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Case Report

Ungulate Impact on Natural Regeneration in Spruce-Beech-Fir Stands in Černý důl Nature Reserve in the Orlické Hory Mountains, Case Study from Central Sudetes

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Abstract: The paper presents the results of a study on tree regeneration of forest stands in the Černý důl Nature Reserve, which is situated in the Orlické hory Mountains Protected Landscape area in the Czech Republic. Research was conducted in a spruce-beech stand with an admixture of silver fir, sycamore maple and rowan on two comparative permanent research plots (PRPs) (PRP 1—fenced enclosure and PRP 2—unfenced). Typological, soil, phytosociological and stand characteristics of the two PRPs are similar. The results showed that ungulate browsing is a limiting factor for successful development of natural regeneration of autochthonous tree species. The population of tree species of natural regeneration on the fenced plot (PRP 1) is sufficient in relation to the site and stand conditions. However, natural regeneration on PRP 2 is considerably limited by browsing. Damage is greatest to fir, sycamore maple and rowan; less severe to beech; and the least to spruce.

Keywords: browsing damage; natural mixed forest; silver fir (*Abies alba* Mill.); European beech (*Fagus sylvatica* L.); Norway spruce [*Picea abies* (L.) Karst.]; admixed tree species

1. Introduction

Autochthonous mixed forests have a high capacity for autoregulation [1–3] and are often considered as model subjects for research on ecological stability and sustainability [4]. Nevertheless, these forest ecosystems are vulnerable to environmental disturbances [5–7], such as wind throw, snow break, water stress and biotic agents, among which ungulate herbivory seems to be one of the most important factors [8,9]. Ungulate browsing can strongly influence forest stand dynamics by compromising the regeneration process in both natural and managed forests [8]. Mountain and submontane forests with overpopulation of deer species are more susceptible to damage than forests at lower elevations [10]. This difference is also the result of the longer regeneration period and slower initial growth of typically shade-tolerant tree species, such as Norway spruce [*Picea abies* (L.) Karst.], European beech (*Fagus sylvatica* L.), and especially silver fir (*Abies alba* Mill.), which have slower tree response after browsing and hence lower growth performance compared to other species [11]. Mountainous areas in the Czech Republic are characterized by high numbers of red deer (*Cervus elaphus* L.) and a higher incidence of deer browsing and bark stripping than elsewhere in the country [12]. Despite this fact there is a lack of long-term field studies and thus the effects of ungulates on forest stands are still relatively unknown.

The approaches to managing forests and wildlife in protected areas should always be based on ecological principles with the long-term goal of ecosystem protection [13,14], but also must acknowledge the carrying capacity of the environment. Over the long term, most protected forests will be left to develop without direct human intervention [15]. Nevertheless, ecological criteria based on present and future autoregulation capacity should be properly selected for these forests [2]. For this purpose, a network of representative forest reserves was selected in the Czech Republic, and appropriate management principles were formulated based on long-term studies [16,17]. Many authors recommend active management during the transition period before the forest is left to be regulated by natural processes [18], which should shorten the time necessary to reach the target state [19–21]. These recommendations are often restricted to silvicultural operations, such as thinning, selection harvest or enrichment planting, while the impact of ungulates is often neglected or underestimated.

The dispersal of many plants, and hence their presence at particular sites, is often related to the activity of large mammals [22]. Thus, plant interactions with biotic vectors of dispersal are highly important for the species composition of plant communities. In contrast, the overpopulation of ungulates, principally deer, was repeatedly found to be a factor suppressing the diversity of understory forest flora and regeneration of the woody plant component of forest ecosystems [8–10,23,24]. Suppression of tree species caused by ungulates in spruce-fir-beech stands—particularly the reduction to elimination of mainly silver fir, sycamore maple and rowan (*Sorbus aucuparia* L.) and to a lesser extent European beech—has been confirmed by many authors [8,25–27]. Ungulates can be the most important driver of meta-community structure in mesic systems without strong abiotic influences in warm temperate forests [28]. An analysis of the global level of browsing revealed that seedlings on south-facing slopes were browsed more intensively than those on slopes with other exposures [29]. Similarly, Cagnacci *et al.* [30] showed that probability of roe deer (*Capreolus capreolus* L.) presence decreased nonlinearly with elevation because the thicker snow cover and lower temperatures in spring reduced ungulate movement and the duration of the period when seedlings are available. Over the long term, game browsing has a considerable impact not only on species composition but also on age and spatial structure of stands [8]. On the other hand, ungulate browsing may help to form some herb layer of forests in the present landscape [28].

Mixed stands of beech, fir and spruce in the area of Orlické Mountains (Central Sudetes) are characterized by a long (350–400 years) cycle of autogenous development [31] with discrete and often rare regeneration events of particular tree species. Regeneration almost exclusively takes place in the shelter of the parent stand with limited canopy openings; coniferous species regenerate in small groups, while beech regeneration tends to cover wider areas and outcompete admixed tree species, such as silver fir and sycamore maple (*Acer pseudoplatanus* L.) [27,32]. Silver fir seems to be the most heavily browsed of the commercially important tree species [9,10,33] in this area, as in other mountain regions.

Almost all fir trees in the Černý důl Nature Reserve belong to large diameter classes, and after subsequent dieback of these trees, new mature trees do not appear. Therefore, the proportion of fir in these stands is further decreasing. In beech-fir forests throughout the Carpathians Vrška *et al.* [34] documented a decreasing share of silver fir in these vast forest reserves. Klopcic *et al.* [35] described the gradual aging of the fir population in Dinaric Mountains along with the insufficient establishment of young fir trees under the stress of game browsing in mixed stands in Slovenia.

To evaluate some of these influence, this study was intended to quantify the impact of ungulates on regeneration of fir, beech, spruce, rowan and sycamore maple and on the height and spatial structure of natural regeneration; and to evaluate the role of ungulates in the shift of tree species composition towards the dominance of beech in the Černý důl Nature Reserve. This conservation area is one of the most valuable natural forest remnants in the Central Sudetes.

2. Materials and Method

2.1. Area Description

The study was conducted on the two permanent research plots (PRPs) in the Černý důl Nature Reserve in the southeastern part of the Orlické Mountains (Czech Republic), ca. 2 km southwest of the border with Poland. The reserve contains 26.37 ha situated at between 740 and 884 m a.s.l. Average annual temperature is 5 °C and annual precipitation is approximately 1200 mm (630 mm during the growing season). The geological bedrock is built of migmatites and orthogneisses of the Gieraltov and Sněžník type. Soil conditions on the crystalline bedrock are fairly heterogeneous. The soils are generally poor in mineral nutrients. Acid modal Cambisols are dominant, Cryptopodzols occur locally and Gleysols to Histosols are found around spring areas. According to local managers, estimated game stocks in Černý důl are as follows: 32 red deer, 77 roe deer and 16 wild boar (*Sus scrofa* L.); however, real game stock numbers are mostly higher: 51 red deer, 45 roe deer and 43 wild boar per 1000 ha.

The main objective for protection of this reserve (declared in 1954) has been to conserve natural remnants of spruce-beech-fir stands at lower ridge locations of the Orlické Mountains. Natural spruce-fir-beech populations occur in the reserve on 32.7% of its area, yet the most valuable stands of the core zone of the reserve occupy only 17.7% of the total reserve area. The core zone consists of herb-rich to acid beech stands and fir-beech stands belonging to the sub-alliance *Acerenion*, *Eu-Fagenion* and the alliance *Luzulo-Fagion* [36]. The remaining approximately two-thirds of the reserve are composed of secondary spruce stands with sporadically admixed beech, sycamore maple, rowan and fir (the alliance *Piceion excelsae*). A rich riparian vegetation community belonging to the alliance *Cardamino-Montion* is found in spring valleys [37].

The location of the PRPs is shown in Figure 1; Table 1 gives the present basic characteristics of each plot. PRP 1 is situated on a moderate slope of southeastern exposure in a game-proof fenced enclosure established in 1985. Prior to installing the fence, local managers considered the regeneration throughout the forest to be insufficient. According to the Czech typological system it is forest type 6S—fresh spruce-beech forest (*Piceeto-Fagetum oligomesotrophicum*), and according to the Zurich-Montpellier School, it is the plant association *Dentario enneaphylli-Fagetum* Oberdorfer ex W. and A. Matuszkiewicz 1960. The soil type is acid mesotrophic modal Cambisol. PRP 2 is located in the same forest and has similar site and stand conditions, but it is outside the game-proof fenced enclosure.

Figure 1. Location of autochthonous mixed stands on permanent research plots (PRPs) 1 and 2 in Černý důl Nature Reserve (PRP 1: 50°12′05″ N, 16°31′10″ E; PRP 2: 50°12′03″ N, 16°31′14″ E).

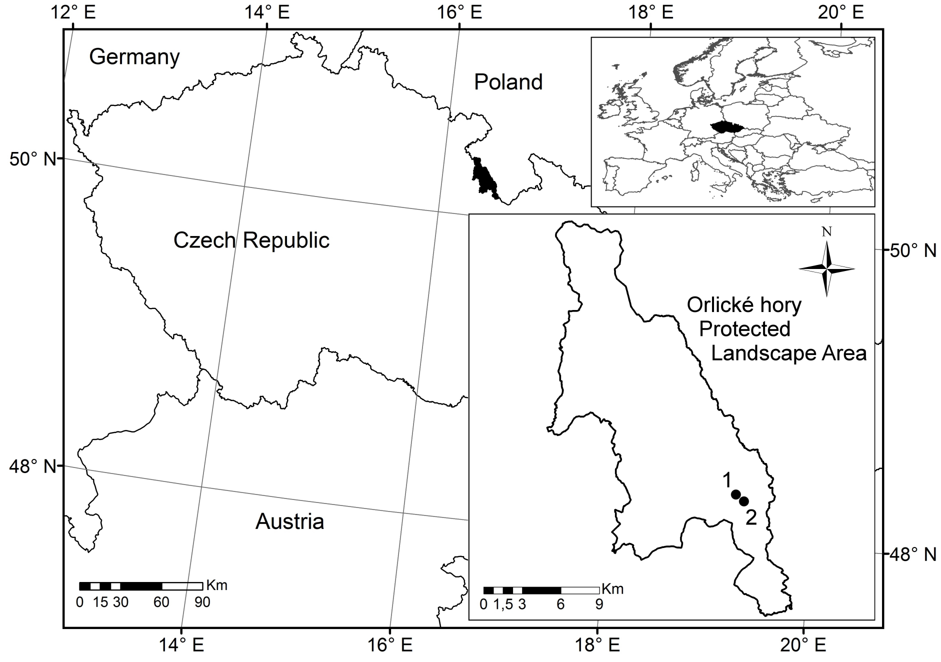


Table 1. Present characteristics of PRP 1 and PRP 2.

Plot number	Plot name	Tree species	Height (m)	Diameter (cm)	Stand volume ($m^3.ha^{-1}$)	Stand basal area ($m^2.ha^{-1}$)	Age of tree layer			Canopy		Altitude (m)	Exposure	Slope (°)	Forest site type
							Lower	Middle	Upper	Mensurational	Biological				
1	Černý důl fenced	beech spruce fir	28 32 33	47 59 65	181 220 78	16.8 21.8 7.0	53	99	185	0.98	1.87	835	SE	13	6S
2	Černý důl unfenced	Beech spruce fir	31 38 38	53 71 72	299 206 50	22.7 18.0 3.4	53	99	185	0.97	1.88	820	SE	11	6S

Notes: Age of tree layer is age of particular tree layers related to the whole forest stand. Height is the mean total height (95% quantile). Canopy description is in Data analysis section. 6S is fresh spruce-beech forest (Picceto-Fagetum oligomesotriphicum) [38].

2.2. Data Collection

FieldMap (IFER-Monitoring and Mapping Solutions Ltd.) was used to establish a 50 × 50 m plot (0.25 ha) in each of the two PRPs to determine the structure of tree layers and composition of natural regeneration of trees. The position of all trees in the understory, midstory, and canopy and in the natural regeneration (recruits with breast-height diameter overbark ≤7 cm) was mapped by this system. Height, height to the base of the live crown, and crown width of the recruits were measured with a hypsometer. In the tree layer, the green crown values and crown projection areas were also measured at ≥4 points along the crown perimeter.

In all recruits, browsing of the terminal apex, lateral browsing, and repeated browsing were investigated by tree species on PRP 2 (unfenced plot). Samples of Norway spruce and European beech in juvenile stages were taken from within and outside of the game-proof fenced enclosure for stem analyses (*i.e.*, five samples of each species at each location). Age and height growth (in 5 cm increments) were determined by counting tree rings at a particular stem height.

2.3. Data Analysis

Horizontal and vertical structure was evaluated in all recruits on the plots. Hopkins-Skellam index [39], Pielou-Mountford index [40,41], Clark-Evans index [42] and Ripley's *K*-function [43] were computed, which represent the mode of recruit distribution on the stand area. The David-Moore index [44] was used to determine distribution based on tree frequency in quadrats. Each PRP contains 25 10 × 10 m quadrats. Horizontal structure of the tree layer and natural regeneration of beech are related to the year 2014.

To calculate characteristics describing the horizontal structure of trees across the plot, the PointPro 2.1 programme was used. The test of the significance of deviations from the values expected for random distribution of points was performed by Monte Carlo simulation. The mean values of the *K*-function were estimated as arithmetic means from *K*-functions computed for 1999 randomly generated point structures. A list of indices of horizontal structure is given in Table 2.

Table 2. Overview of the indices describing the horizontal structure and their common interpretation.

Index	Mean value	Aggregation	Regularity
Hopkins-Skellam	$A = 0.5$	$A > 0.5$	$A < 0.5$
Pielou-Mountford	$\alpha = 1$	$\alpha > 1$	$\alpha < 1$
David-Moore	ICS = 0	ICS > 0	ICS < 0
Clark-Evans	$R = 1$	$R < 1$	$R > 1$

Species diversity was analysed in the framework of species richness [45,46], species heterogeneity [47,48] and species evenness [49,50]. The values of the indices range from 0–1, where 0 denotes minimum species diversity and 1 denoted maximum diversity. Further, biological canopy (sum of crown projections per hectare) and mensurational canopy (crown closure; proportion of a stand covered by the crowns) were calculated.

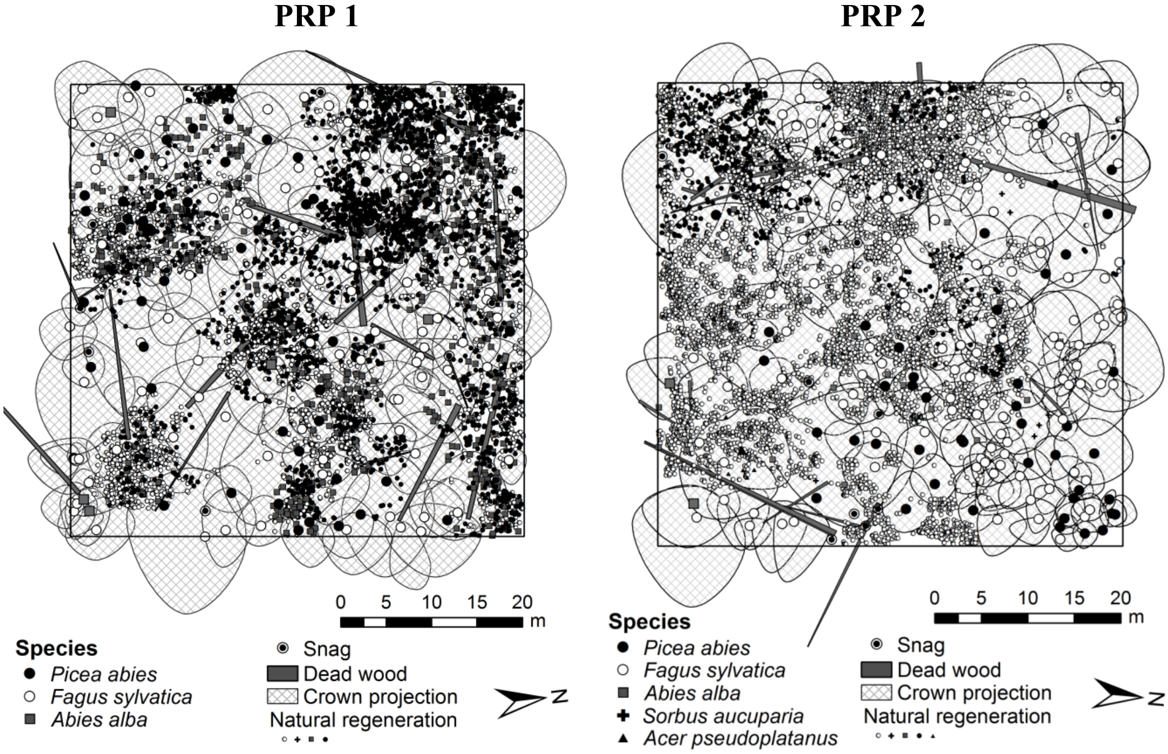
Statistical analyses were processed in the Statistica 12 software. Data were log transformed to acquire normal distribution (tested by Shapiro-Wilk test). Differences in frequencies of height classes, mean height of natural regeneration recruits for each tree species, species diversity and height of recruits not damaged and damaged by browsing between the two PRPs were separately tested by one-way analysis of variance (ANOVA). Significance level was 0.05.

3. Results

3.1. Structure of Natural Regeneration

On the fenced plot (PRP 1), the numbers of recruits per hectare are as follows: 28,680 beech trees, 9412 spruces, 2236 firs and 92 rowans, *i.e.*, 40,420 recruits in total. The horizontal structure of natural regeneration and tree layer on PRP 1 is shown in Figure 2. Beech, spruce and fir recruits are aggregated mostly in distinct and large groups (Figures 2 and 4, Table 3). Rowans are interspersed as single trees (Figure 2, Table 3). On the unfenced plot (PRP 2) the numbers of recruits per hectare are as follows: 30,872 beech trees, 2988 spruces, 104 firs, 52 rowans and 24 sycamore maples*, i.e.* 34,040 trees in total. Beech and spruce recruits are concentrated mostly in groups (Figures 2 and 4, Table 3). Fir and rowan occur as single trees and only very sporadically (Figure 2, Table 3).

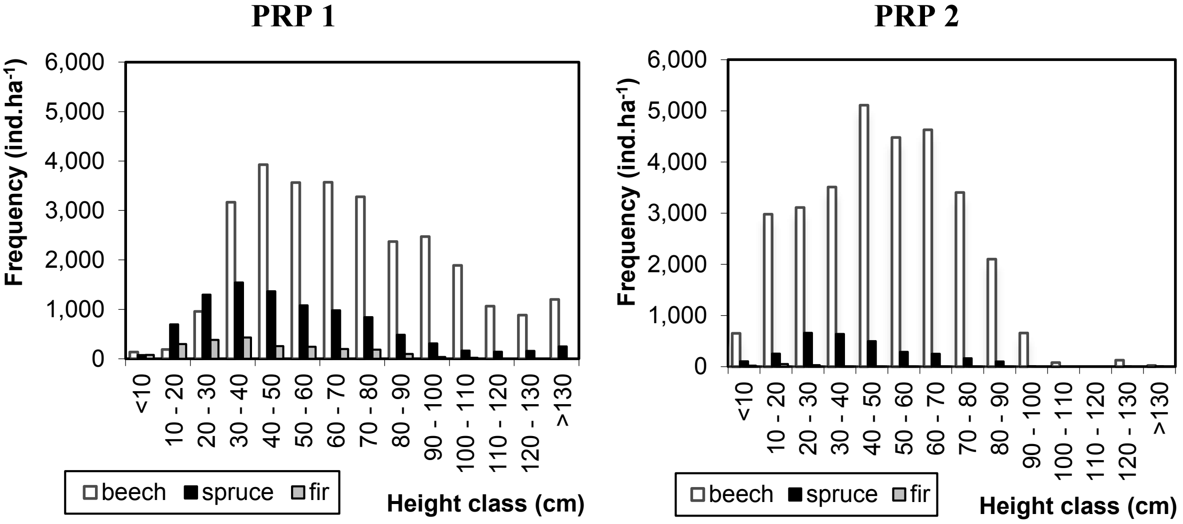
Figure 2. Horizontal structure of the forest stand on PRP 1 (fenced) and PRP 2 (unfenced).



In the representation of recruits by height classes (Figure 3), the frequency of individuals on PRP 1 by height class is only slightly right skewed, approaching the shape of the Gauss curve. This pattern indicates less height differentiation among regeneration in lower size classes compared with PRP 2. Recruits in the height classes of 30–80 cm prevail (4472–5404 trees ha^{-1}). In the other height classes the number of natural seedlings and advance growth recruits is lower. On PRP 2 the height structure of recruits is markedly right skewed. Recruits in the height class of 40–50 cm prevail (4472 trees.ha^{-1}).

Comparing height structure, the mean frequency of all recruits in height classes is similar for both plots ($F_{(1, 30)} = 0.3$, $P > 0.05$), significantly higher for fir ($F_{(1, 30)} = 12.8$, $P < 0.001$) and spruce on PRP 1 ($F_{(1, 30)} = 7.8$, $P < 0.05$) and similar for beech ($F_{(1, 30)} = 0.1$, $P > 0.05$). Comparing height structure of natural regeneration, the mean frequency of all recruits already in height classes >70 cm is significantly higher on PRP 1 ($F_{(1, 16)} = 4.6$, $P < 0.05$).

Figure 3. Histogram of the height structure of recruits on Černý důl PRP 1 (fenced) and PRP 2 (unfenced) as converted per 1 ha.



According to all computed structural indices and Ripley's *K*-function on PRP 1, the recruits are distributed across the plot in a distinctly aggregated manner (Table 3, Figure 4). A more detailed analysis of the horizontal structure by tree species indicates that the distinct formation of spruce, beech and fir groups is the most frequent while rowan is the least frequently grouped. The horizontal structure of rowan is random at a recruit spacing from 5–9 m; at the other distances, it is aggregated ("clumpy"). The *K*-function suggests that this tree species is interspersed as single trees and the aggregation of recruits is substantially smaller than on PRP 2. Furthermore, on the unfenced plot, all computed structural indices indicate the distinctly aggregated distribution of recruits across the plot (Table 3). The aggregated distribution of recruits and/or random distribution of fir (from the spacing of 2 m) according to their distance are expressed by Ripley's *K*-function (Figure 4). Groups of spruce and beech are formed the most frequently and groups of fir the least frequently. Fir distribution is moderately clumpy according to the Clark-Evans index, but according to the other three indices it is fully random, which documents single and sparse occurrence. The spatial pattern of rowan is aggregated according to structural indices, but rather random from the spacing >3 m according to the *K*-function.

Table 3. Indices describing the horizontal structure of mixed spruce, fir and beech stands on PRPs 1 and 2.

Index	Beech	Spruce	Fir	Rowan	all regeneration
			Fenced PRP 1		
Hopkins-Skellam	0.980 *	0.952 *	0.922 *	0.788 *	0.841 *
Pielou-Mountford	15.060 *	11.566 *	6.595 *	2.316 *	10.992 *
David-Moore	28.400 *	7.313 *	1.871 *	−0.042	13.236 *
Clark-Evans	0.572 *	0.694 *	0.702 *	0.569 *	0.742 *
			Unfenced PRP 2		
Hopkins-Skellam	0.937 *	0.956 *	0.651	0.882 *	0.892 *
Pielou-Mountford	31.041 *	11.132 *	1.401	2.936 *	14.132 *
David-Moore	17.417 *	7.983 *	0.063	1.744 *	12.192 *
Clark-Evans	0.568 *	0.536 *	0.845 *	0.281 *	0.692 *

* Statistically significant.

Figure 4. Horizontal structure of the natural regeneration of mixed spruce, fir and beech stand on PRPs 1 and 2, expressed by the *K*-function; the black line represents the *K*-function for real distances of trees on the PRP; the bold blue line represents the mean course for random spatial distribution of trees and the two thinner central curves represent 95% interval of reliability; when the black line of tree distribution on the PRP is below this interval, it indicates a tendency of trees toward regular distribution, and if it is above this interval, it shows a tendency toward aggregation.

Fenced PRP 1 **Unfenced PRP 2**

Beech

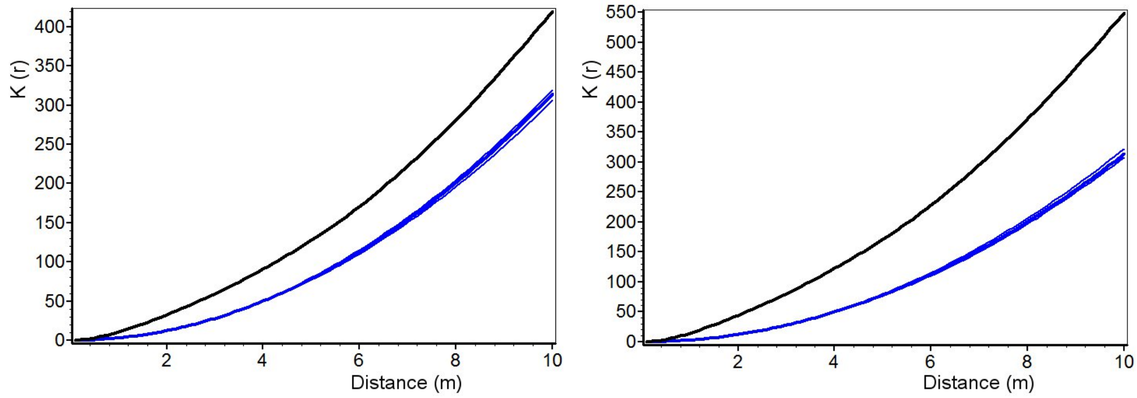
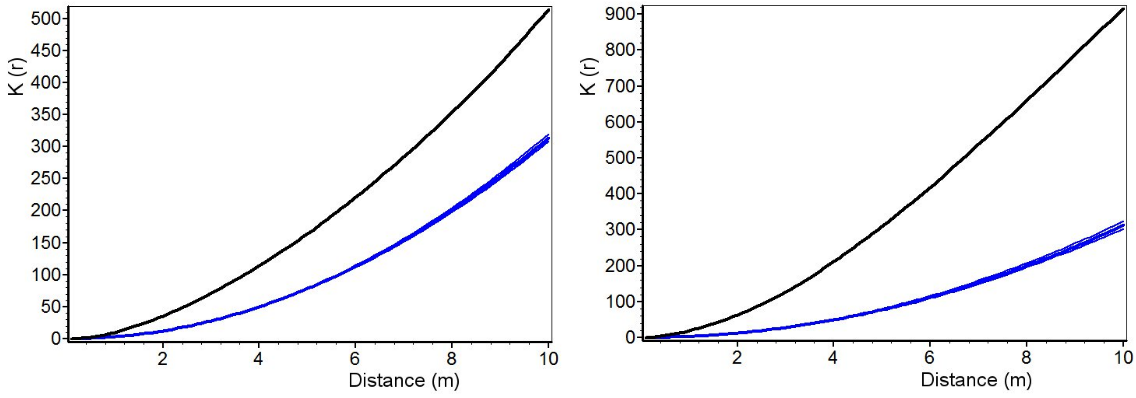
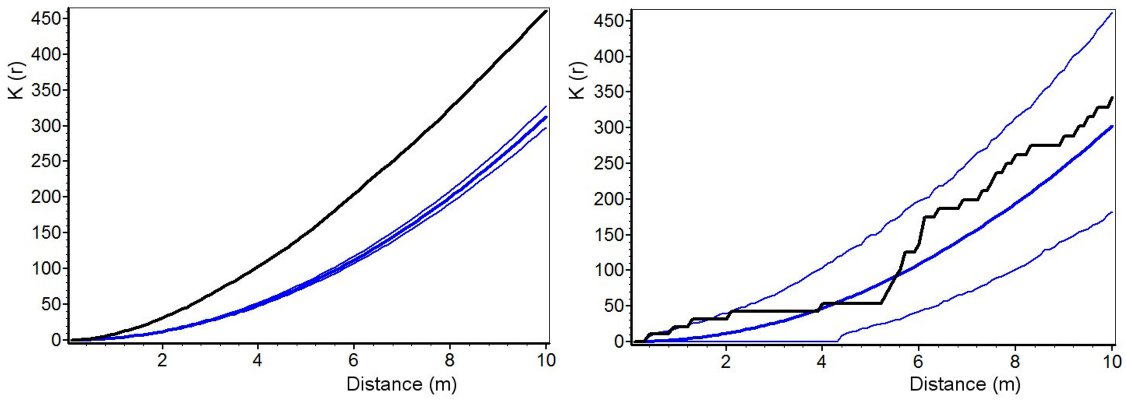


Figure 4. *Cont.*

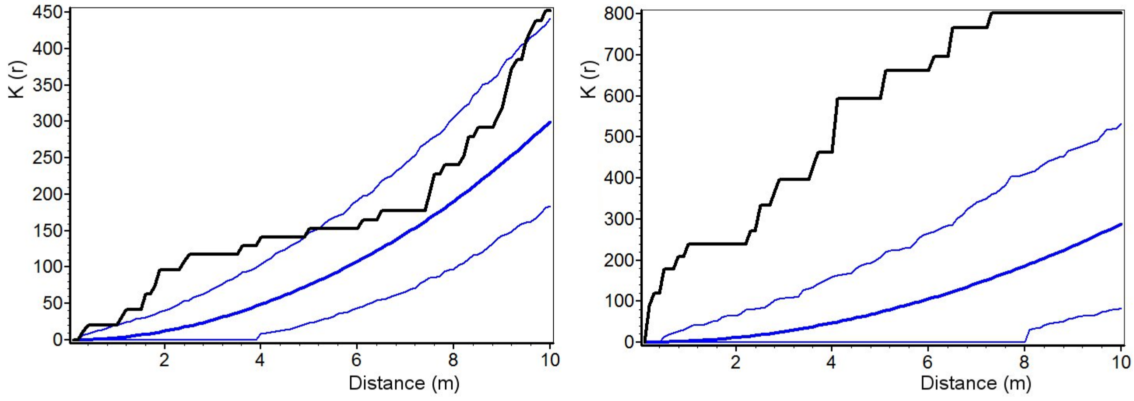
Spruce



Fir



Rowan



3.2. Species Diversity

Species heterogeneity indices indicate medium to high species diversity on PRP 1, and comparatively poor diversity on PRP 2 (Table 4). Similarly, indices of species evenness are often twice as high for PRP 1 as for PRP. Comparing PRPs, indices of tree species heterogeneity and evenness were significantly higher for the fenced plot than for the unfenced plot ($F_{(1,\ 6)} = 12.6$, $P < 0.05$). Indices of relatively poor species richness are similar for both PRPs.

Table 4. Indices describing the species biodiversity of natural regeneration.

PRP	species richness			species heterogeneity		species evenness	
	m	D_1 (*Mai*)	D_2 (*Mei*)	λ (*Sii*)	H' (*Shi*)	E_1 (*Pii*)	E_2 (*Hii*)
1 (fenced)	4	0.283	0.020	0.439	0.756	0.545	0.882
2 (unfenced)	5	0.287	0.021	0.169	0.328	0.237	0.349

Notes: m = species number, D_1 (*Mai*) = Margalef's index, D_2 (*Mei*) = Menhinick's index, λ (*Sii*) = Simpson's index, H' (*Shi*) = Shannon's index, E_1 (*Pii*) = Pielou's index, E_2 (*Hii*) = Hill's index.

3.3. Impact of Ungulates on Natural Regeneration

A comparison of recruit heights of the particular species show a significant difference between the two PRPs ($\mathrm{F}_{(1,\ 18,606)} = 1609$, $P < 0.001$). The difference was most evident in beech ($\mathrm{F}_{(1,\ 14,884)} = 2437$, $P < 0.001$), rowan ($\mathrm{F}_{(1,\ 32)} = 50.1$, $P < 0.001$) and fir ($\mathrm{F}_{(1,\ 581)} = 33.2$, $P < 0.001$) (Figure 5). The mean height of fir is 42.4 cm (± 1.2 S.E.) on PRP 1 and 15.8 cm (± 5.4 S.E.) on PRP 2, where damage to the terminal apex by browsing is observed in 82% of all trees. In recruits taller than 15 cm, the ratio of browsing amounts to 100%. In rowan, the mean height in PRP 2 is 12.6 cm (± 7.7 S.E.; 84% damage to the terminal apex) and in PRP 1, 60.4 cm (± 5.7 S.E.). The smallest difference in heights is recorded in spruce with a difference of 7.9 cm (53.3 cm ± 1.1 S.E. on PRP 1; 45.8 cm ± 1.4 S.E. on PRP 2).

Table 5 summarizes damage caused by ungulate browsing to natural regeneration broken down by tree species on PRP 2. The greatest damage is caused to rowan (browsing of the terminal apex in 85% of trees and of lateral shoots in 60% of trees) and fir (browsing of the terminal apex in 82% and of lateral shoots in 78% of trees). Beech is damaged to a smaller extent (browsing of the terminal apex in 65% and of lateral shoots in 45% of trees) and the smallest damage was observed in spruce (browsing of the terminal apex in 22% and of lateral shoots in 14% of trees). Natural regeneration of sycamore maple is completely browsed by brown hare (*Lepus europaeus* Pallas) every year. In 2013, in the stage of seedlings with cotyledons, there were 3328 trees per hectare on PRP 2 but at the end of the growing season only 27 trees. Hence sycamore maple regeneration is markedly suppressed before the lignification of seedling stems.

Figure 5. Mean height of recruits of particular tree species on PRPs 1 and 2 with 0.95 reliability interval.

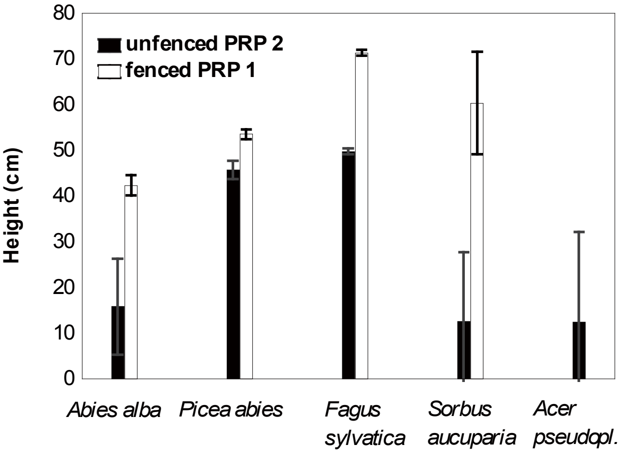


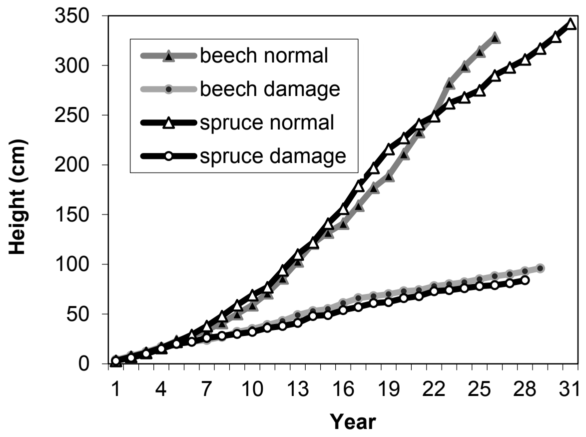
Table 5. Browsing to natural regeneration on unfenced PRP 2 broken down by tree species.

Type of shoot browsing		Beech		Spruce		Fir		Rowan	
		ind. ha^{-1}	%	ind. ha^{-1}	%	ind. ha^{-1}	%	ind. ha^{-1}	%
Terminal	1 time	8,372	27.1	356	11.9	22	21.2	8	15.4
	2–4 times	6,737	21.8	217	7.3	26	25.0	17	32.7
	5+ times	4,832	15.7	96	3.2	37	35.6	19	36.5
	total	19,941	64.6	669	22.4	85	81.7	44	84.6
Lateral		1,945	45.2	416	13.9	81	77.9	31	59.6

Notes: Browsing on sycamore maple for small number of individuals not included; ind. = individuals.

Comparing samples of height growth, the mean height of full growth is significantly higher than reduced growth caused by browsing for both main tree species: beech ($F_{(1, 220)} = 68.4$, $P < 0.001$) and spruce ($F_{(1, 236)} = 77.8$, $P < 0.001$). Figure 6 shows that in the initial stage (by 6 years of age, $F_{(1, 14)} = 8.5$, $P < 0.05$; or by 8 years of age $F_{(1, 14)} = 37.7$, $P < 0.001$) the studied tree species are scarcely damaged by ungulates. However, when they exceed the height of the herb layer, they are periodically browsed for many years; only rarely do they grow up to avoid the impact of game. For example, a beech recruit at this site is about 328 cm (± 15.4 S.E) tall on average at 26 years of age if beyond the reach of game, but under heavy browsing by game it is only 88 cm (± 8.2 S.E.) tall (*i.e.*, 27% of the former height). The situation for spruce is similar. With current height growth its height is 306 cm (± 13.8 S.E) at 28 years of age, but under heavy browsing it is only 84 cm (± 7.7 S.E; *i.e.*, 27% of the former height). A much worse situation is observed in fir, rowan and sycamore maple. Because of their limited number, these tree species were not analysed.

Figure 6. Full height growth of beech and spruce compared to reduced growth caused by browsing.



4. Discussion

The research confirmed clear differences in composition, height and spatial structure of natural regeneration between two study plots. Although the mature stand structure and spatial arrangement are similar, these differences are not the result of different light conditions (Table 1). We do not have detailed quantitative data on seedling and sapling layer conditions prior to fencing, but according to the forest stand inventory conducted for the Agency of Nature Conservation and Landscape (Czech Republic) in 1983, the area was covered by only sparse regeneration not exceeding 40 cm in height (data not published). Today, natural seedlings, advance growth and the initial phase of the early growth stage are obviously damaged by ungulates on PRP 2. Ungulates are a limiting factor for successful regeneration as they severely damage or even fully suppress natural regeneration in unfenced parts of the reserve. In contrast, as a result of the minimum pressure of red deer and other ungulates on PRP 1, natural regeneration with distinct height and diameter differentiation has become established there.

The total number of natural regeneration is 16% lower on PRP 2 than on PRP 1 (34,040 recruits per ha on PRP 2 *versus* 40,420 recruits per ha on PRP 1), with the largest decrease in numbers of silver fir (95% reduction) and rowan (43% reduction). Heavy game browsing negatively influenced the species composition of the understory: in recent years, on the unfenced plot, the shift from lower to higher height classes has been very sporadic for fir, rowan and beech, and less so for spruce. The decreasing proportion of fir is explained mainly by the destructive influence of ungulates on its natural regeneration. In near-natural mixed forests in the Lom forest reserve in Bosnia and Herzegovina Bottero *et al.* [51] found a sharp decrease in the share of fir relative to the share of all trees in the growth and developmental stages studied. Among recruits in the height range of 10–100 cm fir accounted for 67% in the Lom forest reserve, beech accounted for only 17% and spruce for 10%. Among recruits taller than 100 cm and with breast-height diameter ≤7.5 cm beech was dominant with a 74% share, the fir share was only 18% and changes in the spruce share were not significant. A decrease in fir representation in mixed forests was also observed in Slovakia, where fir is replaced in forest stands mainly by European beech [52]. Similar observations of damage caused by ungulates to natural regeneration in comparable mixed stands were reported by Jaworski *et al.* [53], who conducted research in the West Carpathians in Poland. Their results confirmed reduction of fir in the range of 4%–15% in relation to the site within a 10-year period, mainly giving way to beech. Saniga *et al.* [54] also revealed the dominance of European beech in productivity and function at all developmental stages in a virgin forest in the Skalná Alpa National Nature Reserve. A similar trend was observed in mixed forest stands in Dobroč virgin forest in Slovakia [55].

According to Klopcic *et al.* [35] natural regeneration of fir in the unfenced parts of a silver fir-European beech forest (35 years ago) suffered the most damage or even elimination of regeneration by game in the 20–50 cm height classes and no taller recruits were found. In the fenced location, the density of fir recruits was 4.8 times higher than in the unfenced part. In the Černý důl Nature Reserve, the difference in fir density is even more marked; in the game-proof fenced enclosure, the share of recruits is 21.5 times higher than outside the fenced enclosure. Similarly, Vrška *et al.* [25] discussed changes in the environment and development dynamics of the tree species component of ecosystems in the Šumava Mountains natural forests and proposed a solution to the problem of excessive ungulate population levels in relation to the competitiveness of the primary tree species in natural regeneration of silver fir, European beech and Norway spruce. Based on the results of repeated investigations, they concluded that completely fencing virgin forests is currently the only way of ensuring their relatively natural spontaneous development. Research in mixed forests of spruce, fir and beech in Slovenia by Diaci *et al.* [26] showed that a decline in the fir population was caused not only by suppression of natural regeneration by herbivores but also by air pollution (sulfur dioxide emissions), to which the fir is relatively sensitive. Similar observations of the additional influence of air pollution on the health status and decline of silver fir stands in the Krkonoše Mountains of the Czech Republic were reported by Vacek *et al.* [38].

Spatial structure of natural regeneration also clearly differed between fenced and unfenced plots; the latter is characterized by more pronounced aggregation of all recruits irrespective of species than in the fenced area. Comparable findings about the recruit distribution in spruce-fir-beech stands in the Trčkov National Nature Reserve in the Orlické Mountains were reported by Vacek *et al.* [27]. Similar results were obtained by Šebková *et al.* [56] in mixed forests of the Boubín virgin forest in the Horni Vltavice district of the Šumava and Janik *et al.* [57] in mixed fir stands in the Western Carpathians. The research being reported here found that the naturally aggregated pattern in the fenced area was created by a large number of fir recruits, whereas outside the fenced area a limited number of fir recruits (caused by repeated browsing damage with high mortality rates) has led to a random spatial pattern. Similarly, the spatial pattern of silver fir seedlings was random in closed stands in the Krkonoše Mountains, where most of the seedlings were injured by browsing [58]. Conversely, rowans of seed origin in the fenced area are randomly distributed, but on the unfenced plot repeated browsing by ungulates readily creates new individuals by vegetative ways [8] and thus aggregated distribution. Spatial segregation of natural regeneration as a result of browsing damage was also confirmed by Peck *et al.* [59]. The absence of young sycamore maples on the fenced plot is the result of a more distant seed source; on the unfenced plot one mature sycamore maple is present in the overstory.

Nevertheless, we should not be misled by the notion that these areas were free of browsing pressure in the past. Instead, historical evidence shows that the studied spruce-fir-beech stands were influenced by cattle grazing from the 16th to the 18th century [60], which could account for the reduction in the share of shade-tolerant and especially of broad-leaved tree species and for the retention of some light-demanding species [22].

5. Conclusions

The paper confirms the hypothesis about the long-term influence of game on the species mix, spatial and age structure of forests and development of the studied autochthonous populations. However, homogenous initial tree regeneration on the compared plots must be considered when interpreting the present results. Natural regeneration of silver fir, sycamore maple and rowan in particular has not been successful in the study area without protection by fencing to keep out ungulates. Considering the current regulatory situation and the lack of effective methods for reducing ungulate populations to ecologically tolerable levels, it is necessary to find effective methods for the protection of natural regeneration. In many cases, mechanical protection of highly palatable tree species or fencing of whole regenerating forest stands may be justified.

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Author Contributions

Stanislav Vacek and Jiří Remeš planned and designed the experiment; all authors performed the research; Zdeněk Vacek and Jan Král analyzed the data Zdeněk Vacek, Stanislav Vacek and Lukáš Bílek interpreted the results and wrote the paper.

Conflict of Interest

The authors declare no conflict of interest.

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