



The paradox of long-term ungulate impact: increase of plant species richness in a temperate forest

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Nomenclature

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Introduction

The combination of several human-induced factors has recently led to the deterioration of habitat quality and biodiversity in temperate broad-leaved forests. Rackham (2008) identified several ‘modern threats’ that caused accumulation of N and lower light availability in the forest understorey. These processes changed the diversity and composition of plant communities. Potential consequences

Abstract

Questions: Did high densities of wild ungulates cause a decline in plant species richness in a temperate oak wood? How did species composition change after nearly five decades? Did ungulates facilitate the spread of ruderal species and suppress endangered species? Did dispersal strategies play a role in these processes?

Location: Krumlov Wood, SE Czech Republic.

Methods: In 2012, we resampled 58 quasi-permanent vegetation plots first surveyed in the 1960s. Between the surveys, 36 plots were enclosed in a game preserve with artificially high density of ungulates (mostly deer, mouflon and wild boar; ca. 55 animals·km⁻²). We analysed the differences in temporal changes between plots inside and outside the game preserve, focusing on species diversity and composition. We assessed species characteristics relevant to grazing to understand compositional changes.

Results: Ungulates significantly increased α - and γ -diversity and caused significant vegetation homogenization inside the game preserve. Vegetation homogenization and the increase in species richness resulted from massive enrichment with ruderal species. However, richness of endangered species decreased. Species dispersed by animals internally (endozoochory) increased, while species dispersed externally (epizoochory) or by wind (anemochory) decreased.

Conclusions: Contrary to our expectations, our long-term data showed that artificially high ungulate densities substantially increased plant species richness. Apparently, the establishment of ruderal herbs was supported by frequent disturbances and ungulate-mediated dispersal. At the same time, species richness of non-ruderal plants did not change, probably because ungulates hindered the regeneration of woody species and maintained an open forest canopy. In conclusion, high ungulate density led to the spread of ruderal species, which in turn strongly contributed to the observed shift towards nutrient-richer conditions and taxonomically more homogenous communities.

are geographic shifts of populations of native species and the constitution of novel communities.

One of the recent major human-induced factors changing the plant communities of temperate forests worldwide is the increased density of ungulates (Milner et al. 2006; Rackham 2008). These include several species of deer, moose, mouflon and wild boar. The effects of increased densities of ungulates in temperate forests on tree regeneration have been extensively studied (Kuijper et al. 2010;

Habeck & Schultz 2015). Individual species in the understorey may also be negatively affected by increased ungulate pressure (Kirby 2001). Ungulates reduce the abundance of sensitive species and promote the abundance of unpalatable species such as grasses, sedges and ferns (Horsley et al. 2003; Côté et al. 2004; McEvoy et al. 2006). The indirect effects modifying the environment may be as important as those on the trophic level. Ungulates cause soil surface disturbance by trampling, digging for food, etc. (Vavra et al. 2007; Beguin et al. 2011). Animals change the spatial distribution of N supply. While they decrease N input by grazing and remove litter for decomposition, they also provide readily available N from excretion (Singer & Schoenecker 2003). Ungulates are also important vectors of plant diaspores, enabling the spread of many zoochorous species (Heinken et al. 2002; Myers et al. 2004). Deer are more effective in the endozoochorous dispersal of species than wild boar, whereas wild boar excel in the epizoochorous dispersal (Heinken et al. 2002; Schmidt et al. 2004). Wild boar can also support the establishment of quickly dispersing ruderal species by rooting in large areas in a complex spatio-temporal pattern (Welander 2000; Grime 2001; Brunet et al. 2016).

Regarding the effects on forest herbaceous vegetation, ungulates have been reported to have a mostly negative impact on biodiversity in terms of decreasing herb layer cover and species richness (Russell et al. 2001; Rooney et al. 2004; Stockton et al. 2005). Ungulates are also among the main drivers of taxonomic homogenization in temperate forests (Rooney et al. 2004; Rooney 2009; Begley-Miller et al. 2014). However, some effects of ungulate pressure on the diversity of forest herbs can be beneficial. Ungulates may suppress tree regeneration and competitive herbs, thus providing opportunities for low-stature herbs (Kirby 2001; Hédél et al. 2010; Royo et al. 2010). This effect can be particularly relevant in nutrient-rich environments with intensive competition for light (Gough & Grace 1998). However, local disturbances and increased nutrient levels can also lead to the expansion of ruderal and alien species (Chytrý & Danihelka 1993; Kirby 2001; Eschtruth & Battles 2009; Knight et al. 2009; Beguin et al. 2011).

Two topics regarding the impact of ungulates on the vegetation of temperate forests are still poorly understood. First, the vast majority of published studies come from North America, while literature on Europe is scarce (Chytrý & Danihelka 1993; Hédél et al. 2010; Boulanger et al. 2015). Second, most studies are based on short-term comparisons of fenced sites with the surrounding grazed areas (e.g. Kuijper et al. 2010; Suzuki & Ito 2014), while long-term effects remain largely neglected (Nuttall et al. 2014; Boulanger et al. 2015). Due to temporal lags in vegetation response, the long-term effects of ungulates may

have quite different consequences than snapshot comparisons.

In the present study, we compared vegetation changes between two data sets that differ in ungulate density – artificially increased numbers in the game preserve vs ambient numbers outside the preserve. We aimed to assess the long-term effects of high ungulate density on forest understorey vegetation. We tested the hypothesis that long-term ungulate pressure results in a decrease in the diversity of herbaceous vegetation, especially of endangered species, at the plot and at the whole-site level, and that it causes the spread of ruderal species. Besides, we tested whether ungulates caused changes in compositional heterogeneity.

Methods

Study site

Krumlov Wood is a large wood (35 km²) in the southeast of the Czech Republic (16°23' E, 49°03' N; Appendix S1). The elevation of this hilly area varies between 200 and 415 m a.s.l. Climate is subcontinental, relatively warm and dry, with average annual temperature of 8–9 °C and precipitation of 500–550 mm (Tolász et al. 2007). Acidic granodiorite bedrock dominates the area. The prevalent soil types are cambisols and luvisols. Dry acidophilous oak forests with sessile oak (*Quercus petraea* agg.) and mesic oak forests with an admixture of small-leaved lime (*Tilia cordata*), European hornbeam (*Carpinus betulus*) and pedunculate oak (*Quercus robur*) are the dominant vegetation types.

The forest was often mentioned in medieval written sources. It was referred to as Krumlov Wood (Cromauen Wald) already in 1635 (MZA F 119 inv. č. 452). The dominant management type was coppicing, the Wood was regularly coppiced until WW II and nowadays it has the structure of a singled-out coppice. The first game account dates back to 1659, and numbers of killed animals were recorded yearly until the 20th century. Annual fluctuation of culled animals could be considerable (Appendix S2). Prior to modern times, the only known fenced game preserve inside Krumlov Wood existed in the 18th century to keep wild boar (MZA F 177 kniha 34, see also Appendix S1). In 1984, a preserve was established on 2410 ha and high numbers of ungulates were imported, increasing grazing pressure more than ten-fold. Recent game numbers are even higher, and this holds true not only for the preserve. Current grazing pressure outside the preserve is higher than that in the 1960s within the preserve (Table 1). In the preserve, trees do not regenerate successfully. Prior to the reference sampling, the main game was red deer (average density 1.2 km⁻²), roe deer

Table 1. Ungulate densities in the game preserve prior to the original sampling (1963–1967), after the modern preserve was established (1984–2002) and at the time of the resampling (2012), and under ambient conditions in the whole region. The ambient densities are calculated for the area of all woodlands in South Moravia Region. Only males were calculated for the period 1963–1967.

Ungulate Species	Game Preserve, 1963–1967		Game Preserve, 1984–2002		Game Preserve, 2012		Ambient Conditions, 2012	
	Number (km ⁻²)	Weight (kg·km ⁻²)	Number (km ⁻²)	Weight (kg·km ⁻²)	Number (km ⁻²)	Weight (kg·km ⁻²)	Number (km ⁻²)	Weight (kg·km ⁻²)
Red Deer (<i>Cervus elaphus</i>)	1.2	75.8	17.8	1155.8	24.9	1618.1	1.4	90.6
Wild Boar (<i>Sus scrofa</i>)	–	–	2.2	88.9	15.6	622.3	2.7	108.1
Mouflon (<i>Ovis musimon</i>)	0.5	19.8	4.4	166.7	13.8	516.8	1.0	35.7
Roe Deer (<i>Capreolus capreolus</i>)	2.4	33.1	–	–	0.9	12.4	17.6	246.2
Fallow Deer (<i>Dama dama</i>)	–	–	–	–	–	–	1.2	59.9
Total	4.1	128.7	24.4	1411.4	55.1	2956.3	23.8	559.0

(average density 2.4 km⁻²) and mouflon (*Ovis musimon*, average density 0.5 km⁻²) (Table 1).

Data collection

M. Kelbl sampled the vegetation of Krumlov Wood in 1964 and 1968. He recorded plant species composition in 400-m² plots placed subjectively to cover forest vegetation variability. Within each plot, he identified all vascular plants and estimated their cover–abundance. In 2012, we resurveyed 58 plots dominated by sessile oak (*Quercus petraea* agg.). The original plots were not permanently marked in the field. We therefore refer to the plots as quasi-permanent (= semi-permanent; Persson 1980; Kapfer et al. 2016). We resampled 36 plots within the game preserve and 22 plots outside of it. The latter are not completely devoid of grazing impact (Table 1). Two thirds of the plots were in fact part of the game preserve for a decade after its establishment. However, at this time ungulate numbers were relatively low.

We relocated the historical plots using geographic coordinates obtained from an old forestry map with marked plot positions. In the field, we navigated to the approximate plot location with a GPS receiver and then selected the site with corresponding elevation, slope, aspect and tree species composition for resampling (Kopecký & Macek 2015; Kapfer et al. 2016). We assume that the plot re-localization error is < 50 m.

Species characteristics

We focused on three types of species characteristics potentially related to long-term increased density of ungulates: affinity to frequently disturbed habitats, species dispersal mode and endangered status. The preliminary analyses clearly indicated a higher frequency of ruderal species in the game preserve. We therefore analysed data also without ruderal species in order to find out to what extent

ruderals contributed to change. To define ruderal species, we used a data set of 20 467 relevés representing a stratified selection from the Czech National Phytosociological Database. First, we divided the plots into ruderal and non-ruderal habitats according to EUNIS classification provided in Chytrý et al. (2005). Then, we tested whether the species' relative frequency is significantly different between ruderal and non-ruderal habitats. To do this, we used a permutation test implemented through *signassoc* function from *indispecies* package (De Cáceres & Legendre 2009) for R (v 3.3.0; R Foundation for Statistical Computing, Vienna, AT). Finally, we considered all species with significantly ($P < 0.05$, 999 permutation) higher frequency in ruderal habitats to be ruderal species. According to the resulting list, almost 25% of species in our data set were classified as ruderal species. Dispersal strategies (anemochory, endozoochory, epizoochory and hemerochory) were retrieved from the D3 database (Hintze et al. 2013). Endangered species were selected based on the Czech Red List (Grulich 2012).

Data analysis

For data analyses, we focused on herb layer vegetation. The following tests were run separately for two data sets. One data set included all 245 species, the other data set comprised only 182 non-ruderal species.

We tested the hypothesis of species richness differences with two complementary approaches. For plot-level (α -) diversity, we compared the change in the number of species per plot between the sampling periods inside and outside the preserve through the two-sample Monte Carlo test with 999 permutations. For this test, we used *oneway_test* function in the *coin* package for R (Hothorn et al. 2006). For the site level, we explored the difference in species richness with sample-based rarefaction curves. We calculated the expected numbers of species in pooled samples and their 95% CI with Equation 17 from Colwell et al.

(2012). The pooled samples were analysed separately for reference and resurvey plots, inside and outside the game preserve. To assess species richness at the site level (γ -diversity), extrapolations were computed until a hypothetical number of 100 plots was reached, following Equation 18 from Colwell et al. (2012). Rarefactions and extrapolations were calculated using EstimateS v 9.1 (Colwell et al. 2012).

To test for changes in compositional heterogeneity and hypothesized taxonomic homogenization under high ungulate pressure, we compared plot dispersion in multivariate space between surveys through the permutation test developed by Anderson (2006). We performed the test separately for plots inside and outside the preserve and restricted 999 permutations within the blocks defined by plot IDs to account for non-independence between subsequent samples from quasi-permanent plots.

To explore if ungulates affected temporal changes in community composition, we tested the interaction between the survey period and plot position inside/outside the preserve using PERMANOVA (Anderson 2001). As a response variable in PERMANOVA, we used a Bray-Curtis dissimilarity matrix derived from square-root transformed percentage cover data standardized by species maxima and sample totals. A correct test of interaction required split-plot permutations, which were implemented through *shuffle* function from *permute* package (Simpson 2016). Each pair of samples from the same plot was treated as a block, survey period was permuted within the blocks and the position inside/outside the preserve was randomly reshuffled between the blocks. This was repeated 999 times and the resulting distribution of pseudo *F*-statistics was compared to the observed value.

To visualize the compositional changes, we used NMDS performed on the same dissimilarity matrix as was used in PERMANOVA. The NMDS was calculated in two dimensions with several random starts and the final configuration was PCA-rotated and scaled in half-change units through *metaMDS* function from *vegan* package (Oksanen et al. 2016). To facilitate the interpretation of the ordination, we calculated unweighted means of Ellenberg indicator values for each plot (Ellenberg et al. 1992). We visually assessed the extent of the 'mesophication' process by projecting Ellenberg indicator values for light and nutrients into the ordination space through *ordisurf* function from *vegan* R package.

We assessed differences in dispersal strategy between plots inside and outside the preserve using the two-sample Monte Carlo test with 999 permutations restricted to paired samples. Specifically, we focused on the following strategies: dispersal by wind (anemochory), animals (endo- and epizoochory) and human (hemerochory). We

used the same test to evaluate the change in the number of endangered species.

Finally, to identify species with significant change in frequency between surveys, we used multi-level pattern analysis from the *indicspecies* package. This analysis tests the association of each species to the particular group in the data and to all possible combinations of the groups (De Cáceres et al. 2010). In our case, groups were defined by the period of sampling (reference or resurvey) and plot position inside or outside the game preserve. As test statistics, we used group-equalized phi coefficient (Chytrý et al. 2002) and tested its statistical significance with 999 permutation (De Cáceres & Legendre 2009).

Results

Species richness and diversity

Species richness at the plot level increased between the surveys (Fig. 1). Congruent with this result, species richness estimated through sample-based rarefaction showed a general increase between the reference and resurvey samples (Fig. 2). This increase was significantly higher inside the game preserve ($Z = -2.295$, $P = 0.024$). However, this pattern held true only if ruderal species were present in the data sets ($Z = -0.620$, $P = 0.552$; Figs 1 and 2). Therefore, increased ungulate pressure triggered species richness increase through the expansion of ruderal species.

At the whole-site level, the area inside the preserve showed a higher increase in species richness (Fig. 2). The extrapolation to 100 plots indicated an even larger difference: the estimated number of species increased to more than 250 species for the resurvey samples inside the preserve, while for the samples outside the preserve, the increase virtually stopped after 60 plots, before reaching 200 species. The general pattern is similar for the data set with non-ruderal species, but the differences are less distinctive.

Vegetation became homogenized in the game preserve, but not outside of it (Table 2). Taxonomic homogenization inside the preserve was caused by the expansion of ruderal species, because the change in vegetation heterogeneity became insignificant after ruderal species were removed from the data set.

Species composition, functional groups and environmental conditions

Species composition changed significantly both inside and outside the preserve. However, the change was stronger inside the preserve (Table 2, Fig. 3). Interaction between survey period and plot position inside/outside the game

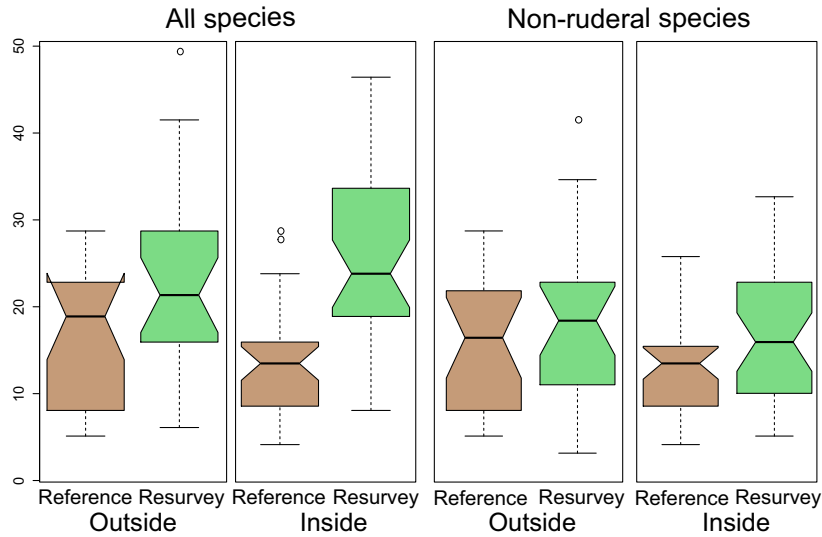


Fig. 1. Comparison of plot species richness between the reference and resurvey data sets, under high ungulate impact inside the game preserve and under ambient conditions outside the preserve. Both data sets (with all species and with non-ruderal species) were used. Heavy lines represent sample medians, boxes encompass 25–75% values, whiskers extend to the most extreme value within 1.5 times the interquartile range and dots are values outside the whiskers. Notches indicate approximate 95% CI for the medians. [Colour figure can be viewed at wileyonlinelibrary.com].

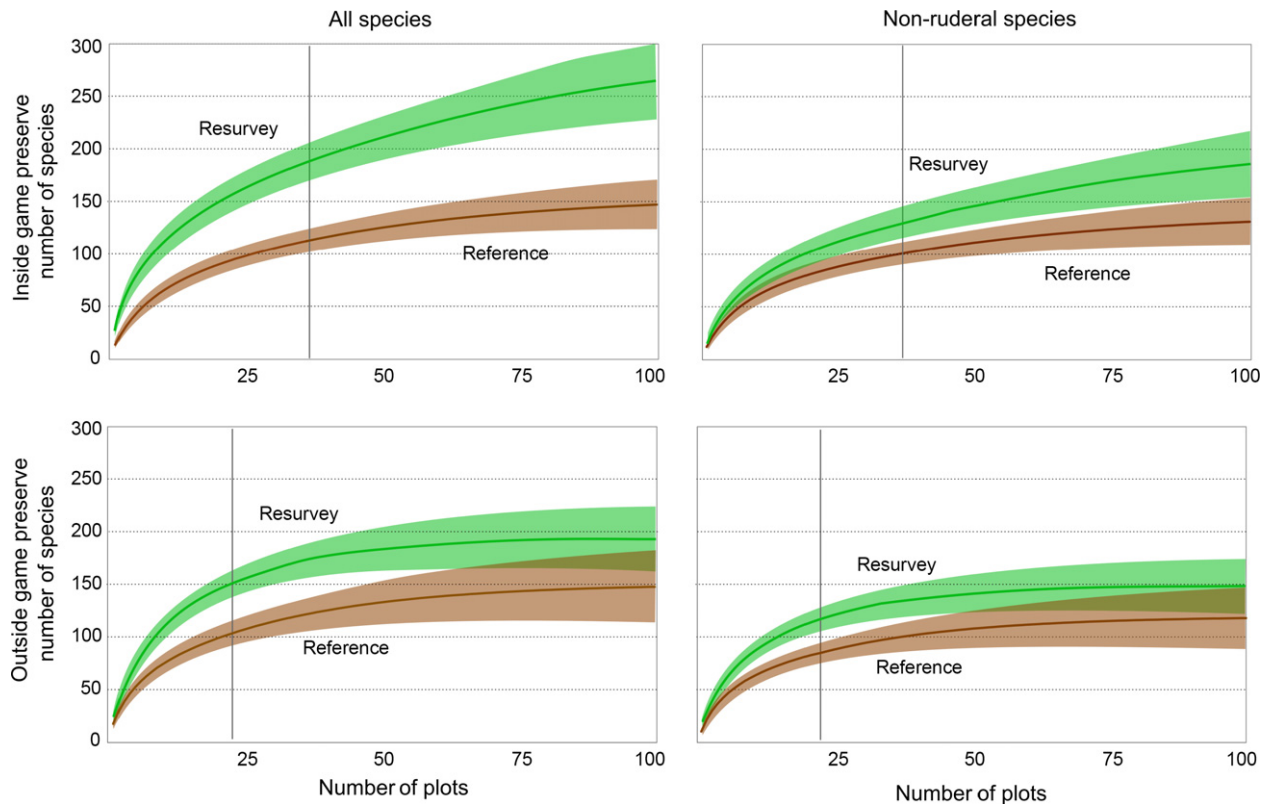


Fig. 2. Sample-based rarefaction curves of reference and resurvey plots inside (top) and outside (bottom) the game preserve. Mean estimated species numbers with 95% CI (transparent filling) are shown. Extrapolations beyond the sample size (vertical grey line) are drawn until reaching 100 plots. Data sets with all species (left) and non-ruderal species (right) were used. [Colour figure can be viewed at wileyonlinelibrary.com].

preserve was statistically significant in the whole data set as well as in the data set without ruderal species. However, variation in species composition explained by the interaction decreased by one third after removing ruderal species (all species: $F = 2.08$, $P = 0.006$, without ruderals:

$F = 1.33$, $P = 0.009$), indicating that the difference was partly caused by the expansion of ruderal species.

Compositional changes were more pronounced inside the game preserve (Fig. 3). Interestingly, when ruderal species were removed from the data set, the magnitude of vegetation change strongly decreased and was similar inside and outside the preserve (Fig. 3).

Ellenberg values indicate a shift towards more light- and nutrient-demanding plant assemblages both inside and outside the game preserve, although the trend was stronger inside the preserve (Appendix S3). Ruderal species have substantially higher Ellenberg values than other species, especially for nutrients (Appendix S4). Therefore, their increase inside the preserve strongly contributed to the observed change.

While few species, mostly species of oligotrophic habitats, were significantly associated with the reference survey, many more, mostly ruderal species, were significantly associated with the resurvey, especially inside the game preserve (Appendix S5). Endangered species significantly

Table 2. Test statistics describing temporal changes in compositional heterogeneity and species composition inside and outside the game preserve. The results are shown for the data sets with all species and for the data sets with non-ruderal species only. Asterisks indicate significant differences according to Monte Carlo permutation tests: $P < 0.05$ (*) and $P < 0.001$ (***)

	All Species		Non-Ruderal Species	
	Inside the Preserve	Outside the Preserve	Inside the Preserve	Outside the Preserve
Compositional Heterogeneity	4.82*	0.01	0.28	0.05
Species Composition	8.59***	2.62***	4.29***	1.77***

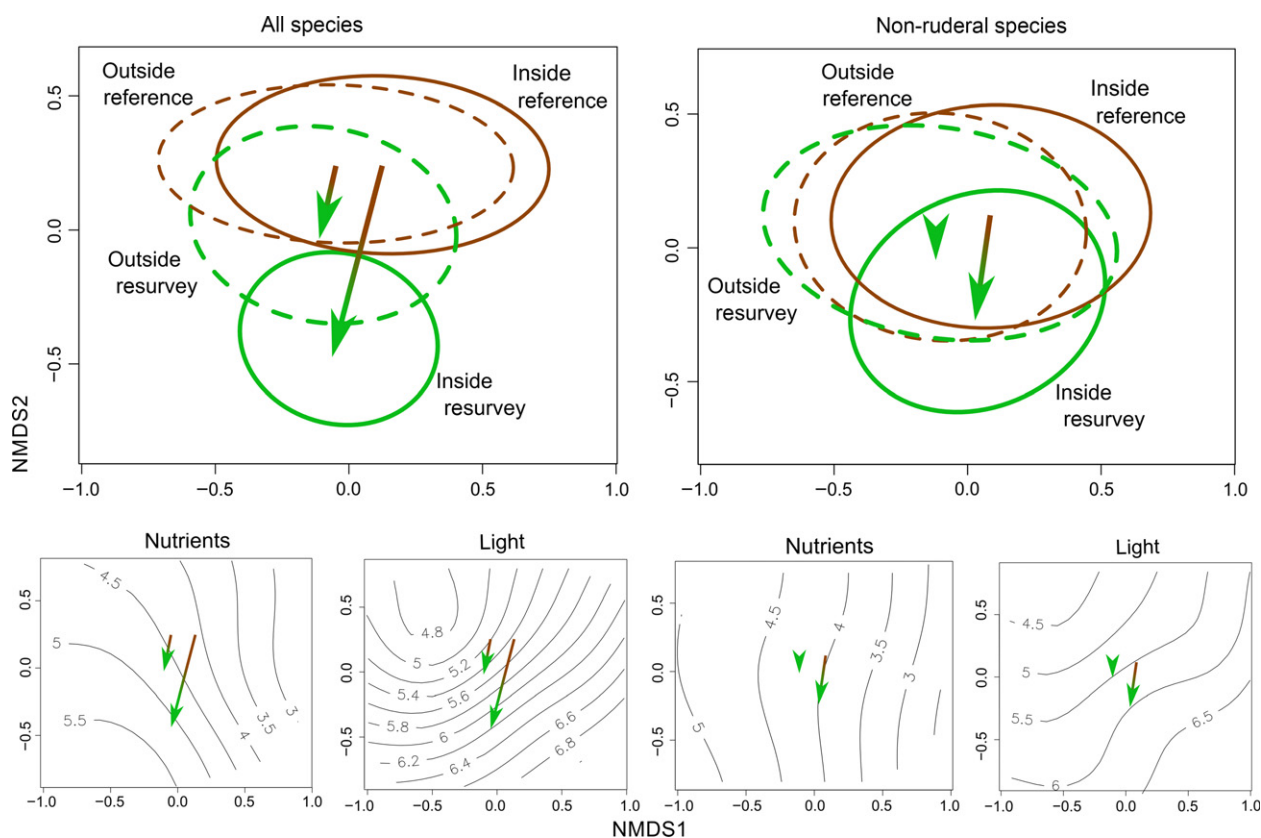


Fig. 3. Change in understory species composition displayed on NMDS diagrams for all species and non-ruderal species. The 95% dispersion ellipses show SD of sample scores of the particular group of plots: inside the game preserve (artificially increased ungulate density) and outside the preserve (ambient density), reference sampling and resurvey sampling. The arrows indicate the direction of the compositional change connecting the centroids of samples from reference to resurvey. Surface plots show isolines of mean Ellenberg indicator values fitted into the NMDS space. Species composition shifted toward more light- and nutrient-demanding species; however, these differences were smaller when only non-ruderal species were used. [Colour figure can be viewed at wileyonlinelibrary.com].

Table 3. Changes in the proportion of seed dispersal strategies inside and outside the game preserve. The test statistics values indicate increase (positive values) or decrease (negative values) of the respective strategy. Asterisks indicate significant differences according Monte Carlo permutation tests: $P < 0.05$ (*) and $P < 0.001$ (***)

	Inside the Preserve	Outside the Preserve
Anemochory	-3.439***	-0.146
Endozoochory	3.436***	0.865
Epizoochory	-2.371*	-0.633
Hemerochory	1.155	0.495

decreased inside the preserve ($Z = -3.306$, $P = 0.001$), while no significant change was observed outside of it ($Z = -1.181$, $P = 0.243$). Inside the preserve, species adapted to endozoochory strongly increased, while species adapted to anemochory and epizoochory decreased (Table 3). Proportions of dispersal strategies outside the preserve did not change.

Discussion

Unexpected increase in species richness

The increase in species richness at both the plot and the whole-site level was an unexpected result of our study. Since this increase was significantly higher in the game preserve than outside of it, we assume that ungulates are the principal drivers of this change. However, most studies from temperate forests observed a decrease in species richness as a result of ungulate impact (Chytrý & Danihelka 1993; Russell et al. 2001; Rooney et al. 2004; Stockton et al. 2005). Increasing species richness was rarely reported from temperate forests (Nessing & Zerbe 2002; von Oheimb et al. 2003). Hegland et al. (2013) suggested that the relationship between grazing intensity and species richness might be unimodal. The authors argued that moderate herbivory resulted in richer herb assemblages through the decreased dominance of woody plants. However, when grazing became too intensive, only herbivory-tolerant or -resistant species remained. Ungulate density at our study site is similar or higher than that in comparable studies (Chytrý & Danihelka 1993; Rooney & Dress 1997; Sage et al. 2003). Nonetheless, we observed a substantial increase in species richness. In the following, we present several possible factors contributing to this result.

Ruderal species as winners

Ruderals, as the most positively affected species, are linked to disturbed habitats with high levels of nutrients (Jansen et al. 2011). After 50 yr, they significantly contributed to species richness at the plot level and also at the whole-site level. Assuming ungulates are not able to migrate freely

across the fenced territory, ruderal species were probably already present in the forest, most likely in open patches and on road margins. Ungulates probably caused the spread of ruderal species to other parts of the forest.

The expansion of ruderal and exotic species corresponds to their life history, in particular their dispersal and establishment strategies (Grime 2001). Indeed, higher invasion success has been observed as a result of ungulate grazing (Eschtruth & Battles 2009; Knight et al. 2009). Compared to typical woodland species, non-forest species are more likely dispersed by ungulates, both by means of endozoochory and epizoochory (Welander 2000; Schmidt et al. 2004; Albert et al. 2015). Endozoochory turned out to be a more successful strategy than epizoochory. Plant diaspores in low-grazed herb layer vegetation are probably not able to reach the fur of animals. Notably wild boar, as an effective vector for epizoochorous dispersal (Heinken et al. 2002, 2006), were present in the study site only in small numbers, at least until 2002. Currently there are 15.6 animals-km⁻². Wild boar populations increased not only in the studied game preserve, but also in the whole region in the past few years (Havránek et al. 2010). This affected vegetation outside the preserve as well. Wild boar probably also supported the establishment of ruderals by soil disturbance, especially through rooting (Brunet et al. 2016).

Contrary to our expectations, neither supplementary feeding nor intensive human and wheeled traffic resulted in an increase of species dispersed by humans. However, feeding represents a quite substantial human intervention in the studied forest ecosystem, because it adds massive amounts of readily available nutrients to the system through animal excrements. According to the local forest manager, the amount of supplementary fodder is currently 23 t of corn, barley, oat and straw per square km yearly.

Why not many losers?

It is widely acknowledged that deer overabundance may pose a threat to indigenous woodland communities (Hobbs & Huenneke 1992; Habeck & Schultz 2015). Despite the high level of ungulate pressure and the related disturbances, the ruderal-free species assemblages of the 1960s remained relatively stable, showing low diversity losses. This effect is more challenging to explain than the above-discussed increase of ruderals.

European lowland woodlands generally changed from light, open-canopy communities towards mesophilous forests with dense shrub layers during the past decades (Kwiatkowska 1994; Hédl et al. 2010; Kopecký et al. 2013). As a result, the forest floor became shadier and moister, leading to the local extinction of light-demanding species. By contrast, shade-tolerant species were able to survive (Kopecký et al. 2013). Interestingly, our results

suggest that the high density of ungulates in Krumlov Wood likely prevented the succession observed in other regions.

By suppressing the regeneration of woody species, ungulates helped light-demanding species to survive. Several authors explained the increased richness of vascular plants as the combined result of grazing and other disturbances, e.g. canopy gaps (von Oheimb et al. 2003; Naaf & Wulf 2007; Royo et al. 2010). In the absence of herbivory, woody plants dominate these places. Improved light conditions can also result in the expansion of unpalatable native species such as *Pteridium aquilinum* or *Dennstaedtia*, out-competing low-stature species (Horsley et al. 2003). The absence of such species in the study area allowed for the occurrence of other species. Neither ruderal nor alien species caused the impoverishment of the original community, as they did at similar sites (Habeck & Schultz 2015). Although species richness increased at the plot level, taxonomic homogenization occurred at the site level. This is in congruence with the hypothesis of homogenization impact of ungulates, observed so far mainly in North American forests (Rooney et al. 2004; Rooney 2009). Interestingly, the main driver of this homogenization was the expansion of ruderal species rather than the loss of rare indigenous species.

Another mechanism potentially underlying no overall species loss can be the relatively free migration of ungulates over the large game preserve. The pressure on the understorey vegetation was not beyond a limit critical for the long-term survival of most species. Such intermittent temporal pattern of herbivory can allow sufficient regeneration of forest plant species (Augustine & McNaughton 1998). Small exclosures established by foresters in the present large preserve to enable tree regeneration might have also served as a refuge for some species. A further possible explanation is the long history of game-keeping at the study site (see Appendix S2). From the evolutionary perspective, communities with a long history of grazing are more resistant to changes (Milchunas & Lauenroth 1993). Grazing in the past centuries probably had the same effect. The vegetation may have already been stabilized in the past, with species sensitive to grazing mostly absent in the original data set (Kirby 2001). Finally, large plot size could be another factor. Plant species were often found in low abundances but still detectable in a 400-m² plot.

'Mesophication' and associated species losses did not occur outside the preserve, either. However, many of the sites sampled outside the preserve were under high levels of grazing impact no more than 20 yr ago. Vegetation composition is probably still partly influenced by past grazing there (Nuttall et al. 2014).

Overall composition of woodland species did not change, but endangered species decreased. Although lower

grazing tolerance could be a factor, originally low abundance of endangered species is the more likely cause. Maintaining high levels of grazing may therefore eventually deteriorate the populations of other rare and sensitive species. Some woodland species may balance on the brink of extinction, soon having to pay their extinction debt (Rogers et al. 2009; Martin et al. 2011). Therefore, in game preserves in acidophilous temperate woodlands, it would be advisable to reduce ungulate numbers or to decrease their impact on plant populations in another way, for example by increasing biomass production by overstorey thinning (Naaf & Wulf 2007; Vild et al. 2013). By making the woodland less dominated by trees with high shade-casting ability, economic demands and nature conservation goals can be simultaneously met.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Location of the study area.

Appendix S2. Numbers of killed animals from 1659 to 1965.

Appendix S3. Distribution of mean Ellenberg values for light and nutrients.

Appendix S4. Ellenberg indicator values for ruderal species and non-ruderal species.

Appendix S5. List of characteristic species.