Chapter 3: **From protégé to nurse plant: establishment of thorny shrubs in grazed temperate woodlands**

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**Abstract**

**Question:** Thorny shrubs play a keystone role in grazed ecosystems by defending non-protected plants against herbivores, but the establishment of thorny nurse plants in grazed ecosystems is poorly understood. Which factors control the establishment of recruits of thorny nurse shrubs in grazed temperate woodlands?

**Location:** Ancient grazed temperate woodlands (52° 32’N, 6° 36’E), the Netherlands.

**Methods:** We surveyed for saplings of thorny nurse shrubs and compared plots with and without saplings for various biotic and abiotic variables. To disentangle the biotic and abiotic factors we performed a transplantation experiment over two growing seasons with nurse shrub saplings (*Prunus spinosa* and *Crataegus monogyna*) planted in two dominant vegetation types - tall unpalatable roughs and short grazed lawns - half of them protected from herbivory via exclosures.

**Results:** Plots with shrubs saplings had taller surrounding vegetation, higher soil pH and higher soil moisture than plots without saplings. Also, plots with shrub saplings predominantly contained unpalatable rough species while plots without saplings mainly contained palatable lawn species. The subsequent transplantation experiment showed that sapling survival was higher in exclosures than in the open, and higher in rough-exclosures than in lawn-exclosures. Sapling growth was higher in roughs than in lawns, higher inside than outside exclosures, and higher for *Prunus* than *Crataegus*, while browsing on saplings was higher in lawns.

**Conclusions:** Unpalatable roughs form essential establishment niches for thorny shrubs in grazed temperate woodlands: they protect against herbivores when the thorniness of the saplings has not fully developed yet, and sapling growth is better due to improved micro-environmental conditions. Once established and thorny, shrub saplings grow out of the protective range of the roughs and will on their turn facilitate tree seedlings, which is essential for the long-term persistence of grazed temperate woodlands. This study shows that nurse plants may start as protégés before becoming a facilitator for other plants in a later life-stage. This finding may be a common phenomenon for other nurse plants in various ecosystems. We argue that an improved understanding of the establishment of nurse
plants and their constraining factors is crucial for effective conservation and restoration efforts in various ecosystems.

3.1 Introduction

Nurse plants improve the micro-environmental conditions for other plants, the protégés, often being seedlings (Callaway 1995). Nurse plants can provide shelter against extreme temperatures and high irradiance, increase water availability, improve nutrient availability, offer physical support, reduce soil compaction and erosion or protect against herbivores (e.g. Flores and Jurado 2003). Nurse-protégé interactions importantly shape a wide range of ecosystems such as deserts (Lortie and Turkington 2008), coastal dunes (Franks 2003), tundra’s (Eskelinen 2008), high mountains (Cavieres et al. 2007), salt marshes (Crain 2008), steppes (Graff et al. 2007), Mediterranean forests (Gómez-Aparicio et al. 2005), savannas (Riginos and Young 2007) and wood pastures (Smit et al. 2006, 2008), and the importance of this type of facilitation for ecosystem structure and functioning is now fully recognized (Callaway 2007). Yet, nurse-protégé interactions have thus far been mostly described for harsh environments with high abiotic stress (e.g. drought, salinity, nutrient limitation), while the crucial role of biotic stress (e.g. consumer pressure or herbivory) has received much less attention in both harsh and benign environments (Smit et al. 2009).

While the strength and importance of nurse-protégé interactions is presently well-known – some species can not establish and survive without the help of others (e.g. obligate facilitation sensu Connell and Slatyer (1977)) – surprisingly little studies have explicitly studied the establishment of these nurse plants in natural communities (Scheper and Smit 2011). Given the key-role of nurse plants for the structure and functioning of ecosystems, it is crucial that viable populations of nurse plant are maintained via consistent and frequent recruitment. Recruitment failure inevitably leads to local extinction of species which, in the case of a nurse plant, may have dramatic consequences for an entire ecosystem: it may not be able to recover after a disturbance and remain in a degraded state for a very long time (e.g. arrested succession (Putz and Canham 1992)). Examples are Mediterranean oak savannas at the Iberian Peninsula where long-term tree recruitment failure is occurring due to a chronic lack of facilitating shrubs (Smit et al. 2008, 2009; Pulido et al. 2010). When the adult oaks die they will not be replaced by recruits and the now typical savanna-landscape will convert to treeless grasslands, with loss of the typical high and unique biodiversity. Hence, an improved understanding of the recruitment of nurse plant is essential for a long-term persistence of ecosystems and for sustainable conservation and restoration practices.

Thorny shrubs are crucial nurse plants in grazed woodlands: they facilitate tree seedling survival and so initiate the establishment of isolated trees or small forest patches in the landscape (Rousset and Lepart 1999, 2000; Bakker et al. 2004; Smit et al. 2005, 2006; Van Uytvanck et al. 2008a). For example, successful recruitment of palatable trees such as Oak (Quercus robur) and Ash (Fraxinus excelsior) is restricted to thorny shrub thickets of Prunus spinosa and Crataegus monogyna as they offer physical protection against large herbivores (Bakker et al. 2004). These findings are in line with the ‘shifting mosaic concept’, predicting a cyclic shifting of grassland – shrub – tree mosaics over space and time in grazed woodlands (Olff et al. 1999). Hence, once these thorny shrubs
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have established they play a crucial role for the dynamics of grazed woodlands by initiating the transition from grassland to forest patches, needed to maintain this landscape. Thus far, the decisive process preceding this transition – the establishment of nurse shrubs in grazed woodlands – has not been studied, while this step is expected to form an important bottleneck for the predicted shifting mosaics in grazed woodlands. This study focuses on the establishment of thorny nurse shrubs in ancient grazed woodlands.

For successful establishment of new (nurse) recruits, various stage-dependent plant limitations exist that need to be passed: 1) source limitation, i.e. insufficient number of seeds at the parent plants; 2) dispersal limitation, i.e. insufficient dispersal of available seeds; or 3) establishment limitation, i.e. insufficient survival of seeds, seedlings (< 1 yr) and saplings (> 1 yr) after dispersal. These limitations are neither mutually exclusive nor independent (Nathan and Muller-Landau 2000). Flowering and fruit production of the thorny nurse shrub *Prunus spinosa* (hereafter *Prunus*) are generally abundant in grazed woodlands in NW Europe, and frequent seed dispersal by frugivorous birds - primarily by thrushes *Turdus merula*, *T. philomelos*, *T. viscivorus*, *T. iliacus* and *T. pilaris* (Sorensen, 1981) suggest that both source and dispersal limitation do not play a major role here. Therefore, in this study we considered establishment limitation of *Prunus* with focus on the seedling and sapling stage. This stage is generally seen as the most sensitive as young woody recruits are particularly vulnerable to the effects of drought, shading, radiation, competition with other species (notably root competition with grasses) or herbivory (Berkowitz et al. 1995; Jurena and Archer 2003; Gómez-Aparicio et al. 2005). For example, while mature *Prunus* is well protected against large herbivores by numerous strong 2-8 cm long thorns, seedlings still lack this defence as the production of protective thorns only takes place after 2-3 years (Rackham 1980). Woody seedlings that grow in short, palatable grass ‘lawn’ vegetation therefore run a high risk of being grazed together with the focal species of the herbivores (Smit et al. 2006). So, we may expect that new *Prunus* recruits can only successfully establish in environments that are, at least temporarily, protected from herbivores. These environments may be formed by the tall unpalatable ‘roughs’ containing low-nutritious and herbivore defended herbaceous species, as predicted by Olff et al (1999). Also, these tall unpalatable roughs may form suitable micro-environmental conditions needed for the initial growth: soil humidity, organic content or nutrient availability may be higher in roughs (non-removed tall vegetation preserves moisture and accumulates litter and/or nutrients), while drought-risk and root competition with dense grasses may be higher outside roughs (in lawns).

In this study we aimed to discover which conditions favor the establishment of recruits of thorny nurse shrubs in grazed temperate woodlands and performed two studies. First we carried out a thorough survey of all recent established saplings of nurse shrubs in the study site and compared environmental conditions in sapling plots with control plots without saplings. Subsequently, we performed a transplantation experiment with shrub saplings planted inside and outside tall unpalatable roughs (in lawns), half of them protected from herbivory, to disentangle biotic (herbivory) and abiotic factors. We expected that 1) shrub sapling plots would differ from plots without saplings in terms of soil pH, humidity, vegetation height and species composition, indicating a specific establishment ‘niche’, and 2) that shrub saplings planted in tall unpalatable roughs would do better than those planted outside these roughs (in lawns), due to combined protection against herbivory and improved micro-climatic conditions.
3.2 Methods

3.2.1 Study site
This study was conducted in the Junner Koeland (100 ha) along the river Overijselse Vecht (52° 32’N, 6° 36’E), one of the very few remnant ancient grazed woodlands in Western Europe, grazed by livestock probably since medieval times (Bakker 2003). The site consists of a mosaic of short grazed lawns (dominated by Festuca rubra and Agrostis capillaris), roughs of tall unpalatable plants (dominated by Juncus effusus, Urtica dioica, Deschampsia cespitosa), shrub thickets (dominated by Blackthorn, Prunus spinosa) and woodlands (predominantly Pedunculate oak, Quercus robur). Trees can only successfully recruit within shrub thickets and not directly in roughs, as these are not tall enough to provide tree recruits sufficient protection against large herbivores to reach the ‘safe’ browse line (~ 1.5 m). During the course of this study the site was grazed year-round by 15 Icelandic horses and varying numbers of cattle, ranging from ca. 20 individuals in January to ca. 60 individuals in July (i.e. 0.4 – 0.8 LU (livestock unit = 600 kg body weight) / ha). Other vertebrate herbivores that occur in the study site are European rabbit (Oryctolagus cuniculus), roe deer (Capreolus capreolus), brown hare (Lepus europaeus), common vole (Microtus arvalis), bank vole (Clethrionomys glareolus) and wood mouse (Apodemus sylvaticus).

3.2.2 Sapling survey
In spring 2007 we made a complete inventory of all established nurse shrub saplings (> 1 yr, > 0.2 m high, notably Prunus spinosa) in our study area by searching systematically placed transects of 20 m wide – running south to north – covering the entire study area. All observed individuals were marked and mapped using GPS. Spatially clumped saplings were checked for connecting rhizomes. Saplings within 5m distance from others and those connected with rhizomes were considered as clonal ramets, hence were not considered as new individuals. Of each individual shrub sapling we measured the height (from ground level to top), the canopy diameter (average of two cross sections) and diameter at stem base. The control plots without shrub saplings were selected at random directions and distance (between 5 and 100 m) from the sapling plots. In all sapling and control plots (1 m in diameter) we measured several vegetation and soil characteristics. Vegetation height was measured by lowering down a polystyrene platform attached to a vertical measuring rod on to the canopy (excluding saplings in sapling plots), averaging four measurements per plot. The cover of occurring plant species was estimated using the Braun-Blanquet scale (Braun-Blanquet 1964). The depth of the organic soil layer was determined with a soil core (until reaching the Pleistocene sand), averaging four measurements per plot. Soil samples of the top 15 cm were brought to the lab where soil pH (by adding demineralised water), pH KCL (by adding KCl, i.e. measure of buffer capacity of soil), water content (weight loss after 12 hrs at 100°C) and organic material (weight loss after 12 hrs at 510°C) were determined. These vegetation and soil characteristics were compared between plots with and without Prunus saplings using the non-parametric Kruskall Wallis tests, as the data violated the assumption of equality of variances.

We analysed the vegetation composition with canonical correspondence analysis in CANOCO for Windows 4.5, using the bi-plot function to test whether the species composition associated with the sapling plots differed from the species composition associated with the plots without saplings. We included the species that occurred in at least 5% of the plots (n = 86) to reduce species numbers (80 in total).
3.2.3 Transplantation experiment

We obtained one-year-old *Prunus spinosa* and *Crataegus monogyna* from a tree nursery (Stichting Bronnen Bomen, the Netherlands) at the end of April 2007. Both species are well known as nurse plants when mature because of their thorniness. The saplings had been grown at the nursery from seed material originating from sites very close to our study site and can therefore be considered as autochthonous.

On 9 May 2007 we transplanted 160 saplings according to a randomized complete block design, with 20 blocks of ca. 16 m² widely distributed over the study site. Each block consisted of eight saplings (four of each species), with equal numbers per species transplanted in patches of tall roughs (4) and in short lawns (4). Both vegetation types co-occur scattered over the study site and were equally distributed within blocks. Tall rough patches measured ca. 40 cm in height at the time of planting (max. height ~100 cm at end of growing season) and were dominated by *Juncus effusus* with co-occurring *Deschampsia caespitosa* and *Urtica dioica* (hereafter called ‘rough’). Short lawns measured < 10 cm in height at the time of planting (and over the entire growing season) and were dominated by palatable grasses *Festuca rubra* and *Agrostis capillaris* (hereafter called ‘lawn’). Half of the transplanted saplings per vegetation type were excluded from vertebrate herbivores by a small exclosure that consisted of a cylindrical iron mesh (20 cm diameter, mesh-width 1 cm²) attached to a wooden pole of 1 m high that was firmly placed in the ground. After transplantation, saplings measured 21.0 ± 3.4 cm in height (means ± sd, n = 160) and ranged from 13.0 – 30.0 cm (measured from top to ground level).

Measurements on the saplings were done on day 3, 34, 87, 139, 283, 365, 500 after transplantation. During each visit we recorded presence-absence of the saplings, their height (from ground up to the highest top or leave) and sapling survival. Saplings without a living stem (brownish color under bark) and those removed by cattle were recorded as dead. At the end of the experiment (500 days after planting) we visually estimated the browsing damage on the unfenced saplings (n = 80) by calculation of the percentage of browsed shoots. We used a browsing index (0-5) where 0 indicates 0% browsed shoots, 1 indicates < 5%, 2 indicates 5 – 25%, 3 indicates 25 – 75%, 4 indicates 75 – 95% and 5 indicates > 95% (Smit et al. 2007). Browsing scores were converted to percentage median values for subsequent statistical analysis.

Sapling survival was analysed at the end of the experiment (500 days after planting) with a logistic regression using stepwise backwards criterion (Likelihood Ration) and block, vegetation type (rough or lawn), planted nurse species (*Prunus* or *Crataegus*) and grazing (grazed or fenced) as explanatory variables. Browsing damage of the non-fenced saplings was analysed with a three-way ANOVA, with block, vegetation type and species as explanatory variables. Relative sapling growth (including surviving saplings only) was calculated by subtracting sapling height at t = x from sapling height at t = 0, divided by sapling height at t = x (where x = day 34, 87, 139, 283, 365 or 500 after transplantation). We used repeated measures ANOVA to test for effects of time (within subject effects) and for the effects of block, vegetation type (rough or lawn), planted nurse species (*Prunus* or *Crataegus*) and grazing (grazed or fenced) and their interactions on relative sapling growth, applying Greenhouse-Geisser adjustment of degrees of freedom when the assumption of sphericity was violated (i.e. unequal variances between groups). When time was significant, we repeated this procedure using three-way ANOVA’s on the relative growth values at the 6 sampling dates.
3.3 Results

3.3.1 Sapling survey
We found 43 individual or small groups of Prunus saplings in the entire study area. We also encountered some saplings of the thorny Crataegus monogyna (1) and Rosa canina (2), but their numbers were too low to be further considered in this study. The Prunus saplings were 96.3 ± 51.8 cm high (mean ± sd, range: 26 – 278 cm), with a diameter of 19.2 ± 10.9 mm (mean ± sd, range: 7 – 57 mm) and a canopy width of 56.1 ± 28.8 cm (mean ± sd, range: 15 – 145 cm). The saplings were widely scattered over the area, but all appeared to occur in a particular environment: sapling and control plots differed significantly in vegetation height, soil pH and soil moisture, all being higher in sapling plots (Table 3.1, Kruskall Wallis test). Depth of the organic soil layer and soil organic matter did not differ between sapling and control plots. The vegetation composition was also different between sapling and control plots. The CCA showed that the first axes had an Eigenvalue of 0.293, with a species-environment correlation of 0.679 and a cumulative percentage variance of 28.5%. The canonical axes were significant (F-ratio: 2.752, P = 0.002, Monte Carlo test after 499 permutations). The T-value Biplot diagram shows that sapling plots were significantly associated with rough species such as Urtica dioica, Juncus effusus, Rumex crispus and Carex hirta (underlined and red, left panel, Fig. 3.1). Control plots were significantly associated with palatable grasses and herb species such as Festuca rubra, Agrostis capillaris, Luzula campestris, Cerastrium arvense and Rumex acetosella (underlined and blue, right panel Fig. 3.1).

Table 3.1 Vegetation height, organic soil layer, soil pH (pH demi), buffer capacity (pH KCL), soil moisture and soil organic matter in plots with and without Prunus spinosa saplings (Kruskall-Wallis test).

<table>
<thead>
<tr>
<th>Variables</th>
<th>means ± std.dev (min – max)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veg. height (cm)</td>
<td>Control plots (n=43)</td>
<td>Sapling plots (n=43)</td>
</tr>
<tr>
<td></td>
<td>16.5 ± 13.8 (7.0–68.0)</td>
<td>40.5 ± 20.7 (9.0–73.0)</td>
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<tr>
<td></td>
<td>Organic layer (cm)</td>
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<tr>
<td></td>
<td>37.4 ± 31.0 (8.0–180.0)</td>
<td>30.4 ± 15.7 (8.0–81.0)</td>
</tr>
<tr>
<td>pH demi</td>
<td>4.99 ± 0.49 (4.06–5.85)</td>
<td>5.40 ± 0.60 (4.24–6.37)</td>
</tr>
<tr>
<td>pH KCL</td>
<td>3.91 ± 0.46 (3.18–4.94)</td>
<td>4.42 ± 0.64 (3.45–5.65)</td>
</tr>
<tr>
<td>Soil moisture (%)</td>
<td>24.29 ± 9.32 (9.53–48.94)</td>
<td>27.79 ± 7.07 (8.16–61.82)</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>4.88 ± 1.39 (1.85–7.68)</td>
<td>4.92 ± 1.40 (1.87–8.16)</td>
</tr>
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3.3.2 Transplantation experiment
At the end of the experiment, i.e. after 500 days, 121 saplings (75.6%) of the original 160 were still alive. 25 saplings (15.6%) had died during the course of this study, of which 7 were pulled out by cattle, 2 died due to drowning (flooding event winter 2007-2008), and 16 died due to undefined causes, most likely due to the combined effects of cattle grazing and drought. Fourteen saplings (8.8%) were excluded from the analyses as the exclosures were demolished by cattle.

Logistic regression with block, vegetation type (rough or lawn), planted nurse species (Prunus or Crataegus) and grazing (grazed or fenced) explained 34.7% of the variation (Nagelkerke R square) in sapling survival. Stepwise backwards selection subsequently removed the (non-significant) factors block, grazing x species, vegetation type and
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species (in 5 steps). The remaining best model contained the factors grazing (Wald: 6.635, df = 1, P = 0.01) and the interaction term grazing x vegetation type (Wald: 3.694, df = 1, P = 0.055), together explaining 30.5% of the variation (Nagelkerke R square). Survival was significantly higher for the fenced saplings (65/66 = 98.5%) than for unfenced saplings (56/80 = 70%). Of these 56 surviving unfenced saplings, more saplings were situated in the roughs (32/56 = 57.1%) than in lawns (24/56 = 42.8%).

Results of the ANOVA on the impact of block, vegetation type and species on browsing of the unfenced saplings (n=68) showed that only vegetation type was significant ($F_{1,45} = 23.734; P < 0.001$), with higher browsing values in lawns (46.5% ± 39.6) than in roughs (9.5% ± 20.9) (means ± sd). Block, species and vegetation type x species interaction had no significant effects on sapling browsing ($F_{19, 45} = 1.165; P = 0.327, F_{1, 45} = 0.319; P = 0.575, F_{1, 45} = 0.762; P = 0.387$, respectively).

Results of the repeated measures ANOVA showed that time significantly affected relative sapling growth ($F_{2.5, 241.2} = 19.015, P < 0.001$; Greenhouse-Geisser adjustment applied). Results of the repeated measures ANOVA showed that vegetation type, species and grazing had significant effects on relative sapling growth over the entire study period (Table 3.2). Relative growth was significantly higher for saplings in roughs than in lawns, for Prunus than for Crataegus, and for fenced saplings than for unfenced saplings. Interactions between these factors had no significant effects on relative growth. The significant differences in relative sapling growth between vegetation types, species and grazing treatment appeared already after 34 days. The differences between species remained until 285 days, the differences between grazing until 365 days and differences between roughs and lawns remained until the end of the experiment (500 days) (figure 2).
Table 3.2 Results of repeated measures ANOVA on relative sapling growth over the entire study period of 500 days (A), and results of ANOVA for each sampling date separately (B-G). V = vegetation (lawn or shrubby), S = planted shrub species (Prunus or Crataegus), G = grazing (grazed or fenced). ***P < 0.001; **P < 0.01; *P < 0.05.
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3.4 Discussion

3.4.1 Sapling survey

The sapling survey shows that the environmental conditions in sapling plots differed significantly from control plots, expressed by a higher soil pH, soil moisture and taller vegetation in the former, while sapling plots contained typical unpalatable rough species and the control plots mostly palatable grasses. These findings strongly suggest that these environments – the tall unpalatable roughs – form suitable establishment niches for *Prunus* saplings, which is in line with our first expectation and predictions from Olff et al (1999). The relative scarcity of *Crataegus monogyna* and *Rosa canina* saplings may have to do with the low number of parent plants in the direct vicinity (pers. obs. CS) suggesting that source limitation could play a role for these species.

![Fig. 3.2](image-url) Relative sapling growth for *Crataegus* and *Prunus* (spec) in lawns and roughs (veg), with and without exclosure (graz) at (A) t = 283, (B) 365 and (C) 500 days. Bars represent means ± 1se.
The diameter frequency distribution of the observed Prunus saplings suggests the existence of a recruitment cohort in the study area. According to the growth-model for individual Prunus ramets by Annema (1997) - that combines measurements on stem diameters and number of growth rings of Prunus saplings to estimate ramet age - the majority of our saplings (83%) established within the last 15 years, hence since 1995. While most environmental conditions remained relatively constant over this period (cattle grazing density, flooding events, precipitation), this recruitment cohort coincides with a strong decline of rabbits in the study area in the early nineties and remaining very low densities until present time (Scheper and Smit 2011; Gleichman unpubl.), most likely due to the arrival and persistence of rabbit hemorrhagic disease (van de Bildt et al. 2006). This is in line with Smit et al (2010) and Bakker et al (2004) who show that rabbits play a crucial role in controlling the clonal expansion of established Prunus spinosa thickets into adjacent grassland. Our data suggest similar strong impact of rabbits on the establishment of new Prunus recruits in the landscape. We acknowledge that the genuine absence or low density of rabbits does not allow drawing strong conclusions about their impact, but we can at least conclude what the realised niches are for Prunus establishment under very low rabbit densities. The fact that Prunus recruitment has been virtually absent during a preceding period with high rabbit densities suggests however that the unpalatable roughs may lose their effectiveness as establishment niche with increasing rabbit densities. Bakker et al. (2004) already remarked that associational resistance or associational avoidance – i.e. reduced herbivory due to a spatial association with a protected neighbor (Milchunas and Noy-Meir 2002; Bakker et al. 2004) – works well when the main herbivore is a large bulk feeder (e.g. cattle) but works poorly when the main herbivore is a small selective feeder (e.g. rabbits). So, we may expect that the tall unpalatable roughs will lose their effectiveness as establishment niche for Prunus saplings in the future when rabbit populations recover from the rabbit hemorrhagic disease.

3.4.2 Transplantation experiment
In line with our second expectation, the performance of the saplings of thorny shrubs was indeed best when protected against herbivores and when growing in tall unpalatable roughs. This indicates that a combination of biotic and abiotic conditions interact in forming the establishment niche for saplings of thorny shrubs. Protection against herbivores via exclosures significantly increased both the survival and relative sapling height. The development of thorns had only just started, so the saplings were still vulnerable to grazing, as found by others (Rackham 1980). Indeed, unfenced saplings were repeatedly browsed or even pulled out, leading to a lower survival and height during the course of this experiment, except for the roughs at the end (t=500). Frequent browsing often leads to death at the early life stages of woody species (Gill 2006), even when they adapted to browsing as mature plants. Indeed, cattle grazing appeared the main cause of death for the shrub saplings. The majority of the dead saplings were unfenced (24/25 = 96%) and most of these were severely browsed (up to 95%) or even completely pulled out. Nevertheless, the overall mortality of our shrub saplings was relatively low (15.6%) as compared to other studies using tree seedlings in similar systems with low-intensity cattle (e.g. 35.8% - 89.4%, Smit et al. 2006; Van Uytvanck et al. 2008b), which may reflect the initiation of herbivore resistance of both species. We expect that the overall mortality rates would have been higher if we had used younger seedlings (< 1yr) instead of 1-year-old saplings, but that the fencing effects would have been stronger due to the higher vulnerability of the seedlings.
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Saplings performed better inside roughs than in lawns, both in terms of sapling survival and in relative height growth, and this effect was consistent over the entire study period. Associational resistance is likely to be responsible for this improved performance. The roughs consisted of relatively unpalatable species such as *Juncus effusus*, *Urtica dioica* and *Deschampsia cespitosa*, all generally avoided by cattle. Indeed, the browsing values of the unfenced saplings were lower in the roughs than in the lawns. Hence, it appears that the saplings in the roughs profited from the unpalatability of their environment. However, sapling performance was also higher in roughs than in lawns within exclosures (until $t=500$), which reflects that besides associational resistance, other factors must play a role. This has probably to do with the differences in the micro-environmental conditions between roughs and lawns as described in the sapling survey: the taller vegetation, higher soil moisture and a higher soil pH in the roughs seem to favor the performance of the shrub saplings. Additionally, the shrub saplings may have profited from growing with *Juncus effusus*, the dominant rough species that is able to release oxygen in the rhizosphere (Visser and Bögemann 2006), but such facilitative mechanisms of the rough species should be tested in more detail.

*Prunus* and *Crataegus* responded similarly to the grazing and vegetation treatments in terms of sapling survival. Yet, the relative growth was higher for *Prunus* than for *Crataegus* until 283 days after transplantation. These initial differences cannot be attributed to cattle preference or differences in thorniness, as the browsing values did not differ between the two species. As the difference in initial growth between the species was consistent in both roughs and lawns, we can not attribute this to different preferences of the two shrub species in terms of soil characteristics. A more likely explanation for the differences in initial growth is the difference in shade tolerance, with *Crataegus* being more shade tolerant than *Prunus* (Grubb et al. 1996). Shade tolerant species generally express lower growth rates, particularly under light conditions, than shade intolerant species (Kobe et al. 1995).

### 3.4.3 Establishment of thorny shrubs in grazed woodlands

Our study shows that establishment of nurse shrubs in grazed woodlands relies on tall unpalatable roughs, which form suitable establishment niches when seeds arrive. This arrival of seeds in these roughs mainly occurs via primary dispersal by birds, notably by *Turdus* species. Here, seeds are relatively safe from predation by rodents, while seeds dispersed by nearby parent shrubs were readily predated by wood mice (Scheper and Smit 2011). Our study suggests that the subsequent survival of these seeds into saplings is increased in these roughs due to combined protection against large herbivores and suitable growing conditions. After 2-3 years the saplings become thornier (Rackham 1980) and will no longer need the protection of the surrounding vegetation. This process was already happening at the end of our study: the difference in growth between fenced and unfenced saplings started to disappear (Fig. 3.2C) suggesting the start of their ‘independence’. The saplings will then further expand in height and grow out of the protective zone of the roughs (>100 cm). In their turn, they will start to facilitate palatable tree seedlings of oak and ash as observed in Bakker et al. (2004). We already observed the first establishments of *Sambucus nigra* and *Quercus robur* within the protective canopy of a larger *Prunus* sapling in our survey, suggesting that this process occurs readily after shrub establishment. Successful tree recruitment directly in roughs, hence without the intervention of nurse shrubs, is generally not possible due to repeated browsing on the tree saplings once they grow out of the protective zone of the roughs.
Establishment niches for thorny shrubs, the tall unpalatable roughs, are generally relatively common at low-intensity grazed sites. Their occurrence in the landscape is probably related to selective grazing behaviour of large herbivores on top of existing small-scale heterogeneity in topography (hollows-hummocks), soil variability and nutrient availability (Olff and Ritchie 1998; Cromsigt et al. 2009). When a patch is not grazed for a short period, the proportion of fibred tissues such as flowering stems will increase which reduces the quality of the forage (high C/N ratio). Such a patch may continue to be avoided by large herbivores and become a larger rough patch, the size and abundance depending on the grazing intensity. As these roughs are generally not the ‘target’ of managers of nature areas, who rather focus on (the recovery of) rarer plant species or vegetation types, there is a tendency to remove these roughs via additional mowing or higher grazing intensities. This however has serious consequences for the dynamics of the ancient grazed woodlands, as roughs are essential establishment niches for new nurse shrubs that are in their turn required for tree recruitment. Eventually, the absence of establishment niches will lead to arrested succession, causing a conversion of the typical savanna-landscape to plain pastures, and a loss of the associated biodiversity. We therefore propose that management of ancient grazed woodlands should consider allowing the occurrence of ‘undesired’ roughs for increased dynamics, heterogeneity and diversity in these systems. A constant relatively low grazing intensity (50-100 grazing days/ha) is probably the best management for maintenance of this landscape (Van Uytvanck 2009).

While our study focused on one particular ecosystem, grazed temperate woodlands, we believe that our findings may have important implications for other ecosystems where nurse plants play a key role. For example, particularly arid ecosystems are presently suffering from degradation and desertification due to intense land-use and climate changes (Millennium Ecosystem Assessment, 2005). Nurse plants are increasingly used to restore these degraded ecosystems (Castro et al. 2002; Gómez-Aparicio et al. 2004) and may play a key role in preventing further degradation (Kefi et al. 2007). However, experimental evidence is thus far scarce and new studies are needed to improve our understanding of the mechanisms. We believe that these studies should focus on the recruitment and establishment of nurse plants and their constraining factors in these degraded systems to allow the prevention of further degradation, and for successful conservation and restoration efforts.

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Herbivore-mediated structural diversity of vegetation