COMPARISON OF FOREST SPECIES- DIVERSITY AND COMPOSITION INSIDE AND OUTSIDE OF THE HOLEDNÁ GAME RESERVE (THE CITY OF BRNO, CZECH REPUBLIC)

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ABSTRACT

The impact of ungulates on the forest vegetation has far-reaching consequences: it decreases species diversity and the production of biomass, causes soil dehydration, erosion and eutrophication the entire forest community. The article addresses the influence of fallow deer and mouflon on the forest vegetation in the Holedná game reserve (western border of the city of Brno, South Moravia, Czech Republic) and compares differences with the forests adjacent to the game reserve. Sixty localities were distributed randomly and phytosociological relevés subsequently recorded there according to the age of the stands inside and outside of the game reserve. The differences in floristic composition were compared for trees, shrubs and herbs, the herb layer species number, the diversity indices and the values of Ellenberg indicators. In the game reserve, a significant difference was found in the coverage of the herb and shrub layer, which was significantly lower than outside the territory. Furthermore, the increased amount of nitrophilous, heliophytes and ruderal herb species inside the game reserve exhibited affiliation to the interior of game reserve. Besides, young trees and woody sapling were less abundant or even missing inside of game reserve. By contrast, the frequency of typical species of oak-hornbeam forests was higher outside game reserve. Due to the higher number of animals and consequent disturbances, nitrophilous plant species dominate in the herb layer of the game reserve, while forest species are more often represented outside it.

Keywords: Game reserve, grazing of animals, change of biodiversity, forest vegetation, herb layer, nitrophytes

INTRODUCTION

Forest biodiversity, nature conservation and forest policy

Three primary attributes of biodiversity are widely recognised as providing a framework for nature conservation and forest policy (1) species/composition; identity and variety of elements (2) structure; physiognomy of forest stand to variation at forest and landscape scale (3) function; ecological and evolutionary processes, including gene flow, disturbances and nutrient cycling (Ferris & Humphrey, 1999; Noss, 1990). Human activity significantly affects biodiversity through its economically oriented intensive management, damages many habitats and increasingly disrupts natural cycles. The dramatic increases in deer populations in many parts of the world over the past century is associated with a shift in forest plant community composition from shrubs and forbs to grasses and with the reduced frequency of many plant species listed as threatened or endangered (Martin *et al.,* 2010). Other examples are habitat fragmentation, the introduction of non-native species, deforestation and also building game reserves (Primack *et al.,* 2011). An important act for the conservation of biological diversity is the protection of individual ecosystems, natural habitats and their all living components. The interaction of the ecosystem components is the basis for their function, i.e. the result is the maintenance of the ecosystem balance, such as nutrient recycling and soil formation (Glowka *et al.,* 1994; Primack *et al.,* 2011). Every change in the ecosystem leads to a reaction that can have unexpected dynamics and consequences for the future (Wohlleben, 2017). The current human impact on forests indicates changes in light availability, soil reactions and nutrient availability, which changes the dynamics of forest species populations and the composition of forest communities compared to the past (Dupouey *et al.,* 2002; Swierkosz, 2003; Hédl, 2004; Howe & Miriti, 2000; Matlack, 2005; Šebesta *et al.,* 2021). The distribution of plants and their presence in habitats is also linked to the activity of large mammals (Heinken & Raudnitschka, 2002; Vellend *et al.,* 2003; Vera, 2000), in game reserves especially. Excessive game activity in forests has been repeatedly found as a factor suppressing species diversity of the herb layer, especially in protected areas (Rooney & Dress, 1997; Kirby, 2001; Chytrý & Danihelka, 1992; Čermák, 1998a). On the other hand, the influence of wildlife or game pressure can currently replace the historic forest pasture, which significantly contributed to the formation of the species composition of forest vegetation of the Central-Europaean landscape (Jakubowska-Gabara, 1996; Vera, 2000; Bellemare *et al.,* 2002). Even the spread of invasive species (and active organisms in general) is, among other things, also related to the activity of large mammals (Adler *et al.,* 2001; Vavra *et al.,* 2007) and generally to soil disturbation (Šebesta *et al.,* 2021). Non-native species usually spread to new habitats due to several factors: herbivory, zoochory and disturbances, with the contribution of nutrient supplementation from emissions or release by decomposition of organic matter just after disturbance (Levin *et al.,* 2003; Couvreur *et al.,* 2007).

Ungulates and their impact on vegetation

The negative impact of high numbers of ungulates on forest vegetation is known and documented in detail. Most of the authors focus mainly on the damage that game causes to the woody component of forest vegetation or natural forest regeneration (Gill, 1992a, b; Ammer, 1996; Fuller & Gill, 2001b). Some investigations are interested in the influence of game grazing on the herb layer (Perea *et al.,* 2014; Martin *et al.,* 2010). The long-term pressure of high levels of game in the herb layer leads to significant changes, which are primarily reflected in the structure and species composition, as well as in its dynamics and functions. Besides, higher presence of deer generally reduce tree recruitment (Rogers & Šebesta, 2019). They also secondarily affect other follow-up groups of organisms, change the nutrients cycle, soil conditions, and microclimatic conditions in the forest ecosystem. These consequent changes appear to be substantial (Putman *et al*., 1989; Fuller & Gill 2001a; Husheer *et al*., 2003; Rooney & Waller, 2003; Côté *et al*., 2004).

The influence of game hurts forest undergrowth, which is also documented/confirmed by several studies in the Czech Republic (Chytrý & Danihelka, 1993; Čermák 1998a, b; Heroldová, 1997; Kočí, 2009; Petřík *et al*., 2009). Chytrý & Danihelka (1993) found out by analyzing repeated phytocoenological relevés in Bulhary and Klentnice game reserve after 38 years this consequences: shrub and lower tree layer of oak and oak-hornbeam forests are disturbed, development of tree seedlings is limited by animal trampling and natural forest regeneration is sporadic. The increase of the soil nitrogen due to animal excrement and high grazing and trampling resulted in extensive ruderalization by the retreat of natural species and the invasion of alien species. Compaction of the soil by the tread and its lower permeability cause the drying of the soil and the replacement of mesophilous by xerophilous species, mostly non-native ones. The impact of ungulates on the shrub layer was investigated by comparing the biomass in both inside and outside of exclosures in game reserve Pálava by Čermák (1998b). The difference between control and comparative plots was more than 40–70 % of the biomass of the control area in 15 and 24 plots respectively. Heroldová (1997) found out in the same locality that ungulates consumed 75 % of the biomass of the shrub layer, the result was mainly due to the bezoar goat (*Capra aegagrus*). In the woodland complex near Dolní Věstonice, roe deers (*Capreolus capreolus*) consumed 19–53 % of the shrubs biomass in two monitored years. Similar results were found by Petřík *et al.* (2009) in the Křivoklátsko and Kočí (2009) in the Radějov game reserve. There is a natural regeneration of woody plants on permanent exclosures, while outside them tree seedlings were grazed; grazing causes a reduction of species richness. On the other hand, the natural regeneration of hornbeam and beech, reduced the cover and species diversity of the herb layer (Petřík *et al*., 2009). The trampling of the animals eliminated the shrubs layer and young trees, which completely prevents natural forest regeneration (Kočí, 2009). Game in forests, unless it is in excessive densities in game reserves, tastes to limit the grows of shrub layer and contributes to maintaining of herb layer diversity. However, depending on their density, the game significantly contributes to the eutrophication of stands and thus to the spread of non-native species (Petřík *et al*., 2009).

Grazing creates a more open forest undergrowth, i.e. it increases the proportion of bare soil and litter at the expense of the herb layer.

Goals

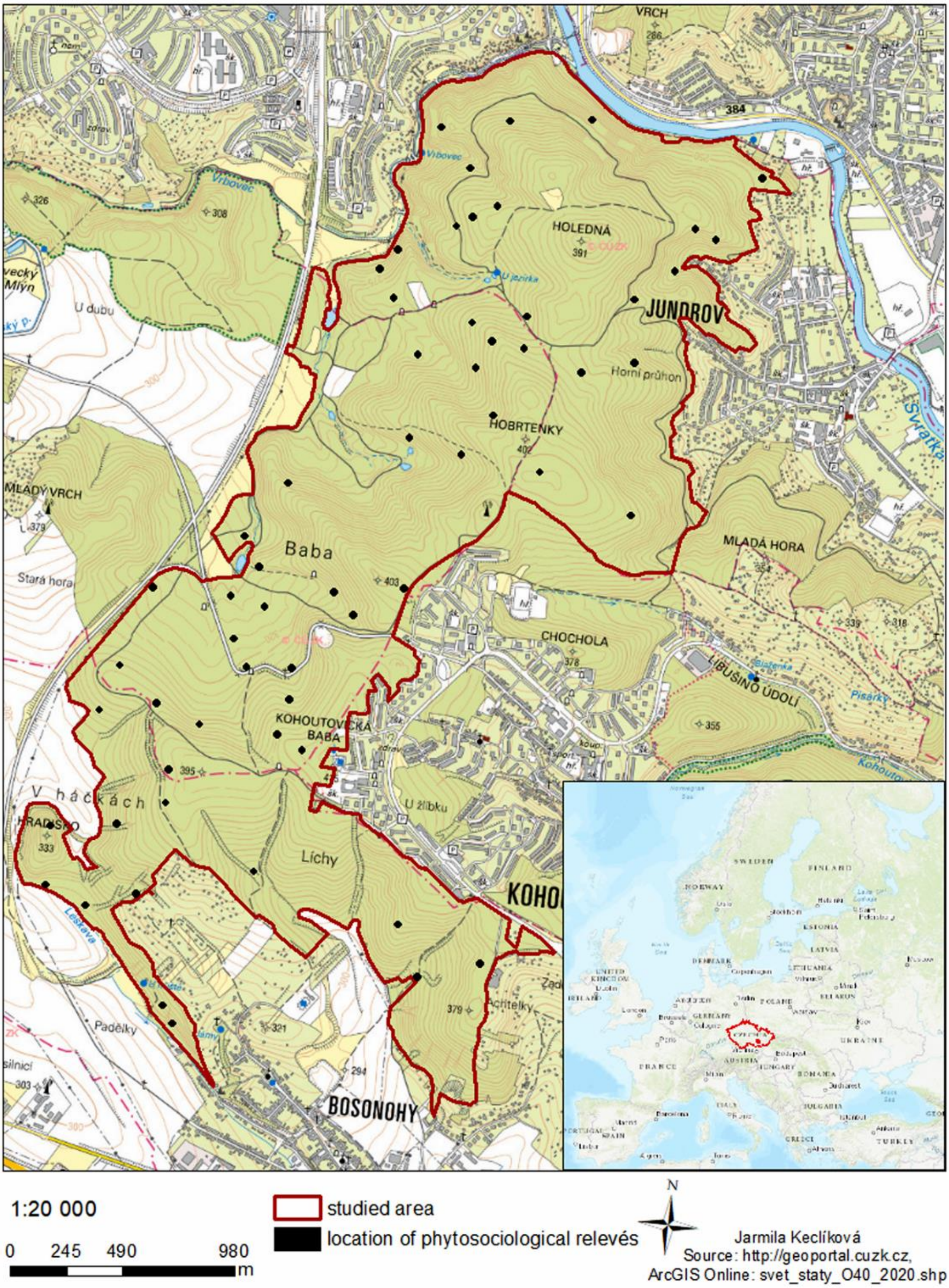
The work aims to describe the differences in species composition and diversity of vascular plants in the Holedná game reserve and the surrounding forest stands. The main task was to prove or refute the presumption of the negative impact of fallow deer and mouflon on all layers of forest vegetation in this area.

METODS

Game reserve Holedná

The Holedná game reserve was declared in 1985, it lies on the forested hill Holedná (+ 396 m), in the cadastral area of Bosonohy, Jundrov and Žebětín on western limit of the city of Brno and it currently covers an area of 323 ha (Fig. 1). Since 1999, the game reserve has been fenced, and since 2003 the forest Enterprise Lesy města Brna has been managing the game reserve (https://regiony.kurzy.cz/katastr/ku/610313/mapa). Most of the area is covered with forest, a small part are fields for game (clover and grain mixtures), meadows and water small bodies. The forest stands are dominated by deciduous trees (*Quercus petraea* agg.), but also woods for increase the usability of the area (*Aesculus hippocastanum, Fagus sylvatica*) (Pačes, 2007). Some parts of the reserve are planted with unsuitable tree species such as *Picea abies, Pinus strobus* and *Quercus rubra*. The area is also declared as SCI for stag beetles (*Lucanus cervus*). The main game in the area are fallow deer (*Dama dama*) and mouflon (*Ovis musimon*), there are also several pieces of Dybowski's red deer (*Cervus nippon dybowskii*). Wild boar (*Sus scrofa*) is bred in a small enclosure on the western border of the area. In 2003, the numbers of ungulates were considerably exceeded, but they were gradually reduced, and currently about 250 ungulates are bred in the area (Pačes, 2007; J. Neshyba, pers. comm.).

Fig. 1: Map of the study area with points of phytocoenological relevés



Plots selection

In the program ArcGIS the forest stands were divided into 5 age groups: (1) 11–30, (2) 31–50, (3) 51–70, (4) 71–90 and (5) 91–120 years, to which tree two-layers stands were added. To these plots were using the Genstratrandompnts (Generate Stratified Random Points) function in the program Geospatial Modelling Environment (http://spatialecology.com) and sixty points (i.e. the centers of phytocelogical plots) were randomly placed in the whole studied area, of which 30 were inside in game reserve and 30 outside of them. Five points were placed in each age group (outside the group (3), in which there were ten points each). A minimum distance of 100 m was done between points in one group to exclude spatial dependencies.

Data collection

In the summer of 2016, phytocenological relevés on a plot of 10 × 10 m were recorded on the generated points. The species were classified into individual layers according to Moravec *et al.* (1994): E_3 (tree layer): woody plants higher than 3 m, to E_2 (shrub layer) woody plants with a height of 1–3 m, to E_1 (herb layer) herbs, semi-shrubs up to a height of 1 m tree seedlings; to E_0 (ground layer) mosses and lichens. Species cover and total cover were recorded as percentages. Nomenclature of vascular plants according to Kubát *et al.* (2002).

DATA ANALYSIS

Analyses were carried out for the complete understorey shrub and tree layers. To reveal the differences between game regime, we calculated frequency for each taxa, for which we used the Juice 7.0 software package (Tichý, 2002). Contrasts in the understorey structure (herb and woody species regeneration) were evaluated with the use of Shannon-Wiener and Simpson diversity indices. Moreover, we counted the species number and cover of vegetation layers present within the plots. To quantify the effects of „animal management“, we calculated Ellenberg indicator values (EIV´s) representing light, temperature, moisture, nutrients and soil reaction for each plot.

To test for differences in diversity, vegetation cover and EIH's between the plots ouside/inside game reserve, independent two-sample t-test. All the statistical analyses were carried out in STATISTICA 12.0 software. The critical level of significance for all statistical tests was $p < 0.05$.

NATURAL CONDITIONS OF THE STUDY AREA

According to the geomorphological division, the studied area belongs to the Hercynian region, to the subsystem of the Brněnská vrchovina Highlands and unit of the IID-2 Bobravská vrchovina Highlands (Demek & Mackovčin, 2014). The area is very rugged, the highest point is the top of the Hobrtenky hill (cota 405 m) and the lowest point is the valley of the Vrbovec stream (225 m a.s.l.). The bedrock is a diverse complex of igneous rocks with a predominance of granodiorites (Chlupáč *et al.*, 2011), in the western part of the territory there are loess and loess clays (www.geology.cz). The most common soil type is cambium soil with several subtypes, near the stream is a fluvial gley soil (https://mapy.geology.cz /pudy/).

The area belongs to two climatic areas, T2 (warm) and MT11 (moderately warm) (Vysoudil, 2004). The average annual temperature (climatic station of the Brno-Tuřany) for the period of years 1961–2010 is 9.1°C, with the highest average monthly temperature in July

(19.3°C) and the lowest in January (–1.9°C). The average annual rainfall is 495.4 mm (range 361.0 to 687.0 mm) (Vesecký, 1961; Dobrovolný *et al.*, 2012).

The predominant potential vegetation is oak-hornbeam forests (ass. *Melampyro nemorosi-Carpinetum*), only in the SW part of the area there is a fragment of the basiphilous oak forest (ass. *Potentillo albae-Quercetum*) (Neuhäuslová *et al.*, 1998). According to the forest typology, the most common is oak-beech vegetation belt is in the area, but a considerable part is also occupied by the beech-oak vegetation belt and the beech vegetation belt is also represented in the small island (Culek *et al.*, 1995). The studied area belongs to the natural forest area No. 33 the foothills of the Bohemian-Moravian Highlands. According to Skalický (1988), the study area belongs to the phytogeographical region Thermophyticum, the province of Pannonian Thermophyticum (Pannonicum) and the phytogeographical district no. 16. Znojemsko-brněnská hill country. A large part of the study area was formerly grown as coppice. Forests outside the boundaries of the game reserve that have been compared are located mainly outside its southern boundaries and only a small part is located at its northern boundaries (Fig. 1).

RESULTS

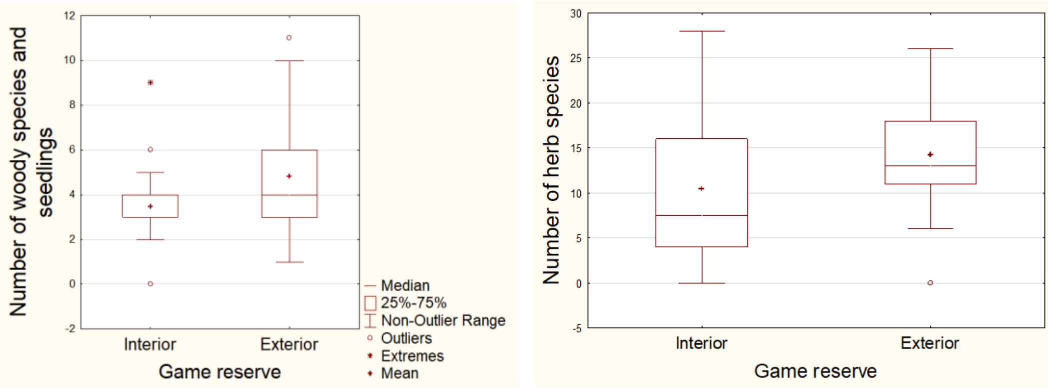
Tree, shrub and herb layer canopy

The differences of tree canopy, shrub and herb layers cover are obvious – see Tab. 1. Although, there is difference between tree layer canopy (E_3) inside and outside game reserve (Tab. 1), the differences is not significant. The variability of tree layer canopy is evident, majority of canopy was about 50 %, maximum 90 % for both management types. Cover of shrub layer (E_2) is almost neglected compared to cover of shrubs in forests out of the game reserve. Most of vegetation samples had no shrubs in the game reserve; on the other hand, some shrubs grew at most of samples with the maximum 45 % cover outside the game reserve. Total cover of the herb layer (E_1) was on average lower in game reserve than out of the game reserve, the difference is significant. The maximum cover of herb layer is only 10 % in the game reserve. Compared with the game reserve, there is 60 % maximum cover of herb layer outside of the reserve.

Table 1: Mean values and standard errors of vegetation layers cover. Game reserve: N = 30; out of game reserve: N = 30. Test statistics (t and p values) for differences between management types are shown. Significant differences are marked in bold

vegetation layer	in game reserve	outside game reserve	t-value	p-value
E_3	49.35 ± 20.77	55.77 ± 18.70	-1.257	0.214
E_2	0.07 ± 0.37	4.67 ± 9.08	-2.773	**0.007**
E_1	4.10 ± 4.85	28.65 ± 18.27	-7.115	< **0.001**

Fig. 2: Comparison of species richness, number of herb and woody species in understorey by game reserve interior and exterior



Diversity

In total, we found 157 herb and 44 woody species in all 60 plots ranging from 1 to 28 species per plot. Forty herb and 8 woody species were unique to plots inside game reserve, conversely 64 herb and 22 woody species were unique to plots outside game reserve. Altogether, only 55 herb and 15 woody species grew inside and outside game reserve, both. The mean number of herb and woody species per plot significantly differ between plot outside/inside game reserve (Tab. 2). The mean number of herbs and woody species was on average higher outside game reserve. On the other hand, Shannon-Wiener and Simpson‘s diversity indices were on average slightly higher outside game reserve but the differences were not significant.

Table 2: The differences is species diversity between the plots inside/outside game reserve. Test statistics (t and p values) for differences between management types are shown. Significantly different cases are bolded

	in game reserve	outside game reserve	t-value	p-value
Number of herbs	10.43 ± 7.97	14.27 ± 5.74	**-2.137**	**0.037**
Number of woody species	3.47 ± 1.59	4.83 ± 2.76	**-2.352**	**0.022**
Shannon - herbs	1.93 ± 0.90	2.28 ± 0.65	-1.742	0.087
Shannon - woody species	1.12 ± 0.42	1.27 ± 0.55	-1.184	0.241
Simpson - herbs	0.80 ± 0.20	0.86 ± 0.11	-1.336	0.187
Simpson - woody species	0.66 ± 0.14	0.64 ± 0.21	0.457	0.650

Ellenberg indicator values

In terms of EIV’s, the localities differed only in light EIV´s with higher values inside game reserve (Tab. 3); EIV´s in nitrogen were higher inside game reserve but the difference was not significant. Overall, in EIV´s for temperature, moisture, nitrogen and soil reaction did not significantly differ.

Table 3: Mean values and standard errors of Ellenberg indicator values (EIV) for herb species of inside and outside game reserve. Test statistics (t and p values) for differences between management types are shown. Significant differences are marked in bold

	in game reserve	outside game reserve	t-value	p-value
light	5.74 ± 1.29	4.81 ± 1.15	**2.964**	**0.004**
temperature	5.09 ± 1.77	5.38 ± 1.07	-0.770	0.445
moisture	4.75 ± 0.99	4.61 ± 0.91	0.598	0.552
nutrients	5.32 ± 1.39	4.90 ± 1.28	1.223	0.226
soil reaction	5.21 ± 1.59	5.38 ± 1.18	-0.476	0.636

Vegetation species composition

Analysis of frequency and fidelity revealed considerable differences in species composition of herb and woody species inside/ouside game reserve (Tab. 4). However, there are common herb and woody species that occur across the game reserve management – *Quercus petraea* (juv.), *Carpinus betulus* (juv.), *Tilia cordata, Luzula luzuloides, Viola riviniana, Moehringia trinervia, Myosotis sylvatica, Hieracium murorum, Calamagrostis epigejos, Viola odorata, Ajuga reptans* etc.

The most pronounced difference is increased incidence of nitrophilous and ruderal herb species inside game reserve as *Urtica dioica* or *Taraxacum* sect. *Taraxacum*. Besides, herb species that prefere disturbation and trampling exhibited affiliation to the interior of game reserve – *Poa annua*, *Stellaria media*, *Cerastium holosteoides*. Moreover, higher frequency of herb species of grassland habitats, such as *Achillea collina, Poa angustifolia, Trifolium repens* was associated with the interior of game reserve. There was only *Larix decidua* as more frequented woody species inside game reserve. By contrast, the frequency of typical species of oak-hornbeam forests was higher outside game reserve (according to frequency), as *Poa nemoralis, Galium odoratum, Convallaria majalis, Pulmonaria obscura, Viola mirabilis, Hieracium sabaudum, Carex digitata, Stellaria holostea, Fragaria moschata, Polygonatum odoratum, Lathyrus vernus* and others. There were many woody species in understorey and shrub layer associated with research plots outside game reserve, as *Acer platanoides, Rosa canina, A. pseudoplatanus, Ligustrum vulgare* and *Sambucus nigra*.

Table 4: Differences in the frequency on understorey species between interior and exterior game reserve plots. Only species with frequency > 13 % are listed in the table. The species are ranked according to the frequency

	Frequency (%)	
	inside game reserve	outside game reserve
Urtica dioica	53	13
Poa annua	43	3
Stellaria media	43	3
Moehringia trinervia	47	23
Cerastium holosteoides	37	3
Luzula campestris	37	10
Hypericum perforatum	27	3
Euphorbia amygdaloides	30	7
Poa angustifolia	23	0
Myosotis sylvatica	33	20
Taraxacum sect. *Ruderalia*	23	10
Agrostis capillaris	23	13
Veronica officinalis	23	13
Sagina procumbens	20	0
Trifolium repens	20	0
Achillea collina	13	0
Larix decidua juv.	13	0
Impatiens parviflora	13	67
Poa nemoralis	27	67
Galium odoratum	0	47
Acer platanoides juv.	0	33
Geranium robertianum	10	50
Convallaria majalis	0	40
Rosa canina juv.	7	37
Pulmonaria obscura	0	33
Geum urbanum	7	37
Carex sylvatica	0	27
Acer pseudoplatanus juv.	7	23
Hieracium sabaudum	10	33
Viola mirabilis	0	23
Carex digitata	3	23
Fragaria moschata	3	23

Stellaria holostea	3	23
Festuca ovina	13	30
Hieracium murorum	17	33
Asarum europaeum	7	23
Luzula luzuloides	27	40
Polygonatum multiflorum	0	20
Brachypodium sylvaticum	3	20
Lychnis viscaria	3	20
Hepatica nobilis	0	17
Ligustrum vulgare juv.	0	17
Campanula persicifolia	0	13
Carex pilosa	0	13
Galeobdolon montanum	0	13
Lathyrus vernus	0	13
Rubus idaeus	0	13
Silene latifolia	0	13
Ribes rubrum	0	13

DISCUSSION

Species composition, canopy

This study reveals a strong difference between the areas with and without ungulates in herb and woody species composition, shrub and herb layers cover. Results show that some plant species were highly preferred and became absent inside the exterior, conversely species that prefere game reserve interior. By comparing research plots, we were able to demonstrate which plant species are more vulnerable to browsing or which are failing to regenerate. Similarly, Perea *et al.* (2014) reveal a strong difference between the areas with and without deer in woody plant composition and diversity after 30–40 years of deer browsing. In our study, after only 35 years this management favouring high fallow deer and mouflons led to a significant decrease of shrub and herb layers cover and specific plant species composition. Rogers & Šebesta (2019) also support our findings and argue that ungulates are making preferential food choices regarding plant consumption which are affecting current understorey communities.

The high number of unique species in the outside the game reserve is mainly related to the higher proportion of species of oak-hornbeam communities, which are in little portion inside game reserve due to pressure of the game for now (*Campanula persicifolia*, *Carex digitata*, *C. sylvatica*, *Convallaria majalis*, *Galium odoratum*, *Geranium robertianum*, *Lathyrus vernus*, *Pulmonaria officinalis* agg. and *Stellaria holostea*). This phenomenon is related to the intensive grazing of the herb layer in the game reserve, but also the soil compaction by trampling of animals and its drying up, where instead of mesophilous perennial forest species, species with drought tolerance take the place. Disturbation the forests soil surface associated with eutrophication supports the spread of ruderal and nitrophilous species (Kočí, 2009; Šebesta *et al.*, 2021).

A similar situation is described by Chytrý & Danihelka (1993) in the game reserves of Bulhary and Klentnice in South Moravia. Also, these authors document the growth of xerophilous ruderals, which oppress surviving more subtle species in the habitat. In the Holedná game reserve, we recorded a minimum of short-lived species (terophytes), which tolerate these conditions and replace perennial forest species. The main reason is decreasing of the usefulness of the game reserve and currently a low food supply due to game overpopulation.

Our results also show the differences in the frequency of individual species (decline) in the game reserve and outside it in percentage, e.g. *Galium odoratum* -47, *Convallaria majalis* -40, *Geranium robertianum* -40, *Poa nemoralis* -40, *Pulmonaria officinalis* agg. -33, *Carex sylvatica* -37, *Hieracium sabaudum* -23, *Stellaria holostea* -20 and *Lathyrus vernus* -13. Chytrý & Danihelka (1993: 243) noted high differences between the frequency of selected species (decline) in the game reserve Bulhary and even higher in the Klentnice game reserve: out of 29 species of E_1 of thermophilous oak forest mentioned by the authors, 17 of them disappeared completely (-100 %), the others show relatively high differences (from -45 to -89 %).

An important or essential species in the studied area is *Euphorbia amygdaloides* with a difference of +23 % in the game reserve, which is ignored by game due to its toxicity; this phenomenon was confirmed by Kočí (2008).

Our findings are concordant with other studies (Perea *et al.*, 2014; Martin *et al.*, 2010) that also shown differences is species composition at high deer densities. We highlight the need for sampling deer-absent areas and game reserves to assess the real impact of deer on herb and woody plant diversity since most studies only address the impact of ungulates browsing in areas with deer only. We found differences in the higher abundance of some nitrophilous species inside game reserve (*Urtica dioica*, *Taraxacum* sect. *Ruderalia*). Also, herb species that prefer disturbation exhibited affiliation to the interior of the game reserve – *Poa annua*, *Stellaria media*, *Cerastium holosteoides*. This affiliation could be explained by the direct and indirect effect of animals (Rogers & Šebesta, 2019). Fallow deer and mouflons take the nutrients to forest environment and they disturb soil surface by trampling and raking. Differences in plant species composition between game reserve exterior and interior are also linked with light conditions, as indicated by the Ellenberg indicator values used in our study. Our results support the claim that animals strongly affect shrub canopy and young trees layer (Martin *et al.*, 2010). Compared to the exterior, a more open shrub layer allows greater light penetration to herb understorey. On the other hand, typical species of oak-hornbeam forests were more abundant outside game reserve (*Poa nemoralis, Hieracium murorum, H. sabaudum*) or even were missing there (*Galium odoratum*, *Convallaria majalis, Pulmonaria obscura, Stellaria holostea, Hepatica nobilis, Lathyrus vernus*). Thus, hunting management that is good for some species may not be appropriate for others.

Young trees

Cover of shrub layer and number of woody species was significantly lower on plots inside of game reserve. The impact of deer on the growth and survival of tree seedlings has been recognized for many years (Martin *et al.*, 2010). Many young trees can not tolerate browsing, so that woody sapling are less abundant (*Acer pseudoplatanus*) or even missing (*A. platanoides, Ligustrum vulgare*) in game reserve. Besides, in our study, young trees under 1.3 m occured often in *scrubby form* in the presence of fallow deer and mouflons, especially when growing in south-oriented slopes. Hence, that damaged young trees do not absent there but they suffer the browsing stress.

Fig. 3: A visible difference in the coverage of the herb and shrub layers inside and outside of the game reserve

The almost complete absence of E_2 in game reserve is an essential fact in our results (Fig. 3). The average value of E_2 coverage in the inside is 0.06 %, in forests outside 6.36 %, which is the result not only of mouflon activity (mostly in winter), but especially of fallow deer (Husák, 1986). This also applies to tree seedlings in E_1, which are continuously bitten by game. Čermák (1997) indicates the hornbeam (*Carpinus betulus*) as well regenerating, the other seedlings of woody species regenerate less and usually die due to drought. The same author also points to species such as *Fraxinus excelsior*, *Sorbus aucuparia* and *Carpinus*, in which there was the greatest difference between individuals of the same age inside and outside the fence (Čermák, 1998 a). Petřík *et al.* (2009) confirmes that the hornbeam (*Carpinus betulus*) was the most active in places without animal activity. In our observation of E_2 in the forest outside the game reserve, the following species weremost represented (according to cover): *Tilia cordata, Carpinus betulus a Fagus sylvatica*, the shrub layer inside the game reserve is missing (Figs. 4 and 5).

Fig. 4: Strongly influenced undergrowth of the oak-hornbeam forest by ungulates in the game reserve Holedná

Fig. 5: Forest structure in the Holedná game reserve: beech stand at the southern border of study area

Diversity

The affiliation and reduced presence of such species inside the game reserve caused a decrease in plant diversity (Tab. 2). However, the test of the species number in both groups shows a significant difference between the inside and the outside game reserve. This is mainly because in the inside of the game reserve more sensitive species have completely receded due to the grazed and trampling of animals and there is a higher proportion of nitrophytes / ruderal species, which in most places outside the game reserve are missing or not represented in such numbers. Similarly, Kočí (2009) and Petřík *et al.* (2009) discovered that grazing causes a reduction in species richness and diversity of undergrowth, there is a significant reduction in cover and height of herb layer, including the number of tree seedlings.

The mouflon is a most important animal for the vegetation of game reserve Holedná, which grazes mainly the herb layer, due to its lips to almost the surface of the bare soil. This fact is supported not only by our results of E_1 coverage in the inside and outside of the game reserve, but also by data from other authors regarding the food preference of mouflon and its pressure on E_1 than other animals (Lochman *et al*., 1979; Chytrý & Danihelka, 1993). In the inside of game reserve, the mean coverage of E_1 was very low (4.2 %), while outside was 28.8 % (Fig. 6). Stockton *et al*. (2005) pointed out a decrease in species richness at the plot scale by 20–50 % in islands with deer in comparison with those without deer.

Fig. 6: Undergrowth of mixed deciduous forest outside the game reserve, under the road to the village of Žebětín

CONCLUSIONS

Analyzes of phytocenological relevés on a randomly generated localities in the Holedná game reserve and in the forests exterior confirmed the following facts:

- there is a significantly lower coverage of the herb and shrub layers of forest vegetation inside the game reserve (Figs. 3–6)
- inside the game reserve is confirmed a significantly lower number of species in the herb layer, both herbs and tree seedlings. The typical species of oak-hornbeam forest are replaced by light demanding, drought-tolerant, trampling tolerant species, and especially nitrophytes, ruderal species or species toxic to game. The increase of nitrophytes is consequence of excrements and soil disturbation by ungulates
- occurrence of light demanding species is caused not only by the lack of shrub layer, which is muted due to ungulates preferences, but also by the lower cover of E_3 in comparison with forests outside the game reserve
- diversity indices, lower inside in comparison with exterior, i.e. in forests outside the game reserve there is a higher species diversity and more typical forest species were recorded with higher cover

- the herb and shrub layers in the game reserve are not able to regenerate due to the pressure of game and thus the usefulness of the game reserve decreases significantly
- our results clearly show a significant negative impact of the game on forest vegetation in the Holedná game reserve.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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