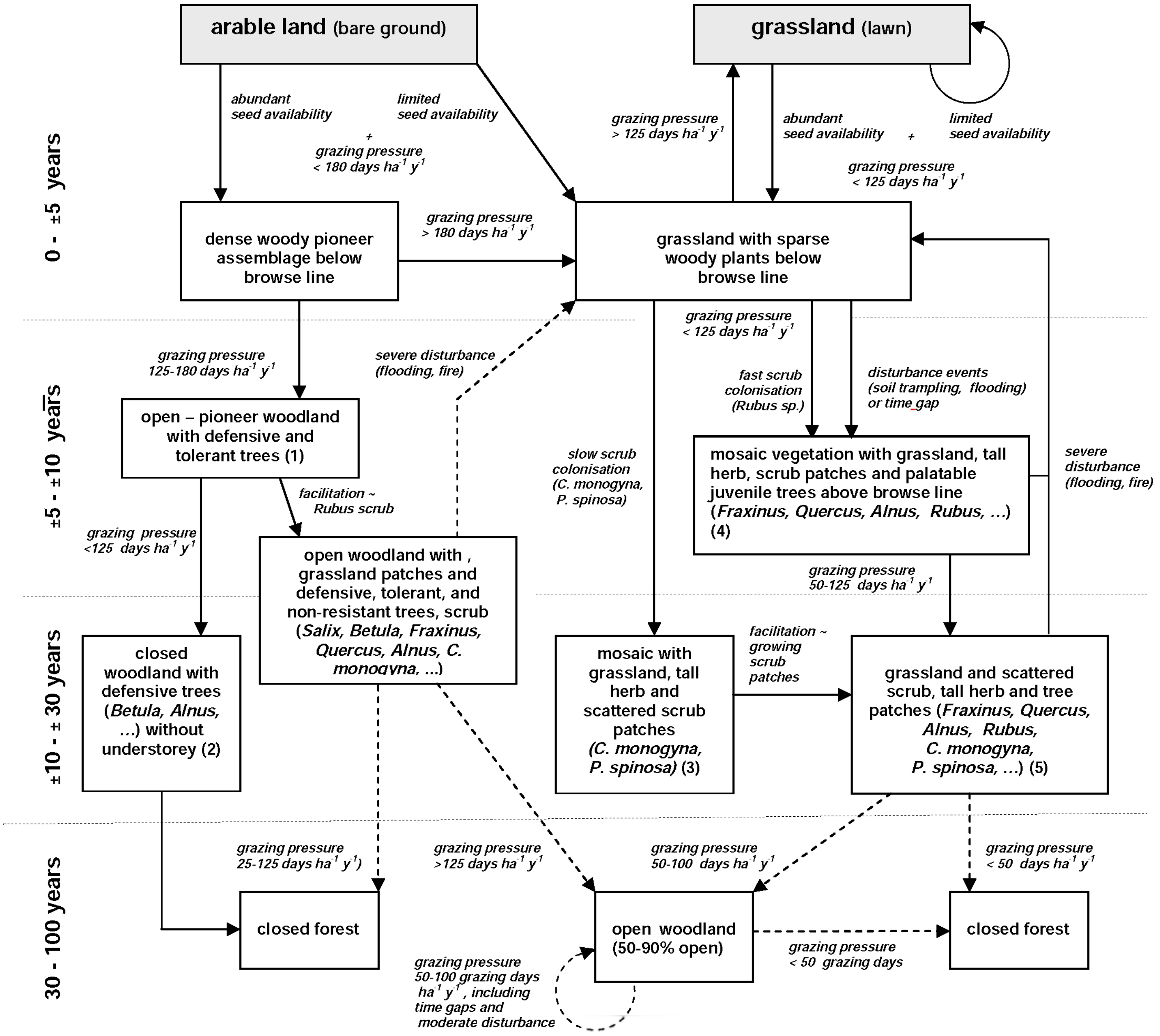


Figure 8.11: conceptual, management oriented state-and-transition model for woodland development on nutrient-rich former agricultural land. boxes: states; arrows: transitions; dotted arrow: predicted (hypothetical) transitions. Numbers between brackets refer to appendix 8.1 (photos).

Transitions starting from arable land lead to different woodland types compared to transitions starting from grassland. When high grazing pressure or limited seed dispersal during the earliest development phase or severe disturbances in later states occur, convergent developments are to be expected. Transitions from one stage to another are determined by seed dispersal, grazing pressure, resistance traits of plants, facilitation, time gaps, and disturbances. Establishment of *Rubus* scrub may not only speed up, but also determine the development of a next state. Some mid and late successional stages may only be reversed by severe disturbances such as fires or extreme floodings. The physiognomics of states on the longer term (i.e. > 30 years) are still hypothetical but the predictions of the demographic models (see 8.6.4.2) give some insight in woodland openness that is to be expected on the longer term.

8.7 General conclusions

8.7.1 Patterns, mechanisms and processes

The regeneration pattern of woody species highly depends on the dynamic pattern of vegetation structure that interacts with large herbivore grazing (direct damage by browsing and trampling, diet, habitat use, nutrient redistribution), presence and distance to potential seed sources and soil disturbances. These interactions cause a series of bottlenecks or opportunities at different life stages of woody species in a grazed environment. Large differences in regeneration patterns in grassland and on former arable land are related to differences in vegetation structure and the woody species' resistance traits against herbivory.

The active mechanisms that favour woodland regeneration are associational resistance (safe sites), release of competition (soil and vegetation gaps) and time gaps in grazing. Associational palatability in preferred patches and increased competition and neighbour contrast susceptibility in avoided patches inhibit regeneration. The balance of facilitation and inhibition will determine the net effects of a community on tree establishment, growth and survival.

Disturbance processes are important in nutrient-rich ecosystems. Individual disturbances may be seen as "ecological reset" mechanisms, creating opportunities for further succession. Grazing rather is a fine-scale disturbance, affecting the successional dynamics after disturbances at a larger scale. Indirect processes such as nutrient redistribution and nutrient depletion have the potential to trigger woodland regeneration, but are probably less important than disturbance processes.

8.7.2 Nature conservation and ecological restoration

Conservation managers have some instruments to handle if woodland regeneration on former agricultural land is desired or needs to be avoided. Grazing intensity thresholds for woody species establishment on former pastures and arable land in relatively nutrient rich environments are ±0.3 AU $ha^{-1}y^{-1}$ and ±0.5 AU $ha^{-1}y^{-1}$, respectively. Both seasonal and year-round grazing allow woody species establishment. Short time gaps in grazing are useful to mimic natural population fluctuations and allow recovery of vulnerable vegetation types, development of vegetation structure gradients and outgrowth of woody species. Natural disturbances often interact with grazing and should be primarily restored. However, a certain degree of uncertainty should be accepted (e.g. unpredictable behaviour of large herbivores, appearance of facilitating scrub, seed dispersal potential).

A conceptual state-and-transition model, based on the observed patterns and the active mechanisms and processes gives insight into the expected vegetation states. It includes a series of (open) woodland types, some of them with high conservation value (mosaic vegetation types), and simultaneously emphasizes needs for future research to test the model (and also comparable models in different habitats and regions).

At present, large herbivore grazing is already involved in a broad range of protected habitats in Europe. However, the need for large areas to fully allow the functioning of mechanisms and natural processes seems to conflict with the static and deterministic approach that is presently designed for the conservation of European habitats in the Natura 200 Network. In my opinion, a broader view on the spatial and temporal functioning of European habitats is needed to develop a sustainable conservation strategy.

8.8 Perspectives for future research

8.8.1 Predictive modelling

The conceptual model presented in this study primarily aims to give conservation managers something to hold on to. Second, it may be used to stimulate new research and management thinking about the interaction effects of large herbivore grazing, episodic disturbances, time gaps and other operating mechanisms in grazed woodland and woodland regeneration in space-limited conditions (as is the case within most of the West-European nature reserve and management dimensions). The conceptual state-and-transition model as presented in this study, could be used as a starting point to develop hypotheses for empirical testing under new research designs that address some of the key knowledge gaps. Refined models should examine the interactions of grazing with other disturbance regimes at spatial extents of interest to conservation managers and under varying grazer densities and they should also consider multiple steady states of vegetation development, including potential threshold effects from herbivory. The a priori construction of such models is important to ensure that they have management utility (Wisdom *et al.* 2006). The ultimate requirement for improved management is new knowledge about large herbivores that addresses the following issues: (i) effects of varying densities of different large herbivore species, such that potential threshold effects can be identified; (ii) the interaction of "issue (i)" with episodic disturbances and temporary decreases or increases in large herbivore densities. However, a major problem will be the conflict between the need for research at the landscape scale and the need for controlled, manipulative experiments, with multiple treatment levels. It will be a challenge to implement for instance experiments with multiple grazing intensity levels, different herbivores and temporary time gaps in grazing and also apply these experiments over large areas with sufficient replication. Therefore, adaptive management methods may be used, i.e. decrease or increase large herbivore densities on targeted landscape types specifically to test result and model predictions from controlled experiments on a smaller spatial scale. Nevertheless, such an approach will be costly, time-consuming, and operationally challenging. It is likely that only a far-reaching cooperation between European grazing-ecologists and conservation organisations may succeed in such a set-up.

8.8.2 Biodiversity

The effects of herbivores on species richness appear to depend on the type and abundance of herbivore species in a particular environment. These effects can be either positive or negative (Olff & Richie 1998). The temporal scale of herbivore effects might also be important, because short-term increases in diversity from herbivory can ultimately disappear owing to herbivory-induced succession to a few defended or tolerant plant species (cf. **chapter 4**). Conversely, periodic grazing effects (e.g. due to cyclic changes in population size) are often not detectable in short term experimental studies and could maintain high plant diversity. These results suggest that the spatial and temporal scale of herbivore effects must be explicitly considered to explain their impact on species richness.

For regenerating woodlands, knowledge on species richness is very scarce. Therefore, there is a great scope for further investigation into the status of biodiversity in relation to the structure of grazed (regenerating) woodland vegetations. This could be approached by experimental manipulation of the structural properties of grazed mosaics or by careful selection of study mosaics stratified in relation to properties to be tested (e.g. configuration, landscape composition, relative covers of landscape elements, development time, land use history). Sampling of biota should be from diverse landscape elements (e.g. grassland, tall herb, scrub, forest patches) and conducted at a scale that allows mosaic level inference (cf. Bennett *et al.* 2006).

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Appendix 8.1
Woodland regeneration views on former agricultural land

(nummers between brackets refer to the stages of Fig. 8.11)

a. on former arable land

(1) Open pioneer woodland with, grassland and tall herb patches and havily browsed tolerant (Salix caprea, in front) and outgrowing defensive (Betula pendula, at the back) tree species (Osbroek, Aalst, Belgium)

(2) In front: ± 12 years grazed grassland without establishment of woody species; at the back: ± 12 years grazed, closed pioneer forest with the defensive Betula pendula dominantly present on a former arable land parcel (Osbroek, Aalst, Belgium).

b. on former pastures

(3) Mosaic with grassland, tall herb and scattered shrubs (C. monogyna, Moenebroek, Geraardsbergen, Belgium).

(4) mosaic vegetation with grassland, tall herb, Crataegus monogyna and bramble scrub patches protecting palatable juvenile trees that grow above the browse line.

(5) Mosaic of grassland, tall herbs, scattered scrub and tree patches (Boelaremeersen, Geraardsbergen, Belgium).

Summary

In this thesis, we focused on woodland regeneration in the presence of large herbivores. We performed field surveys, experiments and elementary modelling to gain insight into the patterns of woody species regeneration, the mechanisms at work and the natural processes that influence woodland regeneration on former agricultural land that is grazed by large herbivores. In general, we aimed to contribute to the understanding of the changing temporal and spatial patterns of the grassland-forest matrix in grazed conditions. We approached the issue of conservation management strategies and landscape openness at the scale of present day, controlled grazing management in fenced nature reserves, but results may also gain insights into mechanisms and processes that are/were active in traditional wooded pastures and wilderness landscapes. The key processes to be understood in grazed ecosystems that include woodland are plant strategies in coping with herbivory, herbivore selectivity, light and nutrient requirements, dispersal and natural disturbances.

We examined the role of (1) the starting conditions of a site (arable land vs. pasture grassland, vegetation structure and abiotic conditions), (2) the surrounding landscape (seed sources), (3) associational resistance, (4) associational palatability, (5) competition of surrounding vegetation, (6) resistance traits against herbivory, (6) time gaps in grazing, (7) natural disturbances (trampling, flooding), (7) nutrient redistribution and depletion, (8) habitat proportion and (9) habitat use and foraging strategy in the regeneration process of woody species. Studies were performed at 13 study sites and one greenhouse-gardencomplex. Sites were situated on former intensively used agricultural land on nutrient rich and heavy soils (sandy loam, loam and clay).

In **chapter 2** we studied the earliest stages of woody species, the seed and emergence stage. We used *Fraxinus excelsior* and *Quercus robur* as model species in an experimental set up to investigate the role of vegetation structure (a successional sequence from grassland to scrub) and the interaction effects of grazing, pre-emergence flooding and trampling on emergence and early growth of seedlings. The studied species represent pioneer species with diverse recruitment strategies (differing in seed characteristics, dispersal vector and seedling light requirements) that establish naturally in grasslands with a natural disturbance regime (e.g. floodplains).

Seedling emergence was enhanced in low swards and sparse vegetation types. Despite different recruitment strategies, the interaction of flooding and trampling of swards enhanced seedling

emergence of both species. Grazing of soft rush and tall sedges enhanced emergence of *F. excelsior.* Short grass swards increased early growth of emerging *Q. robur.* Our results support the hypothesis that natural disturbances of soil and vegetation create microsites for seedling emergence and reduce above ground competition. For subsequent growth and survival, seedlings need protective structures such as tall herb or scrub patches. This implicates the need of a dynamic vegetation structure, offering changing patch qualities in time. Therefore, the recruitment of native tree species assemblages may not only require an appropriate grazing regime, but also the restoration of natural disturbances, such as natural floods.

In **chapter 3**, we examined the role of different vegetation structure types as safe sites for the survival and growth of establishing trees. After dispersal and emergence, survival and growth represent the next bottlenecks in the recruitment process of trees. In an experimental set up with exclosures, we used the same species and vegetation structure types as in chapter 2. Further, we evaluated the effect of time gaps in grazing. We analysed if this offered a window of opportunity for tree saplings to grow out beyond the browse line, which is an important threshold for woodland regeneration and/or the restoration of woodpastures (i.e. open, grazed woodlands with a mosaic of grassland, shrub and tree patches).

Bramble thickets were suitable safe sites for survival and growth of seedlings of both species. Tall sedges, soft rush tussocks and ruderal vegetations with unpalatable or spiny species provided temporal protection, allowing seedlings to survive. Tree regeneration in livestock grazed grassland was highly constrained, but rabbits may undo the nursing effects of bramble thickets. The first year's survival is of major importance for the establishment of trees. Subsequent grazing affects growth rather than survival. A two year's initial time gap before grazing, had positive effects on survival, but did not allow outgrowth of unprotected trees.

In **chapter 4**, we described regeneration patterns of pioneer trees in woodland restoration projects on grazed former arable land. We focused on differences between functional plant groups related to herbivory, i.e. trees with different resistance traits (tolerant, defensive, non-resistant), and the facilitating role of an unpalatable or spiny herb and low shrub layer that quickly develops on the bare ground of ploughed fertile soils. We tested if grazing by large herbivores affected the possibility of the different functional groups to grow beyond the browse line and consequently determined the species composition of pioneer woodland. We further examined if large herbivores are able to increase structural heterogeneity in early pioneer assemblages, which is often a goal in conservation management.

We found a massive and fast colonisation of grazing tolerant and unpalatable woody species in early successional stages. Grazing decreased frequencies and height of the competitively dominant tolerant species (mainly *Salix caprea* L.). After 5-7 years, frequencies of unpalatable and tolerant species were equal, but unpalatable species (mainly *Betula pendula*) were able to grow beyond the browse line. When the cover of unpalatable/spiny vegetation was high enough, it provided suitable nurse sites for tolerant and palatable woody species. In early assemblages, grazing increased horizontal and vertical heterogeneity, resulting in intermediate successional stages. On the longer term, the mechanism of associational resistance will also allow palatable and tolerant species to grow beyond the browse line and promote forest succession and the order of species establishment and replacement. On the longer term, facilitation tends to promote forest succession and the order of species establishment and replacement, though it may not be a conservation goal where intermediate stages are themselves desirable for biodiversity reasons.

In **chapter 5** we studied regeneration patterns of woody species invading grazed grasslands. We described the spatial association of establishing trees and developing vegetation patches. We tested whether these vegetation patches, consisting of grassland, tall herbs and scrub, influenced the regeneration pattern and the conditions that allow trees to grow beyond the browse line. Further, we investigated if increased susceptibility to trampling disturbance by large herbivores on wet soils in floodplains influenced sapling establishment.

Sapling frequencies, with *F. excelsior* as the most common species, were 2-3 times higher in tall herb and scrub patches compared to grassland. Only the spiny *Crataegus monogyna* established in higher frequencies in grassland plots. In floodplains, we found 2-3 times more established woody saplings in wet tall herb plots, compared to moist and wet grassland plots and moist tall herb plots. Browsing reduced sapling height in grassland and tall herb patches. Only in scrub patches did average height (± 2.5m) reach above the browse line. Browsing damage to saplings in scrub was significantly lower than in grassland and tall herbs. Tree establishment occurred in spatial association with non-grassland patches that offer protection against browsing. Fast growing spiny shrubs like *Rubus sp.* offer protection for palatable saplings, allowing establishment and growing out beyond the browse line. Alternatively, increased establishment occurred in wet tall herb patches, where soil disturbance by moderate trampling favoured establishment. Subsequently, saplings were protected at least temporarily against browsing by unpalatable tall herbs.

In **chapter 6**, we assesses the magnitude and direction of nitrogen redistribution in a grazed vegetation mosaic with grassland, wooded pasture and forest. We used nitrogen content of different diet classes, habitat use, foraging and defecation behavior, weight gain and nitrogen losses to explore four different habitat proportion scenarios and two different foraging strategies to calculate net nitrogen balances per habitat. We examined if the spatial redistribution of nitrogen among habitats by cattle could restore nutrient-poor conditions in preferred foraging habitats, and whether such translocation conversely could lead to a risk of eutrophication of vulnerable non-foraging vegetation types. We confronted the results with atmospheric nitrogen deposition and assessed whether the creation of nutrient-poor conditions in grassland could trigger cyclic succession and woody species regeneration.

All scenarios showed a small net nitrogen transport from grassland and wooded pasture to forest habitat. We found that nitrogen redistribution strongly depends on habitat proportion. Nitrogen losses from preferred grassland habitat can be high, given its proportion is small. Depletion is only to be expected at excretion free areas and probably is of minor importance to trigger establishment of woody species, due to feedback mechanisms that promote grazed patches. In general, nitrogen transported by cattle was much lower than input by atmospheric deposition, but grazing can compensate for high N inputs in excretion free areas and maintain mesotrophic grassland types. In none of the scenarios, N transport by cattle resulted in the exceeding of critical nitrogen loads to vulnerable forest ground vegetation.

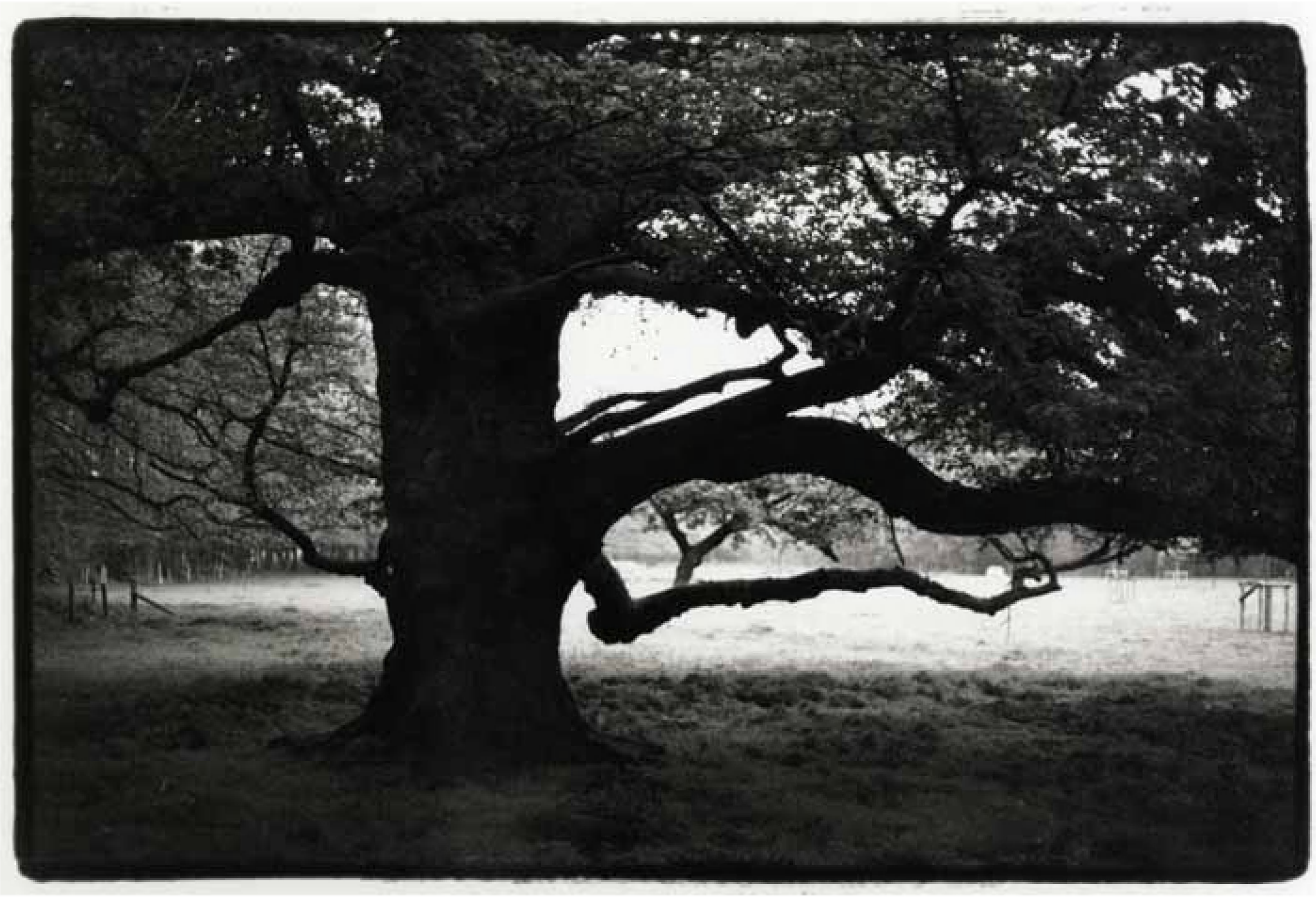
In **chapter 7**, we examined if the introduction of large grazers is a good measure to decrease bramble cover in ancient forests that are included in grazed mosaic landscapes. Expanding bramble cover as a result of increased atmospheric nitrogen deposition and altered forest management is considered as a threat for species rich forest ground flora. We studied the effects of four years of large herbivore grazing on bramble cover, and on cover and flowering of a set of vernal flowering forest forbs. We further conducted experiments to investigate direct effects of grazing and trampling on forest ground flora.

Large *Rubus* thickets had a clear negative impact on cover and flowering of *Anemone. nemorosa* due to competition for light. Four years of cattle grazing reduced bramble cover by more than 50%, but then the limiting factor for *A. nemorosa* cover and flowering shifted to trampling damage. We also found lower cover and flowering of *Hyacinthoides non-scripta* in grazed plots, a direct effect of grazing. The evergreen species *Vinca minor* and *Hedera helix* totally disappeared from the grazed forest. Simulated once-only effects of grazing and trampling had a small and short term negative impact on cover of *A. nemorosa*, but flowering was strongly reduced. Grazing reduced biomass with 25-30 % in the following years. Year-round grazing with large herbivores is an appropriate measure for bramble control in forests, but negative effects on ground flora are possible if grazing pressure is high. A low or moderate grazing pressure should be maintained in landscape mosaics with grassland and forest; or intermittent periods of non-grazing should be provided to maintain forest ground flora diversity.

Finally, in **chapter 8**, we made integrated analyses that summarize the conditions for woodland regeneration on grazed former agricultural land. We deduced a conceptual model for regeneration success on former arable land and grassland with different operating mechanisms and processes (resistance traits, facilitation, natural disturbances, time gaps). The integrated results were made applicable for ecological restoration and conservation management by providing guidelines for grazing pressure, grazing regimes and the use of time gaps. Further, we developed a conceptual, management oriented state-and-transition model that describes the early successional pathways to be expected on grazed former agricultural land.

Co-authors' contributions

Maurice Hoffmann	general supervision, manuscript reading and improvement
Tanja Milotic	fieldwork, manuscript proofreading and improvement
Dirk Maes	manuscript proofreading and improvement, statistical help
Kris Decleer	initial ideas, manuscript proofreading and improvement
Dominique Vandenhaute	fieldwork, preliminary analyses
Anke Van Noyen	fieldwork, preliminary analyses



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