

RESEARCH ARTICLE

Exclusion of large herbivores affects understorey shrub vegetation more than herb vegetation across 147 forest sites in three German regions

Deborah Schäfer^{1*}, Daniel Prati¹, Peter Schall², Christian Ammer², Markus Fischer¹

1 Institute of Plant Sciences, University of Bern, Bern, Switzerland, **2** Silviculture and Forest Ecology of the Temperate Zones, University of Göttingen, Göttingen, Germany

* deborah.schaefer@boga.unibe.ch



Abstract

Background

Many studies have analysed the effect of browsing by large herbivores on tree species but far fewer studies have studied their effect on understorey shrubs and herbs. Moreover, while many studies have shown that forest features and management intensity strongly influence understorey vegetation, the influence of such variation on the effect of large-herbivore exclusion is not known.

This study

In this study, we analysed changes of species richness, Shannon diversity, evenness and cover of understorey herbs and shrubs after excluding large herbivores for seven years on 147 forest sites, differing in management intensity and forest features, in three regions of Germany (Schwäbische Alb, Hainich-Dün, Schorfheide-Chorin). Further, we studied how the effect of large-herbivore exclusion on understorey vegetation was influenced by forest management intensity and several forest features.

Results

As expected, exclusion of large herbivores resulted in highly variable results. Nevertheless, we found that large-herbivore exclusion significantly increased cover and Shannon diversity of shrub communities, while it did not affect herb communities. Forest management intensity did not influence the effect of large-herbivore exclusion while some forest features, most often relative conifer cover, did. In forests with high relative conifer cover, large-herbivore exclusion decreased species richness and cover of herbs and increased Shannon diversity of herbs and shrubs, while in forests with low relative conifer cover large-herbivore exclusion increased species richness and cover of herbs, and decreased Shannon diversity of herbs and shrubs.

OPEN ACCESS

Citation: Schäfer D, Prati D, Schall P, Ammer C, Fischer M (2019) Exclusion of large herbivores affects understorey shrub vegetation more than herb vegetation across 147 forest sites in three German regions. PLoS ONE 14(7): e0218741. <https://doi.org/10.1371/journal.pone.0218741>

Editor: Martin Schädler, Helmholtz Zentrum München Deutsches Forschungszentrum für Umwelt und Gesundheit, GERMANY

Received: January 21, 2019

Accepted: June 7, 2019

Published: July 10, 2019

Copyright: © 2019 Schäfer et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: Data is stored in the BExIS database of the Biodiversity Exploratories program. It is publicly available under the URL <https://www.bexis.uni-jena.de/PublicData/About.aspx> and can be found with the Dataset ID 25366.

Funding: The work has been funded by the DFG Priority Program 1374 "Infrastructure-Biodiversity-Exploratories" (FI 1246/15-2; PR 728/2-3), which was received by DP and MF. The funders had no role in study design, data collection and analysis,

decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Conclusion

We suggest that browsing by large herbivores should be included when studying understorey shrub communities, however when studying understorey herb communities the effects of browsing are less general and depend on forest features.

Introduction

The influence of herbivores on the diversity and composition of Central European forest communities is subject to a long and controversial debate (e.g. [1,2]). Humans have driven many large predators to extinction and hunting does not seem to fully compensate the lack of predators [3,4]. Together with the reduction of natural predators and partly low hunting intensity, an increase in forage quality through agricultural and silvicultural activities (i.e. overall higher nutrient input) and landscape fragmentation has led to a higher number of large herbivores during the last decades [5–7]. These large herbivores, in Central Europe mostly red deer, roe deer, fallow deer and wild boars, can severely damage forest plants by consuming herbs, buds of shrubs and trees, and plant roots. However, the extensive literature on effects of large herbivores in forests mostly focused on tree species [6,8,9], including studies on tree seedlings and saplings [10,11], showing reduced growth [12], shifts in size structure [13,14] and most importantly a homogenisation of tree composition [15,16]. In contrast, the influence of large herbivores on understorey shrubs and herbs has been less studied, even though understorey vegetation is important for abundance and species richness of other organisms, forest succession and ecosystem processes [6,17–20]. Studies that did analyse the influence of large herbivores on understorey vegetation found for example that large herbivores may affect plant cover, species richness or the homogenization of the understorey vegetation (i.e. increase in dominance of some species) [5,9,21–25]. However, previous studies were either not located in Central Europe [25], focused on a single type of forest (e.g. broadleaf forests [21]; ancient forests, [22]) or were constrained in their number of replicates and therefore struggled with the large variation of treatment effects [6,22,23]. Thus, for a comprehensive assessment of the role of herbivory in Central European forests, more multi-site and large-scale studies across different forest types are needed that include shrub and herb species in understorey vegetation.

For several reasons the effect of large-herbivore exclusion on understorey plant communities is expected to be highly variable. Large herbivores differ in their preference for different forest types [26] and plant species [24,27–29], thereby showing pronounced site- and species-specific effects. Whereas the effect of large herbivores is generally considered a disturbance for plants, due to the damage caused while browsing, plants differ in their sensitivity to browsing by herbivores. Some species can tolerate damage by large herbivores [6,13,30] or even overcompensate moderate damage caused by herbivores [6,13,29], thus sometimes shifting entire forest plant communities [6,19]. In addition, large herbivores can affect understorey vegetation positively, for instance by promoting seed dispersal by endo- or epizoochory (endozoochory: [31]; epizoochory: [32–35]). This indicates that the impact of large herbivores strongly depends on their density and their general behaviour, which includes foraging for specific species, creating disturbances, defecating or wallowing [27]. Taken together, the expected large variation of the impact of large herbivores on understorey plants require well-replicated studies for a comprehensive understanding of the influence of large-herbivore exclusion on forest vegetation [6].

Many studies have shown that understorey vegetation is strongly influenced by different forest features and management intensity [36–38]. An effect of large-herbivore exclusion on

understorey vegetation may differ systematically in different forest types, characterised by different forest features and management intensity, either due to the different composition of the understorey vegetation or the influence on the density or identity of large herbivores. For example, the effect of browsing by ungulates on understorey vegetation in low light conditions of a dense forest stand differs from the effect in more open conditions where understorey vegetation cover and species richness is much higher. Even though previous studies suggested that forest features and management possibly influence responses of understorey vegetation to browsing and prevent a final conclusion whether browsing decreases or increases species richness in understorey vegetation (Hester *et al.* 2006), the influence of forest features and management over a large gradient has not been thoroughly tested so far. The two studies that analysed the influence of management on browsing effects on plants differentiated between categories of management only (i.e. clear-cuts and uncut [39]; or clear-cuts, thinning, uncut [40]). It remains important to disentangle how a gradient of different forest features and management intensities influences the possibly context-dependent effect of large herbivores on understorey plants. A large gradient of forest sites with differing forest features, species composition and management intensity needs to be studied.

We experimentally tested the influence of large-herbivore exclusion on forest understorey vegetation, focusing on shrub and herb species, across a wide range of different Central European forests. We excluded large herbivores with fences for seven years on 147 forest sites within the Biodiversity Exploratories program. The forest sites were located in three different regions of Germany (Schwäbische Alb, Hainich-Dün, Schorfheide-Chorin) and included the main forest and management types typical for Central Europe [41]. In particular, we asked i) Do large herbivores reduce the species richness, evenness and vegetation cover of shrubs and herbs? ii) Does the influence of large herbivores on shrub and herb communities depend on certain forest features or management intensity?

Materials and methods

Study sites

We conducted this study within the framework of the Biodiversity Exploratories program. The Biodiversity Exploratories program serves as an open research platform to perform long-term and large-scale studies about the relationships between land-use intensity, biodiversity and ecosystem functioning. Our 147 forest study sites are located in three regions of Germany; the Schwäbische Alb (southwestern Germany, 50 sites), the Hainich-Dün (central Germany, 47 sites) and the Schorfheide-Chorin (northeastern Germany, 50 sites). The size of the forest sites is 100 m x 100 m and they are distributed over an area of approximately 30 km x 30 km in each region (S1 Fig). They represent a range of management practices typical for the respective regions, including managed, even-aged forest with conifers replacing the natural vegetation, managed even-aged and managed uneven-aged forests with natural species and formerly managed forests left unmanaged for decades. More information on the sites, the management and the geology, topography and climate of the three regions can be found in [41,42].

Forest management and structure

To characterize forest management intensity, we used two previously developed indices, the Forest Management Intensity Index ForMI [43] and the Silvicultural Management Index SMI [44]. The ForMI includes the proportion of harvested tree volume, the proportion of tree species that are not part of the natural forest community and the proportion of dead wood that showed signs of saw cuts. The three proportions were then summed, resulting in the ForMI ranging from 0 to 3. The SMI considers an age- and species-specific risk component of stand

loss and a density component as a deviation of natural self-thinning. The two components were then summed, resulting in a SMI ranging from 0 to 1.1. The two measures of forest intensity were highly correlated ($r = 0.796$).

In addition to forest management intensity, we included seven forest features to describe differences among our forest sites. The first forest feature captured variation in soil conditions, as higher nutrient availability might promote regrowth of plants after damage by large herbivores [45]. For this, we used the first axis of a PCA on soil variables, which included the concentrations of nitrogen, carbon, phosphorous, sulphur, soil texture, pH and the content of water and stones (hereafter called PC1 soil). The second feature was the cover of herbs and shrubs, measured independently with vegetation records on 20 m x 20 m plots on each forest site. Higher cover of residential plants may attract more herbivores, thereby potentially increasing browsing levels [46]. Further features were relative conifer cover in percentage of the whole canopy, total canopy cover and the mean diameter at breast height (DBH) of the 50 largest trees measured on each forest site, which are expected to affect understorey vegetation and the density of large herbivores. Details on the measurements of these forest features are in the [S1 Material](#). Variation of the forest features for the different regions are shown with density curves in [S2 Fig](#).

Large herbivore exclusion experiment

On 150 forest sites, we established a herbivore exclusion experiment in 2008. For this, we fenced an area of 12 m x 12 m with a fence of 190 cm height. The mesh size of the fence increased with increasing height. The mesh size was 5 cm x 15 cm up to 80 cm height, 10 cm x 15 cm from 80–110 cm height and 15 cm x 15 cm above 110 cm. In 2013, we selected and permanently marked two 5 m x 5 m plots on 147 forest sites, one within the fenced area (fenced plots) and one outside the fenced area (unfenced plots). Out of 150 forest sites from the Biodiversity Exploratories program we excluded one forest site due to a severe logging event, making comparisons to other sites impossible and two other forest sites consisting of such a dense thicket that prevented fieldwork. Within forest sites, the fenced and unfenced plots were separated by no more than 5 m and were selected to be similar in terms of tree and shrub layer. As a measure of herbivore pressure, we counted the number of saplings, i.e. trees higher than 20 cm, with a diameter at breast height of less than 7 cm, and recorded the percentage of browsed saplings in the unfenced plots of each site in early spring 2014.

In August 2015 and April 2016, we identified all vascular plant species growing in the fenced and unfenced plots and estimated the cover percentage of each species. With these data we calculated species richness S , exponential of the Shannon index of diversity as $\exp(H)$ using the R package 'vegan' version 2.4–3 [47] and evenness as $evar$ calculated with the R package 'codyn' version 2.0.2 [48]. Additionally, we estimated the total cover percentage of the herb layer (non-woody plants) and shrub layer (woody plants smaller than 5 m). To cover the summer and spring aspects, we recorded the vegetation in summer 2015 and spring 2016, but used the higher cover estimates whenever a species was present in both records.

Statistical analysis

To analyse the effect of large-herbivore exclusion on understorey vegetation, we calculated the difference (fenced-unfenced) and log response ratio (lnRR) ($\ln(\text{fenced/unfenced})$) of species richness, evenness, $\exp(H)$ and total cover separately for herbs and shrubs. We excluded forest sites without any herbs or shrubs on both fenced and unfenced plots. Furthermore, for species that occurred on more than 15 plots, we also calculated the difference and lnRR of their cover. Positive differences and lnRR of larger than zero indicate a positive effect of the exclusion of large herbivores on understorey plants.

To test whether the herbivore pressure, and hence the magnitude of herbivore exclusion, differed among forest sites we calculated a model with regions, forest features and browsing percentage in the unfenced plots as a response variable. Additionally, as we expected large variation in response to herbivore exclusion, we also tested whether the variance of our response variables changed with increasing browsing pressure. Thus, we compared the variance of the response variables among plots below and plots above the median of browsing percentage with an F-test.

We used the differences and lnRRs as response variables in several linear models to test how forest management or forest features influence the effect of large-herbivore exclusion. First, we calculated a linear model with the intercept only, which would indicate an overall significant difference of the vegetation between the fenced and unfenced plot, hence a treatment effect. Second, we calculated linear models containing the region, either of the two forest management indices (SMI or ForMI), and their interaction with region, and browsing percentage as a co-variable. Third, we calculated linear models containing the forest features and the browsing percentage. In that case, we simplified models by minimizing the Akaike information criterion (AIC). We tested all models graphically for normality and heteroscedasticity of the residuals and checked whether correlations between response variables were lower than 0.7 to avoid multicollinearity (S3 Fig).

Ethics statements

The forest sites are partly owned by private persons and partly owned by the state. Fieldwork permits were issued by the responsible state environmental offices of Baden-Württemberg, Thüringen, and Brandenburg (according to § 72 BbgNatSchG). No rare species were sampled during the fieldwork.

Results

In the unfenced plots, we found that the percentage of browsed saplings increased from 10% in the Schwäbische Alb and 12% in Hainich-Dün to 28% in Schorfheide-Chorin (S4 Fig). Furthermore, browsing increased in forests with higher cover of herbs (estimate: 0.06) and relative conifer cover (estimate: 1.46). We found traces of browsing on one sapling in each of only two fenced plots in Hainich-Dün. Overall, this indicates that our large-herbivore-exclusion treatment effectively reduced herbivory in our forest sites.

On a relative scale, large-herbivore exclusion increased the total cover and Shannon diversity of shrubs significantly, whereas it did not significantly affect species richness or evenness of shrubs and any measures of herbs (Table 1). On an absolute scale, large-herbivore exclusion increased the cover of shrubs by 13 percentage points, whereas all other measures were not affected significantly (Fig 1, Table 1).

Among the 152 herb species, 30 species were present on more than 15 forest plots. Three of these were affected by the exclusion of large herbivores. Large-herbivore exclusion increased the abundance of *Arum maculatum* L. (found on 15 plots, lnRR estimate: 0.51, p-value: 0.008) and *Anemone ranunculoides* L. (found on 33 plots, lnRR estimate: 0.81, p-value: 0.09) and decreased the abundance of *Viola reichenbachiana* Boreau (found on 56 plots, lnRR estimate: -0.23, p-value: 0.027).

We also found that the variance of treatment effects was influenced by browsing percentage for some response variables. In forest sites with high browsing percentage the variance of the difference of cover of herbs between fenced and unfenced plots (df: 51, 51; F-value: 2.15) and shrubs (df: 51, 51; F-value: 3.00), and the difference (df: 49, 50; F-value: 4.80) and lnRR (df: 51, 51 F-value: 2.04) of Shannon diversity of herbs was increased.

Table 1. Influence of forest features on absolute and relative treatment effects.

	Δ (inside—outside the fenced area)							
	Herbs				Shrubs			
	Species richness	Diversity	Evenness	Cover [%]	Species richness	Diversity	Evenness	Cover [%]
Intercept only	-0.336	-0.043	0.024	-1.544	0.129	0.149	0.082	2.974 *
Region	-	-	-	-	-	-	-	*
Mean Schwäbische Alb	-	-	-	-	-	-	-	0.56
Mean Hainich-Dün	-	-	-	-	-	-	-	1.59
Mean Schorfheide-Chorin	-	-	-	-	-	-	-	6.68
PC1soil	-	-	-	-	-	-	-	-
Cover herbs	-	-0.007 *	-0.001	-	-	-	-	-
Percentage cover conifers	-3.211 *	-	-	-13.149 *	-	-	-	-
Mean DBH max 50	-0.075 *	-	-	-	-	-	-	-
Canopy cover	-	-	-	-	-	-	0.610 *	-
Number sapplings	-	-	-	-	-	-	-	-
Browsing percentage	-	-0.007	-	-	-	-	-	-
	lnRR (inside /outside the fenced area)							
	Herbs				Shrubs			
	Species richness	Diversity	Evenness	Cover [%]	Species richness	Diversity	Evenness	Cover [%]
Intercept only	-0.014	-0.001	0.055	0.040	0.117	0.062 *	0.109	0.241 *
Region	-	-	-	*	-	-	-	-
Mean Schwäbische Alb	-	-	-	0.01	-	-	-	-
Mean Hainich-Dün	-	-	-	-0.13	-	-	-	-
Mean Schorfheide-Chorin	-	-	-	0.28	-	-	-	-
PC1soil	-	-	-	0.200	-	-	-	-0.071 *
Cover herbs	-	-	-	-	-	-	-	-
Percentage cover conifers	-0.352	0.241 *	-	-	-	0.241 *	-	-
Mean DBH max 50	-0.009	-	-	-	-	-	-	-
Canopy cover	-	-	-	-	-	-	0.596 *	-
Number sapplings	-	-	-	-	-	-	-	-
Browsing percentage	-	-	-	-	-	-	-	-

Results originate from linear models on the change in species richness, diversity, evenness and cover of understorey herbs and shrubs based on the difference (top) and the log-response ratio (lnRR, bottom) of the values inside vs. outside the fenced area in response to region and different forest features. The percentage of browsed tree sapplings was also included as a co-variable. Intercept only indicates results from a null-model containing only the intercept. Estimates of the linear models are given after model simplification minimizing AIC. Asterisks indicate significant effects at $p < 0.05$, the respective estimates are written in bold letters and marginally significant effects at $p < 0.1$ are written normally). For significant region effects, we show the mean value of each region.

<https://doi.org/10.1371/journal.pone.0218741.t001>

Both forest management indices did not significantly influence the treatment effects and therefore explained little variation in the effects of large-herbivore exclusion. This indicates that the effects of large-herbivore exclusion were largely independent of management intensity (S1 and S2 Tables).

Some forest features influenced the treatment effects (Table 1, Figs 2 and 3). When considering the difference (inside–outside the fenced area) we found that in forests with high relative conifer cover, large-herbivore exclusion decreased the species richness and cover of herbs, while in forests with low relative conifer cover large-herbivore exclusion increased the species richness and cover of herbs (Fig 2A and 2C). In forests with large mean DBH large-herbivore exclusion decreased the species richness of herbs, while in forests with small mean DBH large-herbivore exclusion increased the species richness of herbs (Fig 2B). In forests with high cover

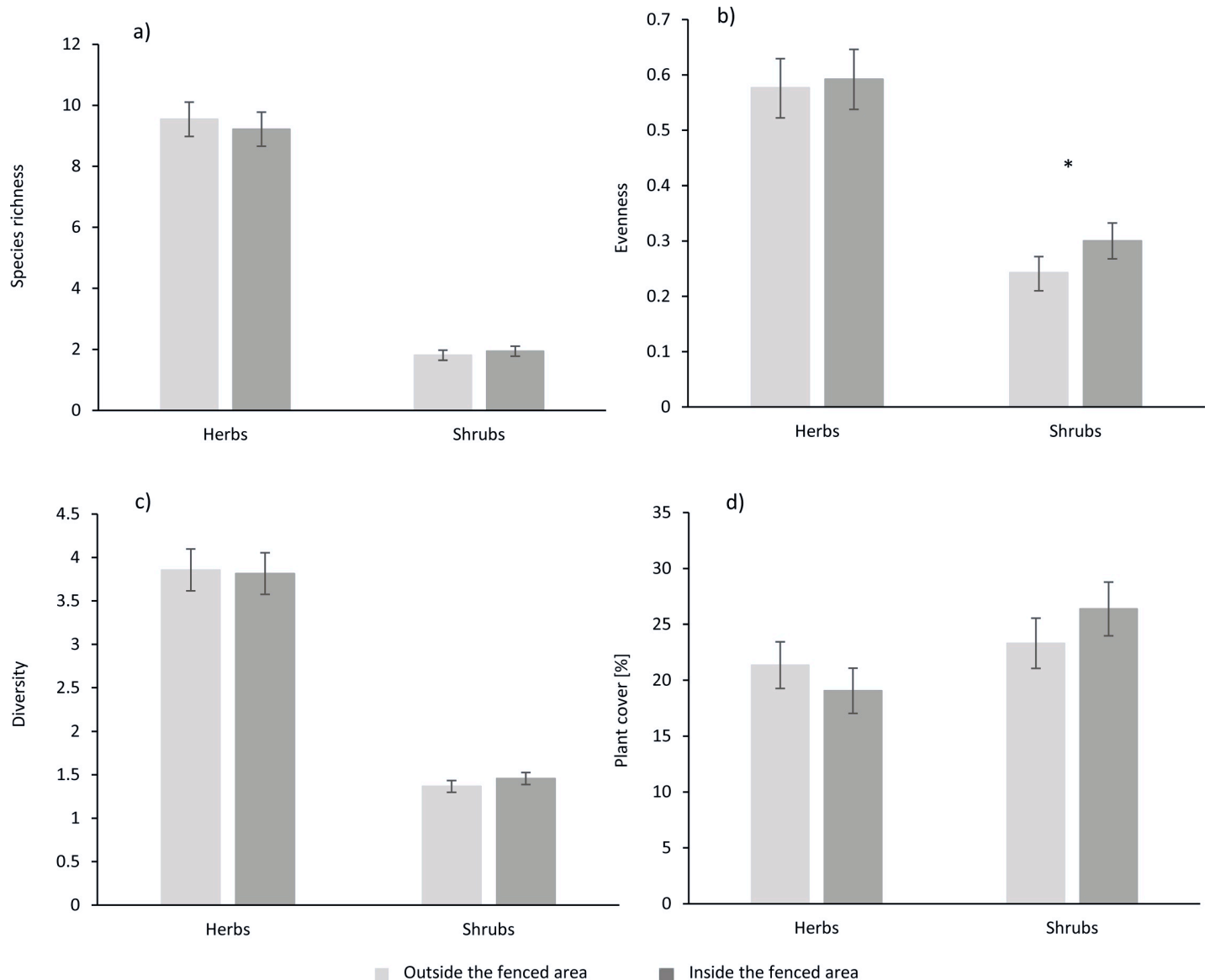


Fig 1. The effects of herbivore exclusion on (a) species richness, (b) evenness, (c) diversity ($\exp(H)$), (d) plant cover of herbs and shrubs in 147 forest sites of three regions in Germany (means \pm standard error). A star indicates significant difference between the 5 m x 5 m plots outside the fenced area (bright grey) and the 5 m x 5 m plots inside the fenced area (dark grey).

<https://doi.org/10.1371/journal.pone.0218741.g001>

of herbs, large-herbivore exclusion decreased the Shannon diversity of herbs, while in forests with low cover of herbs large-herbivore exclusion increased the Shannon diversity of herbs (Fig 2D). In forests with high canopy cover, large-herbivore exclusion increased the evenness of shrubs (Fig 2E), while in forests with low canopy cover large-herbivore exclusion decreased the evenness of shrubs.

When considering relative values ($\ln RRR$ (inside/outside the fenced area)) instead of the difference, we found that in forests with high relative conifer cover, large-herbivore exclusion increased the Shannon diversity of herbs (Fig 3A), while in forests with low relative conifer cover large-herbivore exclusion decreased the Shannon diversity of herbs. In forests with high relative conifer cover, large-herbivore exclusion more strongly increased the Shannon diversity

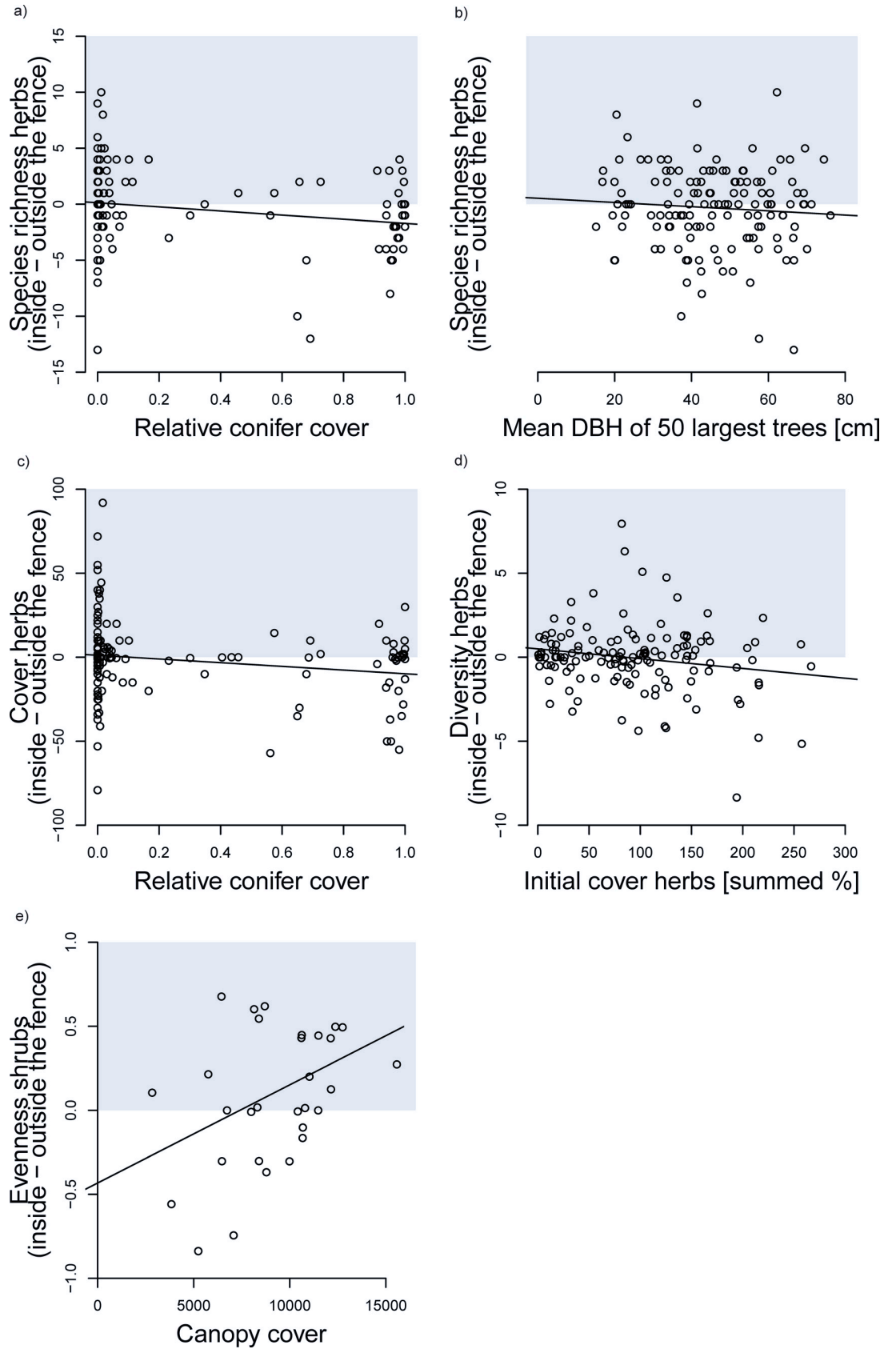


Fig 2. Significant influences of forest features on absolute treatment effects (inside the fence–outside the fence), i.e. the difference. Positive values indicate increased response variables in fenced plots, negative values indicate decreased response values in fenced plots. We see (a) difference of species richness of herbs against relative conifer cover (b) difference of species richness of herbs against mean DBH of the 50 largest trees (c) difference of cover of herbs (all herbs estimated together) against relative conifer cover (d) difference of diversity of herbs (exp(H)) against initial cover of herbs (summed cover of individual species measured independent of treatment plots in spring and summer, resulting in values ranging from 0.1–266) (e) difference of evenness of shrubs against canopy cover.

<https://doi.org/10.1371/journal.pone.0218741.g002>

of shrubs than in forests with low relative conifer cover (Fig 3B). In forests with high canopy cover, large-herbivore exclusion increased the evenness of shrubs (Fig 3C), while in forests with low canopy cover large-herbivore exclusion decreased the evenness of shrubs. Moreover, we found that in forests with high PC1 soil values (nutrient rich, clayey soils) large-herbivore exclusion increased shrub cover less strongly than in forests with low PC1 soil values (Fig 3C).

Discussion

Despite the large variability of responses to the exclusion of large herbivores, we found a general increase in Shannon diversity and cover of shrubs after large-herbivore exclusion. The absence of browsing damage allowed for unhindered growth of the shrubs that were already present and increased the Shannon diversity of shrubs. This most likely indicates plant-specific preferences of herbivores, most likely roe deer, which has been shown to have more selective browsing habits than other large herbivores [21,49]. Such selective browsing leads to specific shrub species suffering more than others and those browsing-sensitive shrubs increase in cover when herbivores are excluded. Thus our multi-site study is in agreement with the conclusions of Coté et al. [6] who show in their review that high levels of browsing decreased abundance and complexity in understorey plant communities. Our results are also in line with [50], who found that biodiversity decreased if herbivore changes the dominance structure of the plant community.

In contrast to the shrubs, the exclusion of large herbivores showed no overall effects on species richness, Shannon diversity, evenness and cover of herbs. The absence of any consistent effect of the exclusion of large herbivores on understorey herbs can have several reasons. On the one hand, large variation in our results suggests that the influence of forest features and management intensity on understorey herbs might be stronger than the effect of large herbivores and therefore mask an effect of large-herbivore exclusion on understorey herbs. On the other hand, previous studies have shown that persistent seed banks in forests are scarce [51–53], and that many herbaceous species are dispersal-limited [54] and rely on dispersal by large herbivores [33,55]. The herbaceous plants that we found on our forest sites may display different strategies (resistance or tolerance) to survive under the increasing pressures of large herbivores since the beginning of the twentieth century [5,7,56]. Less defended plants could not be introduced in the fenced plots or did not establish during the seven years of large herbivore exclusion. Alternatively, a negative effect of large herbivores on herbaceous plants via browsing may have been compensated by a positive effect of seed dispersal by large herbivores in the unfenced plots [57] or a negative effect of increased shrub cover on light availability for herbs in the fenced plots [24,58]. Further, the effect of non-migratory, large herbivores on forest vegetation might be strongest in winter when food is limited, which could at least partly explain the lack of an effect on understorey herbs. Instead, seven years of herbivore exclusion may have been too short for inducing major shifts in the understorey vegetation as previous studies also suggested that overbrowsing by large herbivores can push plant communities into an alternative stable state, from which recovery is expected to be slow [19,59,60]. The increase in

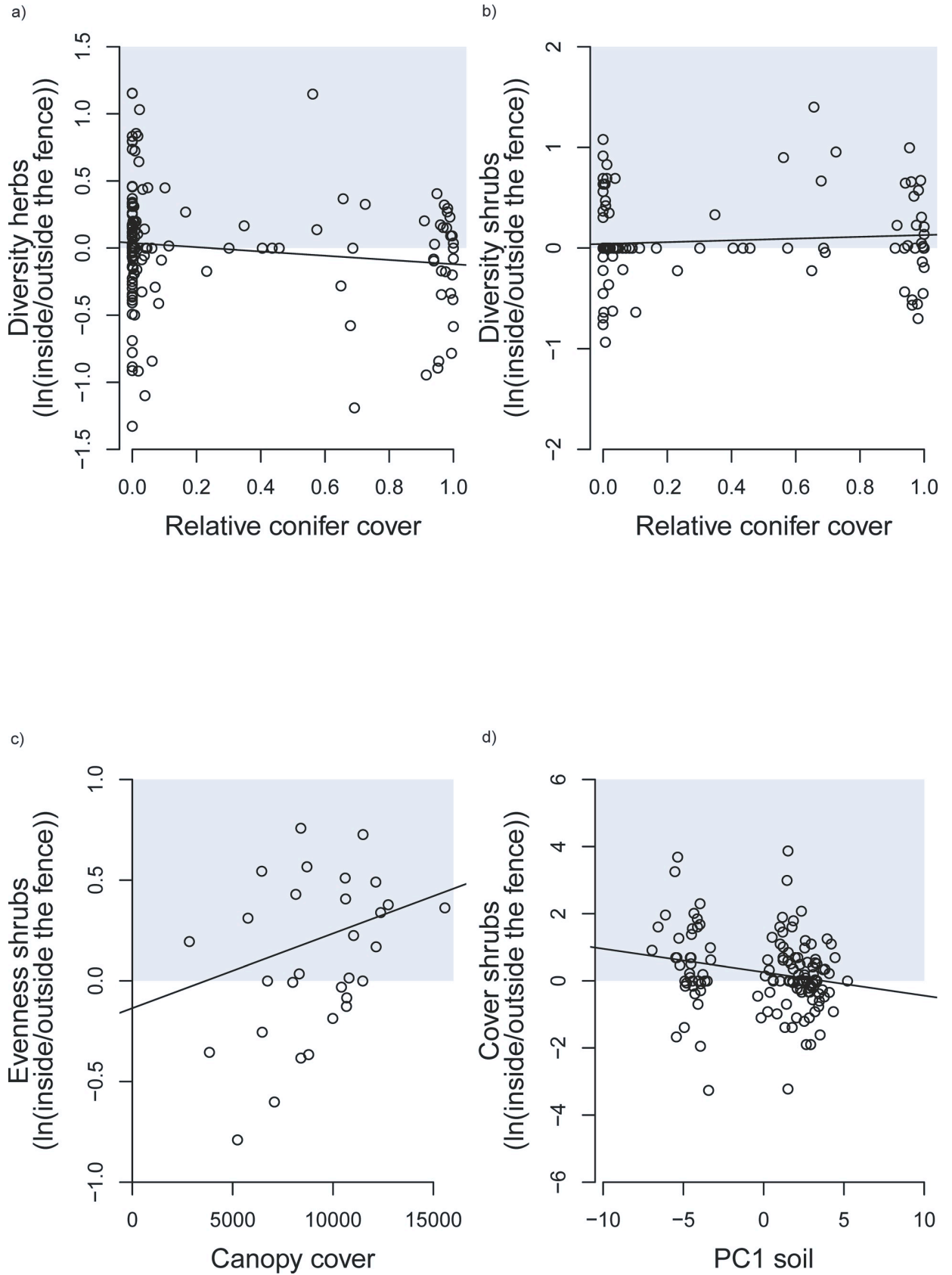


Fig 3. Significant influences of forest features on relative treatment effects (ln(inside the fence/outside the fence)), i.e. lnRR. Positive values indicate increased response variables in fenced plots, negative values indicate decreased response values in fenced plots. We see (a) lnRR diversity (exp(H)) of herbs against percentage cover of conifer (b) lnRR diversity of shrubs against percentage cover of conifers (c) lnRR evenness of shrubs against canopy cover (d) lnRR cover shrubs against PC1 soil (low values indicate clay, alkaline soils, rich in organic nitrogen and high values indicate sandy, acid soils, poor in organic nitrogen).

<https://doi.org/10.1371/journal.pone.0218741.g003>

shrub cover together with the lack of seed dispersal and a generally slow recovery of the understorey plant community after large-herbivore exclusion could explain our findings.

We found little evidence that different forest features or management intensity influenced the effects of large-herbivore exclusion on the understorey vegetation. For example, relative conifer cover, the forest feature that most often influenced treatment effects, correlated positively with browsing percentage, initial shrub cover and a more diverse understorey. Thus, the influence of relative conifer cover on the treatment effects could partly be caused by an underlying increase of herbivore pressure, which increased with relative conifer cover or a stronger increase of the Shannon diversity of shrubs and herbs in fenced plots, because competitive species, that are undefended, could increase their cover more in more diverse understoreys. Many previous studies reporting that forest features and management have strong influences on understorey vegetation have not considered effects of large herbivores [36,38,61,62]. Our study suggests that the effects of large herbivores on understorey vegetation, especially of herbs, is indeed relatively small compared with the effects of forest features and management intensity and can be dependent on them.

Consequences of excluding large herbivores strongly depend on the actual density of large herbivores and their general behaviour at the sites, such as foraging for specific species, creating disturbances, defecating or wallowing [6]. All these behaviours may affect plant communities, but are highly context-specific. We therefore expected large variation in our treatment effect, which can make it difficult to find significant effects, even if sample size is large [27]. Part of the large variation found in our data could also be caused by small-scale differences of the fenced and unfenced areas, which could not be quantified by our explanatory variables, which were partly measured on a larger scale. Nevertheless, our results support that variation in treatment effects was higher at sites with higher browsing percentage.

Conclusion

We advocate the implementation of long-term studies over several decades to analyse the potentially very slow recovery of understorey plant communities after large-herbivore exclusion. We also stress the need of large-scale studies to account for the large variation in the response of understorey plant communities after exclusion of large herbivores. Despite large variation in the response of understorey plants to large-herbivore exclusion, we found some consistent changes in shrub cover and Shannon diversity, most likely caused by selective browsing of herbivores. However, we did not find changes of herbaceous vegetation seven years after large-herbivore exclusion, indicating that, compared with other forest features and management intensity, browsing is not strongly influencing understorey herbs. Lastly, we suggest that browsing should generally be considered when studying understorey shrubs. When studying understorey herbs browsing seemed to be generally of lesser importance, however it should still be considered in interaction with other forest feature and management.

Supporting information

S1 Material. Details on the measurements of the forest features.
(DOCX)

S1 Fig. Maps showing the location of the three study regions Schwäbische Alb, Hainich-Dün and Schorfheide-Chorin within Germany and the distribution of the 147 forest sites within the regions. The three main forest types conifer managed, broadleaf managed and broadleaf unmanaged are marked with different colours.
(DOCX)

S2 Fig. Density curves of the eight different forest features showing the range and variation within and between the three different study regions. The three different study regions are indicated with different colours. Schwäbische Alb in yellow, Hainich-Dün in red and Schorfheide-Chorin in blue.
(DOCX)

S3 Fig. Spearman correlation of all explanatory variables included in our study and the cover of herbs and shrubs and species richness of herbs and shrubs on the unfenced 5 m x 5 m plots from 147 forest sites. Red squares indicate negative correlations and blue squares indicate positive correlations. The more intense the colour the stronger the correlation.
(DOCX)

S4 Fig. Browsing intensity in 147 forest sites. Browsing intensity is indicated as the percentage of browsed saplings on a 5 m x 5 m plot in three regions in Germany (mean \pm standard error).
(DOCX)

S1 Table. Influence of ForMI (forest management intensity index) on absolute and relative treatment effects. Results originate from linear models on the change in species richness, diversity, evenness and cover of understorey herbs and shrubs based on the difference (top) and the log-response ratio (lnRR, bottom) of the values inside vs. outside the fenced area in response to region and ForMI. On the left hand side, the response variables are from the herb layer and on the right hand side, the response variables are from the shrub layer. Estimates of the linear models are given for significant results only. Stars indicate the p-value (***) $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$, $0.05 < p < 0.1$). For significant region effects, we show the mean value of each region.
(DOCX)

S2 Table. Influence of SMI (silvicultural management intensity index) on absolute and relative treatment effects. Results originate from linear models on the change in species richness, diversity, evenness and cover of understorey herbs and shrubs based on the difference (top) and the log-response ratio (lnRR, bottom) of the values inside vs. outside the fenced area in response to region and SMI. On the left hand side, the response variables are from the herb layer and on the right hand side, the response variables are from the shrub layer. Estimates of the linear models are given for significant results only. Asterisk are given to indicate the p-value (***) $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$, $0.05 < p < 0.1$). For significant region effects, we show the mean value of each region.
(DOCX)

Acknowledgments

We thank Judith Hinderling and Torsten Meene for their excellent help during fieldwork, Caterina Penone for discussions about and advice on the choice of forest features and Marion Schrupf and Ingo Schöning for providing information on the soil parameters. We further thank the managers of the three Exploratories, Kirsten Reichel-Jung, Swen Renner, Katrin

Hartwich, Sonja Gockel, Kerstin Wiesner, and Martin Gorke for their work in maintaining the plot and project infrastructure; Christiane Fischer and Simone Pfeiffer for giving support through the central office, Michael Owonibi for managing the central data base, and Eduard Linsenmair, Dominik Hessenmöller, Jens Nieschulze, Ingo Schöning, François Buscot, Ernst-Detlef Schulze, Wolfgang W. Weisser and the late Elisabeth Kalko for their role in setting up the Biodiversity Exploratories program. Fieldwork permits were issued by the responsible state environmental offices of Baden-Württemberg, Thüringen, and Brandenburg (according to § 72 BbgNatSchG).

Author Contributions

Conceptualization: Deborah Schäfer, Daniel Prati, Markus Fischer.

Data curation: Deborah Schäfer, Peter Schall.

Formal analysis: Deborah Schäfer.

Funding acquisition: Daniel Prati, Markus Fischer.

Investigation: Deborah Schäfer.

Methodology: Deborah Schäfer, Daniel Prati, Peter Schall, Christian Ammer.

Supervision: Daniel Prati, Markus Fischer.

Validation: Deborah Schäfer, Daniel Prati.

Visualization: Deborah Schäfer.

Writing – original draft: Deborah Schäfer.

Writing – review & editing: Daniel Prati, Peter Schall, Christian Ammer, Markus Fischer.

References

1. Vera FWM. Grazing ecology and forest history. Wallingford: CABI; 2000.
2. Mitchell FJG. How open were European primeval forests. Hypothesis testing using palaeoecological data. *Journal of Ecology*. 2005; 93: 168–177. <https://doi.org/10.1111/j.1365-2745.2004.00964.x>
3. Milner JMOS, Bonenfant C, Mysterud A, Gaillard J-M, Csányi S, Stenseth NC. Temporal and spatial development of red deer harvesting in Europe. Biological and cultural factors. *The Journal of Applied Ecology*. 2006; 43: 721–734. <https://doi.org/10.1111/j.1365-2664.2006.01183.x>
4. Kuijper DPJ. Lack of natural control mechanisms increases wildlife–forestry conflict in managed temperate European forest systems. *European Journal of Forest Research*. 2011; 130: 895–909. <https://doi.org/10.1007/s10342-011-0523-3>
5. Jedrzejewska B, Jedrzejewski W, Bunevich AN, Milkowski L, Krasinski ZA. Factors shaping population densities and increase rates of ungulates in Białowieża Primeval Forest (Poland and Belarus) in the 19th and 20th centuries. *Acta Theriologica*. 1997; 42: 399–451.
6. Côté SD, Rooney TP, Tremblay J-P, Dussault C, Waller DM. Ecological Impacts of Deer Overabundance. *Annual Review of Ecology, Evolution, and Systematics*. 2004; 35: 113–147. <https://doi.org/10.1146/annurev.ecolsys.35.021103.105725>
7. Burbaitė L, Csányi S. Red deer population and harvest changes in Europe. *Acta Zoologica Lituanica*. 2010; 20: 179–188. <https://doi.org/10.2478/v10043-010-0038-z>
8. Gill RMA. A review of damage by mammals in north temperate forests: 1. deer. *Forestry*. 1992; 65: 145–169. <https://doi.org/10.1093/forestry/65.2.145>
9. Newman M, Mitchell FJG, Kelly DL. Exclusion of large herbivores. Long-term changes within the plant community. *Forest Ecology and Management*. 2014; 321: 136–144. <https://doi.org/10.1016/j.foreco.2013.09.010>
10. Andren H, Angelstam P. Moose Browsing on Scots Pine in Relation to Stand Size and Distance to Forest Edge. *The Journal of Applied Ecology*. 1993; 30: 133. <https://doi.org/10.2307/2404277>

11. Welch D, Staines BW, Scott D, French DD, Catt DC. Leader Browsing by Red and Roe Deer on Young Sitka Spruce Trees in Western Scotland I. Damage Rates and the Influence of Habitat Factors. *Forestry*. 1991; 64: 61–82. <https://doi.org/10.1093/forestry/64.1.61>
12. Kullberg Y, Bergström R. Winter Browsing by Large Herbivores on Planted Deciduous Seedlings in Southern Sweden. *Scandinavian Journal of Forest Research*. 2001; 16: 371–378. <https://doi.org/10.1080/02827580152496768>
13. Anderson RC, Loucks OL. White-Tail Deer (*Odocoileus virginianus*) Influence on Structure and Composition of *Tsuga canadensis* Forests. *The Journal of Applied Ecology*. 1979; 16: 855. <https://doi.org/10.2307/2402859>
14. Potvin F, Beaupré P, Laprise G. The eradication of balsam fir stands by white-tailed deer on Anticosti Island, Québec. A 150-year process. *Écoscience*. 2016; 10: 487–495. <https://doi.org/10.1080/11956860.2003.11682796>
15. Gill RMA, Beardall V. The impact of deer on woodlands: the effects of browsing and seed dispersal on vegetation structure and composition. *Forestry*. 2001; 74: 209–218. <https://doi.org/10.1093/forestry/74.3.209>
16. Ammer C. Impact of ungulates on structure and dynamics of natural regeneration of mixed mountain forests in the Bavarian Alps. *Forest Ecology and Management*. 1996; 88: 43–53. [https://doi.org/10.1016/S0378-1127\(96\)03808-X](https://doi.org/10.1016/S0378-1127(96)03808-X)
17. Horsley SB, Marquis DA. Interference by weeds and deer with Allegheny hardwood reproduction. *Canadian Journal of Forest Research*. 1983; 13: 61–69. <https://doi.org/10.1139/x83-009>
18. Hobbs NT. Modification of Ecosystems by Ungulates. *The Journal of Wildlife Management*. 1996; 60: 695. <https://doi.org/10.2307/3802368>
19. Stromayer KAK, Warren RJ. Are overabundant deer herds in the eastern United States creating alternate stable states in forest plant communities. *Wildlife Society Bulletin*. 1997; 25: 227–234.
20. George LO, Bazzaz FA. The fern understory as an ecological filter. emergence and establishment of canopy-tree seedlings. *Ecology*. 1999; 80: 833–845. [https://doi.org/10.1890/0012-9658\(1999\)080\[0833:TFUAAE\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1999)080[0833:TFUAAE]2.0.CO;2)
21. Kirby KJ. The impact of deer on the ground flora of British broadleaved woodland. *Forestry*. 2001; 74: 219–229. <https://doi.org/10.1093/forestry/74.3.219>
22. Watkinson AR, Riding AE, Cowie NR. A community and population perspective of the possible role of grazing in determining the ground flora of ancient woodlands. *Forestry*. 2001; 74: 231–239. <https://doi.org/10.1093/forestry/74.3.231>
23. Stockton SA, Allombert S, Gaston AJ, Martin J-L. A natural experiment on the effects of high deer densities on the native flora of coastal temperate rain forests. *Biological Conservation*. 2005; 126: 118–128. <https://doi.org/10.1016/j.biocon.2005.06.006>
24. Ammer C, Vor T, Knoke T, Wagner S. *Der Wald-Wild-Konflikt. Analyse und Lösungsansätze vor dem Hintergrund rechtlicher, ökologischer und ökonomischer Zusammenhänge*. Göttingen: Univ.-Verl.; 2011.
25. Rooney TP. Deer impacts on forest ecosystems: a North American perspective. *Forestry*. 2001; 74: 201–208. <https://doi.org/10.1093/forestry/74.3.201>
26. Heinze E, Boch S, Fischer M, Hessenmöller D, Klenk B, Müller J, et al. Habitat use of large ungulates in northeastern Germany in relation to forest management. *Forest Ecology and Management*. 2011; 261: 288–296. <https://doi.org/10.1016/j.foreco.2010.10.022>
27. Bergström R, Edenius L. From twigs to landscapes—methods for studying ecological effects of forest ungulates. *Journal for Nature Conservation*. 2003; 10: 203–211. <https://doi.org/10.1078/1617-1381-00020>
28. Heurich M, Brand TTG, Kaandorp MY, Šustr P, Müller J, Reineking B. Country, cover or protection. What shapes the distribution of red deer and roe deer in the Bohemian Forest Ecosystem. *PLoS ONE*. 2015; 10: e0120960. <https://doi.org/10.1371/journal.pone.0120960> PMID: 25781942
29. Augustine DJ, McNaughton SJ. Ungulate Effects on the Functional Species Composition of Plant Communities. *Herbivore Selectivity and Plant Tolerance*. *The Journal of Wildlife Management*. 1998; 62: 1165. <https://doi.org/10.2307/3801981>
30. Knight TM. Effects of herbivory and its timing across populations of *Trillium grandiflorum* (Liliaceae). *American Journal of Botany*. 2003; 90: 1207–1214. <https://doi.org/10.3732/ajb.90.8.1207> PMID: 21659221
31. Oheimb G von, Schmidt M, Kriebitzsch W-U, Ellenberg H. Dispersal of vascular plants by game in northern Germany. Part II. Red deer (*Cervus elaphus*). *European Journal of Forest Research*. 2005; 124: 55–65. <https://doi.org/10.1007/s10342-005-0053-y>

32. Agnew ADQ, Flux JEC. Plant Dispersal by Hares (*Lepus Capensis* L.) in Kenya. *Ecology*. 1970; 51: 735–737. <https://doi.org/10.2307/1934057>
33. Sorensen AE. Seed Dispersal by Adhesion. *Annual Review of Ecology, Evolution, and Systematics*. 1986; 17: 443–463. <https://doi.org/10.1146/annurev.es.17.110186.002303>
34. Schmidt M, Sommer K, Kriebitzsch W-U, Ellenberg H, Oheimb G von. Dispersal of vascular plants by game in northern Germany. Part I. Roe deer (*Capreolus capreolus*) and wild boar (*Sus scrofa*). *European Journal of Forest Research*. 2004; 123: 167–176. <https://doi.org/10.1007/s10342-004-0029-3>
35. Heinken T, Schmidt M, Oheimb G von, Kriebitzsch W-U, Ellenberg H. Soil seed banks near rubbing trees indicate dispersal of plant species into forests by wild boar. *Basic and Applied Ecology*. 2006; 7: 31–44. <https://doi.org/10.1016/j.baae.2005.04.006>
36. Boch S, Prati D, Müller J, Socher S, Baumbach H, Buscot F, et al. High plant species richness indicates management-related disturbances rather than the conservation status of forests. *Basic and Applied Ecology*. 2013; 14: 496–505. <https://doi.org/10.1016/j.baae.2013.06.001>
37. Gossner MM, Schall P, Ammer C, Ammer U, Engel K, Schubert H, et al. Forest management intensity measures as alternative to stand properties for quantifying effects on biodiversity. *Ecosphere*. 2014; 5: art113. <https://doi.org/10.1890/ES14-00177.1>
38. Kumar P, Chen HYH, Thomas SC, Shahi C, Gilliam F. Linking resource availability and heterogeneity to understorey species diversity through succession in boreal forest of Canada. *Journal of Ecology*. 2017; 19: 716. <https://doi.org/10.1111/1365-2745.12861>
39. Tremblay J-P HUOT J, Potvin F. Density-related effects of deer browsing on the regeneration dynamics of boreal forests. *The Journal of Applied Ecology*. 2007; 44: 552–562. <https://doi.org/10.1111/j.1365-2664.2007.01290.x>
40. Horsley SB, Stout SL, deCalesta DS. White-tailed deer impact on the vegetation dynamics of a northern hardwood forest. *Ecological Applications*. 2003; 13: 98–118. [https://doi.org/10.1890/1051-0761\(2003\)013\[0098:WTDIOT\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2003)013[0098:WTDIOT]2.0.CO;2)
41. Fischer M, Bossdorf O, Gockel S, Hänsel F, Hemp A, Hessenmöller D, et al. Implementing large-scale and long-term functional biodiversity research. *The Biodiversity Exploratories*. *Basic and Applied Ecology*. 2010; 11: 473–485. <https://doi.org/10.1016/j.baae.2010.07.009>
42. Schall P, Schulze E-D, Fischer M, Ayasse M, Ammer C. Relations between forest management, stand structure and productivity across different types of Central European forests. *Basic and Applied Ecology*. 2018; 32: 39–52. <https://doi.org/10.1016/j.baae.2018.02.007>
43. Kahl T, Bauhus J. An index of forest management intensity based on assessment of harvested tree volume, tree species composition and dead wood origin. *Nature Conservation*. 2014; 7: 15–27. <https://doi.org/10.3897/natureconservation.7.7281>
44. Schall P, Ammer C. How to quantify forest management intensity in Central European forests. *European Journal of Forest Research*. 2013; 132: 379–396. <https://doi.org/10.1007/s10342-013-0681-6>
45. Wilson SD, Tilman D. Plant Competition and Resource Availability in Response to Disturbance and Fertilization. *Ecology*. 1993; 74: 599–611. <https://doi.org/10.2307/1939319>
46. Müller A, Dahm M, Bøcher PK, Root-Bernstein M, Svenning J-C. Large herbivores in novel ecosystems—Habitat selection by red deer (*Cervus elaphus*) in a former brown-coal mining area. *PLoS ONE*. 2017; 12: e0177431. <https://doi.org/10.1371/journal.pone.0177431> PMID: 28505192
47. Oksanen J, Guillaume Blanchet F, Friendly M, Kindt R, Legendre P, McGlenn D, et al. *Vegan: Community Ecology package*. R package version 2.4–3. 2017.
48. Smith B, Wilson JB. A Consumer's Guide to Evenness Indices. *Oikos*. 1996; 76: 70. <https://doi.org/10.2307/3545749>
49. Ohse B, Seele C, Holzwarth F, Wirth C. Different facets of tree sapling diversity influence browsing intensity by deer dependent on spatial scale. *Ecology and Evolution*. 2017; 7: 6779–6789. <https://doi.org/10.1002/ece3.3217> PMID: 28904759
50. Koerner SE, Smith MD, Burkepile DE, Hanan NP, Avolio ML, Collins SL, et al. Change in dominance determines herbivore effects on plant biodiversity. *Nature Ecology and Evolution*. 2018; 2: 1925–1932. <https://doi.org/10.1038/s41559-018-0696-y> PMID: 30374174
51. Bierzychudek P. Life histories and demography of shade-tolerant temperate forest herbs: A review. *New Phytologist*. 1982; 90: 757–776. <https://doi.org/10.1111/j.1469-8137.1982.tb03285.x>
52. Hyatt LA, Casper BB. Seed bank formation during early secondary succession in a temperate deciduous forest. *Journal of Ecology*. 2000; 88: 516–527. <https://doi.org/10.1046/j.1365-2745.2000.00465.x>
53. Whigham DF. Ecology of Woodland Herbs in Temperate Deciduous Forests. *Annual Review of Ecology, Evolution, and Systematics*. 2004; 35: 583–621. <https://doi.org/10.1146/annurev.ecolsys.35.021103.105708>

54. McEuen AB, Curran LM. Seed dispersal and recruitment limitation across spatial scales in temperate forest fragments. *Ecology*. 2004; 85: 507–518. <https://doi.org/10.1890/03-4006>
55. Heinken T. Dispersal of plants by a dog in a deciduous forest. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie*. 2000; 122: 449–467.
56. Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1–131.1, <https://CRAN.R-project.org/package=nlme>. 2018.
57. Small CJ, McCarthy BC. Spatial and temporal variability of herbaceous vegetation in an eastern deciduous forest. *Plant Ecology*. 2003; 164: 37–48. <https://doi.org/10.1023/A:1021209528643>
58. Oheimb G von, Ellenber H, Heuvelodp J, Kriebitzsch WU. Einfluss der Nutzung unterschiedlicher Waldökosysteme auf die Artenvielfalt und -zusammensetzung der Gefäßpflanzen in der Baum-, Strauch- und Krautschicht unter besonderer Berücksichtigung von Aspekten des Naturschutzes und des Verbissdruckes durch Wild. In: Scholz F, Degen B, editors. *Wichtige Einflussfaktoren auf die Biodiversität in Wäldern*. Mitteilungen der Bundesforschungsanstalt für Forst- und Holzwirtschaft, 195. Wiedebusch, Hamburg: Verlag M.; 1999. pp. 279–450.
59. Webster CR, Jenkins MA, Rock JH. Long-term response of spring flora to chronic herbivory and deer exclusion in Great Smoky Mountains National Park, USA. *Biological Conservation*. 2005; 125: 297–307. <https://doi.org/10.1016/j.biocon.2005.03.027>
60. Collard A, Lapointe L, Ouellet J-P, Crête M, Lussier A, Daigle C, et al. Slow responses of understorey plants of maple-dominated forests to white-tailed deer experimental exclusion. *Forest Ecology and Management*. 2010; 260: 649–662. <https://doi.org/10.1016/j.foreco.2010.05.021>
61. Paillet Y, Bergès L, Hjältén J, Odor P, Avon C, Bernhardt-Römermann M, et al. Biodiversity differences between managed and unmanaged forests. Meta-analysis of species richness in Europe. *Conservation Biology*. 2010; 24: 101–112. <https://doi.org/10.1111/j.1523-1739.2009.01399.x> PMID: 20121845
62. Chaudhary A, Burivalova Z, Koh LP, Hellweg S. Impact of Forest Management on Species Richness. *Global Meta-Analysis and Economic Trade-Offs*. *Scientific Report*. 2016; 6: 23954. <https://doi.org/10.1038/srep23954> PMID: 27040604