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Changes in lower layers biodiversity caused by Polish pony in old growth Scots pine stands

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Abstract: The study was conducted in coniferous and deciduous old growth forests in two forest complexes located in: i) the fenced area of the Popielno Research Station of the Polish Academy of Sciences, with free-living Polish pony [Polish primitive horse (*Equus ferus caballus*)], and ii) open Maskulińskie Forest District managed (harvested) forest, without horses. The impact of forest animals on ground cover layer as well as on understory shrub layer and undergrowth, in i) area (horses and other forest animals) was compared with the results in ii) area (forest animals without horses). Very significant differences in the understory and undergrowth (above 0,5 m) layer vegetation communities structure between both areas and type of stands were found. The results suggest that the presence of the Polish horse substantially changed the species composition and increased the species diversity of the ground layer and shrub layer both on coniferous forest and in the deciduous forest habitats. The height of the shrub layer trees was lower by 30% in the area with the Polish horse.

Keywords: Polish primitive horse, forest site type, herb layer, undergrowth layer, understory layer, biodiversity.

1. Introduction

Pro-ecological model of modern forestry in Poland, close-to-nature [1,2], provides locally usage of natural forest farming methods, also with the use of livestock or a Polish horse or Tarpan. It is assumed that adequate management of natural populations will support restoring rare habitats, e.g. luminous oak stands, which occurred numerously in Poland in the past due to the existence of goats, pigs and ponies in the forest. However, the impact of herbivorous megafauna on the biological diversity of forests, including natural complexes, has been debated for many years, and is still open. In the opinion of some authors [3,4,5], large herbivorous mammals were the factor affecting the appearance of primary forest communities, as a result of their pressure, forests were formed as a mosaic of a patches dominated by trees and open grass areas. According to Vera [4], along with the development of farming, free-living aurochs (*Bos primigenius*) and tarpans (*Equus gmelini*) were replaced by domesticated animals, such as cows, horses and pigs. Most studies describing the effect of large herbivores on forests focus on ruminants – the red deer (*Cervus elaphus*), the roe deer (*Capreolus capreolus*), the fallow deer (*Dama dama*), the European bison (*Bison bonasus*) or the elk (*Alces alces*) [6]. However, little is known about the effect on free-living non-ruminant ungulate species, such as Exmoor pony (*Equus caballus* Linnaeus 1758), the Eurasian wild horse Tarpan (*Equus caballus gmelini* or *Equus ferus ferus*), the Przewalski's Horse (*Equus ferus przewalskii*). To the above mentioned group of non-ruminants belongs also the Polish primitive horse Konik Polski (*Equus ferus caballus*) [7,8].

In general, *Equidae* contains species mainly related to open land structures, where they stay for most of the year (pastures and meadows), they may also use available tree stands [9], where there can be seen mostly in winter and spring [10,11]. The diet of horses and cervids is significantly different, since the woody species (shrubs and trees) account for 40% to 90% in the deer diet [12], while primitive horses are first of all grass-eaters, only to an insignificant degree feeding on arborescent species [9,10,13,14]. As non-ruminants, horses obtain metabolic energy from both enzymatic digestion and bacterial fermentation [15,16]. The alimentary tract of horses is both anatomically and functionally adapted for small portions of woody plant food [11].

As mentioned before equids use forest habitats relatively seldom [10,17,18,19]. In case of the Polish primitive horse, it may use the forest not only as a feeding place, but also as protection against vexing insects or heat [17,20,21]. The grazing of the Polish primitive horse, like the Exmoor pony, is increasingly more frequently used as a natural means to maintain the non-forested status of the areas that are not agriculturally used. Free-living horses help to preserve vast meadow and pasture or dune areas, contributing to preservation of semi-natural open communities, just like large herbivorous mammals in the past [10,22,23,24,25]. Polish primitive horses were used, for instance, not only to restrain the succession of forest species in grass areas and moors, but also to contribute to maintaining their biodiversity [26,27]. Grazing on mid-forest meadows in extensive conditions favours the diversity of the undergrowth structure and the dispersed dung left by horses contributes to the herb layer diversification. This results in increasing the number of plant species [28,29].

Apparently, so far the only place from which the information has been provided concerning the diversified effect of the herbivorous megafauna in Europe on the forest environment is New Forest in England. However, this area, apart from the cervids and non-ruminants represented by the pony is also inhabited by other mega-ruminants, i.e. cows [30]. In Poland, Polish primitive horses are bred in two different systems, in stables and in nature reserves – in the wild. Breeding primitive horses in reserves is currently popular not only in Poland, but also in Germany, the Netherlands and France [7,21,31,32,33]. Reintroduction to the tree stands of the Polish primitive horse, a mammal which lived in a wild state in the Białowieża Primeval Forest until about 1780 [34], becomes a part of the activities undertaken for preserving the endangered species *ex situ*, but it requires evaluation of its impact on the environment, especially on plant communities.

At moderate level of invasive or late-succession plants occurrence, large herbivores stops their expansion and consequently promotes increasing biodiversity and structural variability of the landscape [3,35,36,37,38,39]. According to Borkowski [40] and Klich [7], the Polish primitive horse can affect trees and shrubs by nibbling shoots, browsing and debarking seedlings and branches. However, the bark of trees and shrubs is a marginal component in the horse diet, used only in the winter period [10, 18, 41] reported that horses can cause significant damages in tree stands while feeding only with a large concentration of animals.

The aim of the present study was to evaluate the impact of the Polish primitive horse on forest plants. We compared vegetation condition in two adjoining areas with ungulate communities differing in the presence of the Polish primitive horse. The main hypothesis of the research undertaken is an assumption that the presence of free-living Polish primitive horses in tree stands of limited forest use in the Popielno Research Station (RS) will significantly change the plant composition, and increase the degree of plant diversity in the forest ground cover layer (forest vegetal cover, undergrowth and understory) in comparison to the tree stand that is used for economic purposes in Maskulińskie Forest District (FD), depending on the tree species composition in the stand (deciduous or coniferous).

2. Materials and Methods

2.1. Research areas

The research was carried out in 2013 in two adjacent areas: i) the experimental forest of the Popielno Research Station of the Polish Academy of Science (RS), and ii) managed [harvested] stands of Maskulińskie FD. Both forest complexes located in the north-eastern part of Poland, in the area managed by the Regional Directorate of State Forests in Olsztyn, belonging to the Land of the Great Mazurian Lakes and Mazurian Plain mesoregions [42]. The examined areas are distinguished by a high number of pine stands, unique on the European scale for the age of the pine trees, which are even up to 230 years old.

The Popielno RS covered an area of 1628 ha (including 57 ha of meadows, pastures and hunting grounds). This is a reserve, inhabited by four herds (46 animals) of free-living Polish primitive horses [43<http://popielno.pl/koniki-polskie/>]. The density of horses is about 3 animals per 100 ha. Control areas were established in the Maskulińskie FD, where the Polish primitive horse does not occur. The area of the Maskulińskie FD is characterized by similar natural and forest conditions. The objects under examination are inhabited by a natural community of ungulates, including cervids and in the area of Popielno additionally by the Polish primitive horse.

A habitat of mixed coniferous forest (over 61%) prevails in the Popielno RS, with the Scots pine *Pinus sylvestris* L. as the dominant species (about 89%), of the average age of 131 years, with an addition of the European white birch *Betula peudula* Roth. and the European larch *Larix decidua* Miller. Other species growing here include the Norway spruce *Picea abies* (L.) Karst., the European oak *Quercus robur* L., the European beech *Fagus sylvatica* L. and a young generation of pine. The Popielno forest, with soil of higher fertility rate, is occupied by fresh mixed forest [16% share) and fresh forest [2% share of the habitat). The neighbouring Maskulińskie FD occupies similar habitats, with prevailing fresh coniferous (47%) and mixed coniferous stands (25%), while fresh mixed forests occupy 15% of the forest division area. The species composition is dominated by the pine [85%) with an average age of 116 years, and the share of other species (spruce, birch, alder, oak) does not exceed several percent.

Both areas are inhabited by red and roe deer. In both areas the deer are controlled and their density (estimated on the basis of pellet group counts) is around 7 ind. and 2 ind. of red and roe deer, respectively (Borkowski, personal communication).

2.2. Methods

In 38 sub-compartments in the Popielno RS, and in 29 in the Maskulińskie FD, both in coniferous and in deciduous habitats, 67 sample plots were established, 40 x 40 m size (the area of 1,600 m²). In each case the inventory of understory and undergrowth (higher 0.5 m) was carried out, describing number and species composition. Additionally, five round plots, each of an area of 0.5 m², in each of the 67 sub-compartments were distributed, with at least 5 m distance from large gaps (about 1,000 m²) in the tree stand canopy, where the inventory of undergrowth (lower 0.5 m) and the herb layer were carried out. All plants in the undergrowth were counted, specifying their species composition and height (to an accuracy of 1 mm). In case of grasses, the percentage of surface coverage was specified instead of individual's numbers.

The Shannon-Wiener index of plant biodiversity [$H' = -\sum(p_i \ln p_i)$; where p_i is the relative coverage of species i] was calculated for each sample plot with the use of the BioDiversity Pro software [44]. The mean values of the index, as well as the height of plants for the research areas were compared using Anova Two-way analysis of variance, after testing the normality of distribution and evaluating the insignificance of differences for variances between the compared groups. In two cases, where differences between means were significant, the comparison was carried out using a post-hoc Tukey's test, taking into account two fertility groups of habitats and the presence or absence of the Polish primitive horse. Additionally, a Bray and Curtis [45] coefficient of qualitative and quantitative similarity of the community of plants was evaluated. Dendrograms which compared individual branches for each of the two layers of plants, taking into account plants of the herb layer and

undergrowth, as well as the plants of the shrub and herb layer, were constructed in the BioDiversity Pro software.

3. Results

3.1. The herb layer and undergrowth (lower 0.5 m)

For the herb layer species, a certain variability of occurrence was observed, related to the type of the forest habitat and presence or absence, of the Polish primitive horse (Table 1).

3.1.1. Deciduous forests (DF)

In habitats with a higher fertility rate, where the Polish primitive horse did not occur [No H DF), 42 species in the layer of undergrowth and undergrowth were observed. The most numerous (with the share of over 6%) included: *Carpinus betulus* L., *Oxalis acetosella* L., *Pleurozium schreberi* (Willd.) Mitten. In more fertile habitats, despite the absence of the horses, the least numerous (below 3%) included: *Asarum europaeum* L., *Equisetum sylvaticum* L. *Luzula pilosa* (L.) Willd. It should be emphasized that the above mentioned species were not found with the presence of the Polish primitive horse.

In deciduous forests with the presence of Polish horses (H DF), 51 species were observed, with the most numerous being *Dryopteris carthusiana* (Vill.) H. P. Fuchs, *Mycelis muralis* L. and *Oxalis acetosella* L. In the absence of Polish horses, the following species were not observed: *Dryopteris filix-mas* (L.) Schott, *Hylocomium splendens* (Hedw.) Schimp. in Bruch, Schimp. and W.Gümbel, *Poa nemoralis* L., *Prunus serotina* (Ehrh.) Borkh. (the above mentioned species scarcely occurred), *Viola reichenbachiana* Jordan ex Bor. (the species was numerous, above 6%).

3.1.2. Coniferous forests (CF)

In poor habitats, without the presence of Polish horses (No H CF), 30 species were observed in the herb and undergrowth layer, including the most numerous: *Deschampsia flexuosa* L., *Pleurozium schreberi* (Willd.) Mitten., *Vaccinium myrtillus* L. Species occurring in poor habitats without the Polish horses [and absent where the Polish horse occurred), included: *Dryopteris filix-mas* (L.) Schott, *Luzula pilosa* (L.) Willd., *Poa nemoralis* L., and *Urtica dioica* L.

Poor habitats where the Polish horse was present [H con) were characterized by the occurrence of 40 species, including the following species observed in great numbers: *Deschampsia flexuosa* L., *Hylocomium splendens* (Hedw.) Schimp. in Bruch, Schimp. and W.Gümbel, *Pleurozium schreberi* (Willd.) Mitten. The species growing in sub-compartments where the Polish horse was present [and non-occurring with the absence of Polish horse) include: *Calamagrostis arundinacea* L. Roth., *Juniperus communis* L., *Potentilla erecta* [L.) Rausch.

The differences between number of species observed in the herb layer on both habitats are presented in Figure 1.

Table 1. The share of species in the herb layer and undergrowth lower 0.5 m in the areas of various fertility, with the presence or absence of the Polish primitive horse. The '+' symbol denotes a

percentage share of the entire number of existing species not lower than 3%, the ++ symbol denotes the share between 3% and 6%, and the '+++' symbol denotes the number of the species exceeding 6%. The absence of the symbol - absence of the species. All species in the column account for 100%.

Species	Deciduous forest		Coniferous forest	
	No horse	Horse	No horse	Horse
<i>Acer campestre</i> L.				+
<i>Acer platanoides</i> L.	+	++		
<i>Acer pseudoplatanus</i> L.		+		
<i>Aegopodium podagraria</i> L.	+	+		
<i>Anthericum ramosum</i> L.	+		+	
<i>Asarum europaeum</i> L.	+			
<i>Athyrium filix-femina</i> (L.) Roth		+		
<i>Betula pendula</i> Roth.	+	+	+	+
<i>Calamagrostis arundinacea</i> L. Roth				+
<i>Calluna vulgaris</i> L.			+	+
<i>Campanula rotundifolia</i> L.		+		+
<i>Carex digitata</i> L.	+	+	+	++
<i>Carpinus betulus</i> L.	+++	++		+
<i>Convallaria majalis</i> L.	+	++	+	++
<i>Corylus avellana</i> L.		+		
<i>Crataegus monogyna</i> Jacq.		+		
<i>Dactylis polygama</i> Horv.		++		+
<i>Daphne mezereum</i> L.	+	+		
<i>Deschampsia flexuosa</i> L.	+	+	+++	+++
<i>Dicranum polysetum</i> Sw.		+		+++
<i>Dicranum scoparium</i> (L.) Hedw.	+	+	++	+
<i>Dryopteris carthusiana</i> (Vill.) H. P. Fuchs	+	+++		++
<i>Dryopteris filix-mas</i> (L.) Schott		+	+	
<i>Equisetum sylvaticum</i> L.	+			
<i>Fagus sylvatica</i> L.	+	+		
<i>Ficaria verna</i> Huds.	+	+		
<i>Fragaria vesca</i> L.	+	+	+	+
<i>Fraxinus excelsior</i> L.		+		
<i>Geranium robertianum</i> L.	+	+	+	
<i>Hepatica nobilis</i> Mill.	++	++		+
<i>Hylocomium splendens</i> (Hedw.) Schin Bruch, Schimp. & W.Gümbel		+		+++
<i>Impatiens noli-tangere</i> L.	++	+		
<i>Juniperus communis</i> L.				+
<i>Larix decidua</i> Mill.				+
<i>Luzula pilosa</i> (L.) Willd.	+		++	

Table 1. Cont.

Species	Deciduous forest	Coniferous forest
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	No horse	Horse	No horse	Horse
<i>Lycopodium clavatum</i> L.			+	
<i>Maianthemum bifolium</i> (L.) F. W. Schmidt	+++	++	+	+
<i>Melampyrum pratense</i> L.	+	+	+	+
<i>Milium effusum</i> L.			+	+
<i>Mnium undulatum</i> (L.) Hedw.		+		++
<i>Mycelis muralis</i> L.	+++	+++		+
<i>Oxalis acetosella</i> L.	+++	+++	+++	++
<i>Paris quadrifolia</i> L.	+	+		
<i>Picea abies</i> L.				+
<i>Pinus sylvestris</i> L.	+	+		+
<i>Pleurozium schreberi</i> (Willd.) Mitten.	+++	++	+++	+++
<i>Poa nemoralis</i> L.		+	+	
<i>Polygonatum multiflorum</i> (L.) All.		+		
<i>Polygonatum odoratum</i> (Mill) Druce		+	+	+
<i>Polypodium vulgare</i> L.				+
<i>Polytrichum commune</i> Hedw.	+		+	
<i>Populus tremula</i> L.	+			
<i>Potentilla erecta</i> (L.) Raeusch				+
<i>Prunus serotina</i> (Ehrh.) Borkh.		+		
<i>Pteridium aquilinum</i> (L.) Kuhn	+	+		+
<i>Quercus robur</i> L.	+	+	+++	+
<i>Quercus rubra</i> L.	+			+
<i>Rubus caesius</i> L.		+		
<i>Rubus chamaemorus</i> L.	+			
<i>Rubus idaeus</i> L.	++	+	+++	+
<i>Rubus saxatilis</i> L.		++	+	++
<i>Sorbus aucuparia</i> L.	+	+	+	+
<i>Tilia cordata</i> Mill.	+	+		+
<i>Tilia platyphyllos</i> L.	+			
<i>Trientalis europaea</i> L.	+	+	++	+
<i>Ulmus minor</i> Mill.	+	+		
<i>Urtica dioica</i> L.	+	+	+	
<i>Vaccinium myrtillus</i> L.	++	+++	+++	+++
<i>Vaccinium vitis-idaea</i> L.		+	+++	++
<i>Veronica officinalis</i> L.			+	
<i>Vinca minor</i> L.			+	
<i>Viola hirta</i> L.	+			
<i>Viola reichenbachiana</i> Jordan ex Bor.		+++		
Total	42	51	30	40

The evaluation of qualitative floristic similarity dendrogram indicates higher similarities for this layer of divisions according to the fertility groups of habitats than in relation to the presence

and feeding of the Polish primitive horse. The upper part of the dendrogram shows more sub-compartments occupying deciduous sites, which means that more sub-compartments in those sites demonstrated a relatively low similarity of the species composition, while the lower part of the dendrogram tends to present sub-compartments with coniferous forests, which proves a higher similarity between these areas as regards the species composition (Figure 1).

Bray-Curtis Cluster Analysis (Single Link)

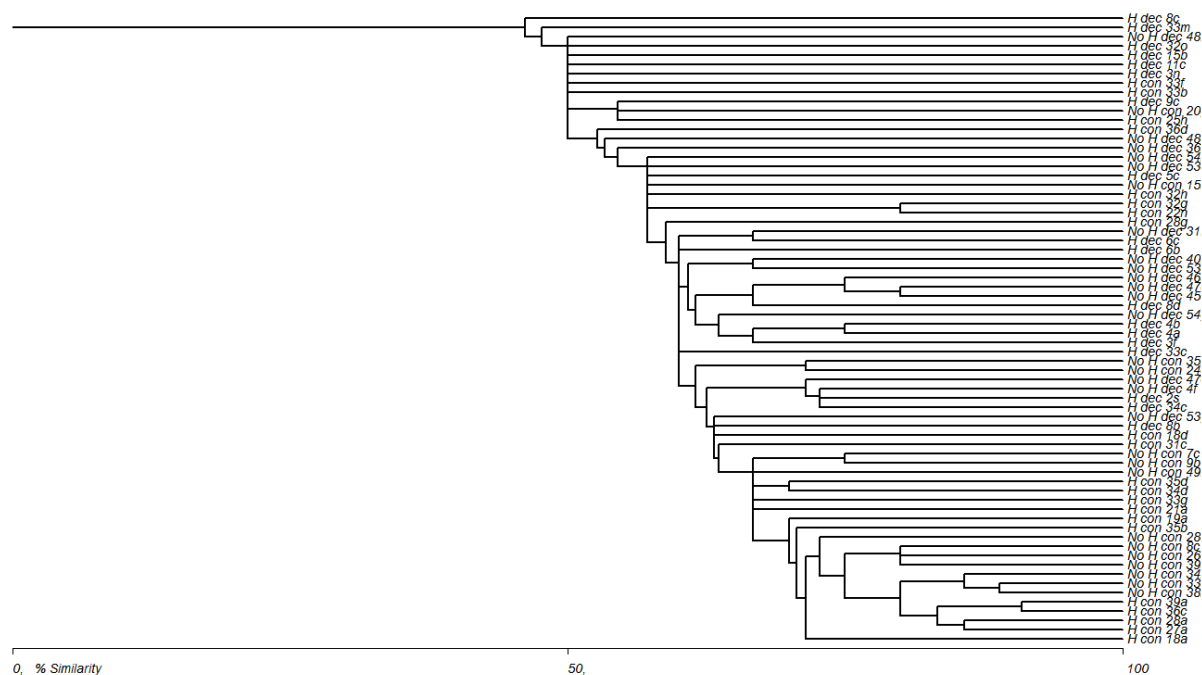


Figure 1. The similarity for clusters of species in herb layer and undergrowth lower than 0.5 m growing in all areas under examination; (H – horse present, No H – horse absent, con– coniferous forests (CF), dec – deciduous forests (DF); 7c, 54g – codes of the forest sub-compartment.

The average height of the herb layer depended on the habitat ($F_{1,64} = 5.249$, $p = 0.025$), while the herb layer growing in poorer habitats [coniferous sites) was 20% taller than the herb layer occurring in deciduous habitats (Table 2). The presence of the Polish primitive horse did not affect the height of the herb layer ($F_{1,64} = 2.678$, $p = 0.106$) and the effect of integration of those two factors was also insignificant ($F_{1,64} = 0.006$, $p = 0.934$).

Table 2. The values of the Shannon-Wiener index (\pm SE) and average heights (\pm SE) calculated for the lower layers of forest vegetation (undergrowth higher than 0.5 m, and understory layers), in relation to the habitat fertility group and the presence of the Polish primitive horse. The means with statistically significant differences ($p < 0.05$) are marked with a different letter. The *ns* abbreviation means that statistically significant differences were not observed

Effect	N	Shannon-Wiener index (\pm SE)	Average height of the layer (\pm SE) [cm]
The herb layer and undergrowth (lower 0.5 m)			
<i>Horse effect</i>			
No H	29	0.823 \pm 0.038ns	14.28 \pm 0.98ns
H	39	0.855 \pm 0.031ns	17.07 \pm 1.19ns
<i>Habitat effect</i>			
DF	34	0.878 \pm 0.035ns	13.95 \pm 0.94b
CF	34	0.805 \pm 0.033ns	17.70 \pm 1.24a
<i>Interaction horse x habitat</i>			
No H x DF	18	0.879 \pm 0.043ns	12.46 \pm 1.26ns
H x DF	15	0.865 \pm 0.058ns	15.18 \pm 1.32ns
No H x CF	21	0.835 \pm 0.045ns	16.22 \pm 1.35ns
H x CF	14	0.779 \pm 0.047ns	18.69 \pm 1.86ns
Understory and undergrowth (higher 0.5 m) layer			
<i>Horse effect</i>			
No H	25	0.441 \pm 0.034ns	80.02 \pm 4.49a
H	39	0.517 \pm 0.036ns	56.35 \pm 7.21b
<i>Habitat effect</i>			
DF	32	0.414 \pm 0.033a	68.96 \pm 6.40ns
CF	32	0.561 \pm 0.036b	62.23 \pm 5.33ns
<i>Interaction horse x habitat</i>			
No H x DF	12	0.441 \pm 0.053b	75.72 \pm 12.19ns
H x DF	19	0.397 \pm 0.043b	64.91 \pm 7.29ns
No H x CF	12	0.441 \pm 0.045b	83.99 \pm 8.46ns
H x CF	19	0.640 \pm 0.045a	47.34 \pm 4.41ns

3.2. Understory and undergrowth layer higher than 0.5 m

The highest species diversity (21 species) was observed in deciduous forests where the Polish primitive horse was present and the lowest (13 species) was in coniferous sites without the presence of the horse (Table 3).

Table 3. The share of species in the understory and undergrowth higher 0.5 m layer with the presence or absence of the Polish primitive horse. Abbreviations – see Table 1.

Species	Deciduous forests (DF)		Coniferous forests (CF)	
	No horse	Horse	No horse	Horse
<i>Acer campestre</i> L.		+		
<i>Acer platanoides</i> L.	++	++	+	+
<i>Acer pseudoplatanus</i> L.	+			
<i>Alnus glutinosa</i> [L.] Gaertn.		+		
<i>Betula pendula</i> Roth.		++	++	+++
<i>Carpinus betulus</i> L.	+++	+++	+++	++
<i>Corylus avellana</i> L.	+++	+++	+++	+++
<i>Crataegus monogyna</i> Jacq.				+
<i>Daphne mezereum</i> L.		+		
<i>Euonymus verrucosus</i> Scop.	+			
<i>Fagus sylvatica</i> L.	+	++		++
<i>Frangula alnus</i> Mill.	+	+	+	+
<i>Juniperus communis</i> L.			+	+
<i>Larix decidua</i> Mill.	+	+		+
<i>Picea abies</i> [L.] H. Karst	++	+	+++	+++
<i>Pinus sylvestris</i> L.	+	+	+	++
<i>Populus tremula</i> L.	+	+		+
<i>Prunus cerasifera</i> Ehrh.		+		+
<i>Prunus domestica</i> L.	+			
<i>Quercus robur</i> L.	++	++	+++	+++
<i>Quercus rubra</i> L.	+	+	+	
<i>Robinia pseudoacacia</i> L.		+		+
<i>Rubus idaeus</i> L.	+	+	+	+
<i>Sorbus aucuparia</i> L.	++	++	++	+++
<i>Tilia cordata</i> Mill.	++	+++	++	+
<i>Ulmus glabra</i> Huds.		+		
Total	17	21	13	18

3.2.1. Deciduous forests (DF)

In fertile habitats, where the Polish primitive horse did not occur (No H DF), 17 species were observed in the shrub and undergrowth layer, two of which were found in high numbers (above 6% of all the species occurring in this site and in this variant). These were: *Carpinus betulus* L., and *Corylus avellana* L. Three species occurred sparsely with the absence of the Polish primitive horse: *Acer pseudoplatanus* L., *Euonymus verrucosus* Scop., *Prunus domestica* L.

In fertile sites, where the Polish primitive horse was present (H DF), 21 species in the shrub and understory layer were observed, with *Carpinus betulus* L., *Corylus avellana* L., *Tilia cordata* Mill.

occurring in the highest numbers. Species related only to the occurrence of the Polish primitive horse in this site included: *Acer campestre* L., *Alnus glutinosa* (L.) Gaertn., *Betula pendula* Roth., *Daphne mezereum* L., *Prunus cerasifera* Ehrh., *Robinia pseudoacacia* L., *Ulmus glabra* Huds (all of them in small numbers).

3.2.2. Coniferous forests (CF)

In poorer habitats, where the presence of the Polish primitive horse was not observed (No H CF), 13 species were recorded in the shrub and understory layer, of which: *Carpinus betulus* L., *Corylus avellana* L., *Picea abies* (L.) H. Karst, *Quercus robur* L. occurred in high numbers. Only one species in these habitats was exclusively related to the absence of the Polish primitive horse and it was *Quercus rubra* L.

In poorer sites with the presence of the Polish primitive horse (H CF), 18 species were found in the shrub and understory layer. The following species were observed in great numbers: *Betula pendula* Roth., *Corylus avellana* L., *Picea abies* (L.) H. Karst, *Quercus robur* L., *Sorbus aucuparia* L. Five species occurred in sites inhabited by the Polish primitive horse [and did not exist when the horse was absent]: *Crataegus monogyna* Jacq, *Fagus sylvatica* L., *Populus tremula* L., *Prunus cerasifera* Ehrh., *Robinia pseudoacacia* L.

The dendrogram of qualitative floristic similarity (Figure 2) shows higher similarities of the shrub and undergrowth layer in tested habitats (CF or DF) than in relation to the presence or lack of feeding by the Polish primitive horse. The upper part of the dendrogram shows more areas occupying more fertile habitats (DF), and characterized by a relatively lower similarity of the species composition of the shrub and understory layer. On the other hand, the lower part of the dendrogram tends to show areas with poorer habitats (CF), which proves a higher similarity of the species composition between those areas (Figure 2). In this dendrogram four larger groups of similarities can be distinguished: 1) groups of stands in more fertile habitats with the presence of the Polish primitive horse (the upper part), 2) groups of stands in more fertile habitats without the occurrence of the Polish primitive horse (the upper and the central part), 3) groups of stands in poorer habitats, without the presence of the Polish primitive horse (the central part), 4) a group of stands in poorer sites, with the presence of the Polish primitive horse (most often in the lower and the central part of the dendrogram). However, it should be noted that a certain irregularity as regards the cluster occurrence in the dendrogram was observed for some areas.

Bray-Curtis Cluster Analysis (Single Link)

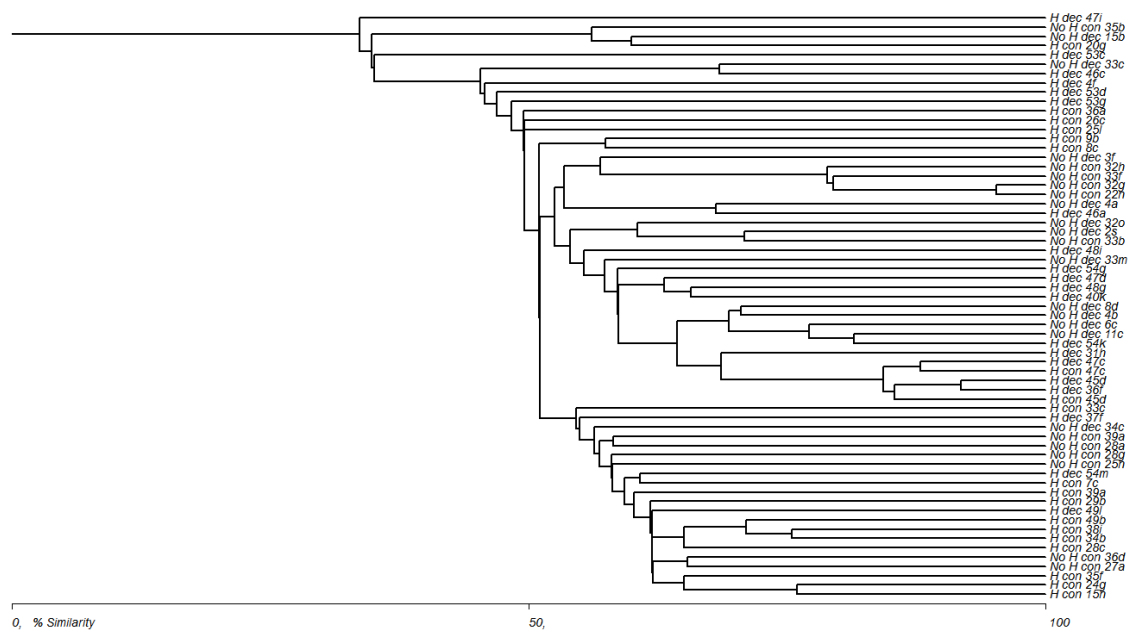


Figure 2. The similarity for clusters of shrub and understory layer species growing in all areas under examination; (H – horse present, No H – horse absent, con – coniferous forests (CF), dec – deciduous forests (DF); 7c, 54g – codes of the forest sub-compartment).

The average height of the woody understorey vegetation >0.5 m was by 30% lower in the stands with the horse, than in those without horse ($F_{1.58} = 8.913$, $p = 0.004$) (Table 2). The quality of the habitat did not affect the height of the plant layer ($F_{1.58} = 0.341$, $p = 0.561$) and the interaction between the effect of the habitat and the effect of the presence of the horse was not significant ($F_{1.58} = 2.644$, $p = 0.109$).

Species that occurred in areas with the Polish primitive horse affected an increase the the biodiversity of the shrub and understory layer, including: *Acer campestre* L., *Alnus glutinosa* (L.) Gaertn., *Crataegus monogyna* Jacq., *Daphne mezereum*, *Prunus cerasifera* Ehrh., *Robinia pseudoacacia* L. and *Ulmus glabra* Huds. (Table 3).

3.3. Evaluation of the biodiversity in plant communities

The evaluation of floristic complexes did not show the negative impact of the Polish primitive horse on the layer of herb and undergrowth (lower 0.5 m). The results of the two-way analysis of the Shannon-Wiener biodiversity index (S-W index) variance ($F_{1.64} = 0.523$, $p = 0.472$) did not reveal significant differences between the areas (Table 2). The presence of the Polish primitive horse did not affect the biodiversity of the herbs and undergrowth (below 0.5 m) between habitats ($F_{1.64} = 1.718$, $p = 0.194$), as well as interaction between these two factors was not significant ($F_{1.64} = 0.826$, $p = 0.367$).

In the understory and undergrowth (higher 0.5 m) layer, both in deciduous and coniferous habitats where the Polish primitive horse was present, a higher species diversity in comparison to the control areas was found. S-W index for understory and undergrowth was significantly higher in deciduous stands than in coniferous ones ($F_{1.58} = 6.221$, $p = 0.015$). The interaction “habitat x horse

presence” was also statistically significant ($F_{1,58} = 6.195$, $p = 0.016$), and the higher value of the S-W index was calculated for coniferous sites with the presence of the Polish primitive horse (Table 2).

The values of the Shannon – Wiener index for the shrub layer ranged from 0.2 to 0.8 in the Popielno RS area, and from 0.2 to 0.6 in the Maskulińskie FD. In this layer, the S-W index values were higher in the area inhabited by the Polish primitive horse (Figure 3). The spatial analysis of this index indicated that in the area inhabited by the horse is (insignificantly) higher than in the control area, with the absence of the horse impact (Table 2).

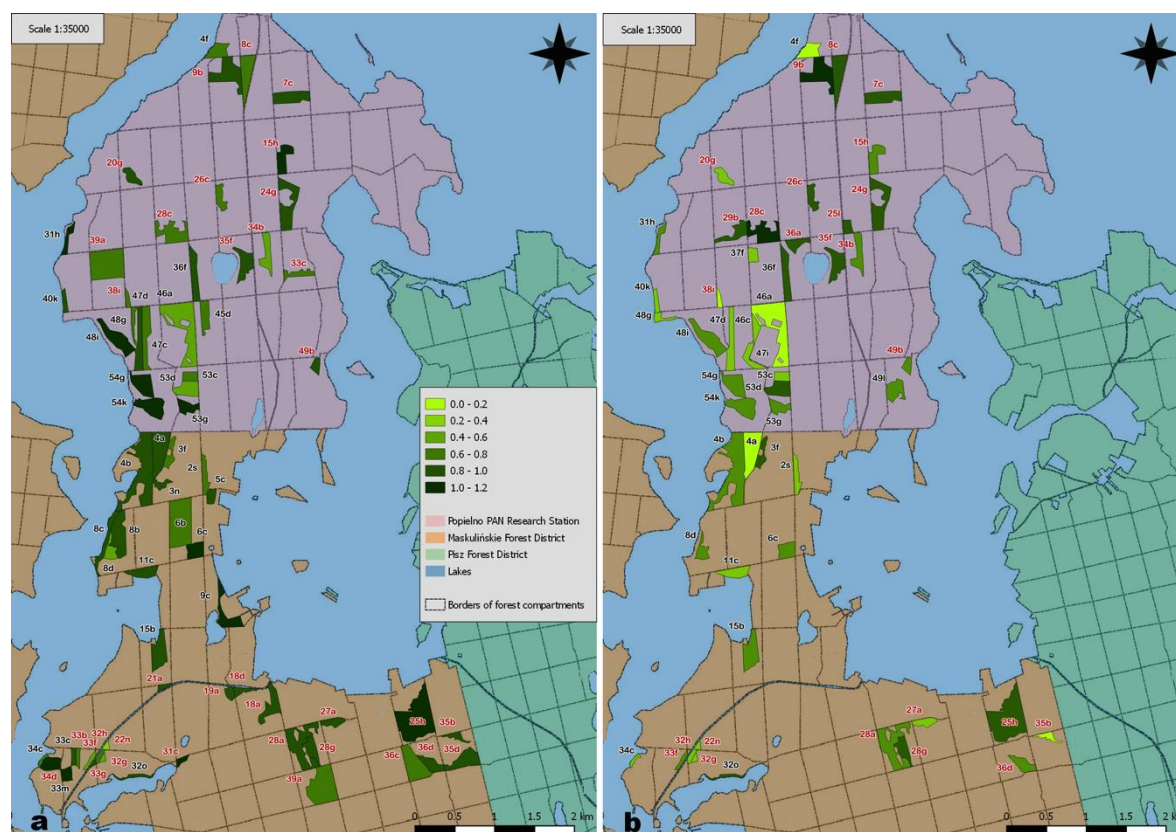


Figure 3. The location of evaluated forest sub-compartments and the graphical interpretation of Shannon-Wiener coefficient for undergrowth below 0.5 m, and herb layer a), and undergrowth higher than 0.5 m shrub layer b); description in the legend.

4. Discussion

The obtained results indicate some differences in the species composition as regards the vegetation in the herb layer and the undergrowth both below 0.5 m and higher 0.5 m and understory layer in the areas where the Polish primitive horse was present, in comparison to the tree stand without the horse. For the herb and undergrowth (below 0.5 m) layer in deciduous forests, the species diversity in areas with the horse was by 9 species higher, and in coniferous forests – by 10. Free-living horses, with forest and meadow communities situated within the boundaries of their habitat, do not demonstrate a negative pressure on natural restoration (undergrowth below 0.5 m), as they treat it first of all as the place of protection and rest. Since horses do not feed in a dense forest, they prefer habitats as well as forest edges and areas of little density of trees. Skiwski and Klich [46] also show that the spatial use of forest by the Polish primitive horses is limited not only by the food base quality. Pépin et al. [47], Haggi and Ingersoll [48], and Taraborelli et al. [49] indicated that a

dense tree stands does not provide an attractive environment for them also in view of the strategy of avoiding predators and difficult escape and due to a longer period of adaptation to the dense forest conditions. In Popielno RS this situation did not take place due to loose old trees.

Chodkiewicz and Stypiński [33] reported that by biting the species forming the shrub and understory layer, the cervids create an opportunity for less numerous, often protected species to grow. As their food, horses use species of plants that are typically avoided by other species or breeds of animals. The alimentary tract of animals contributes to the distribution of diaspores of many species of plants. This can affect the maintenance of species diversity in ecosystems of meadows and forest clearings. Sobczak et al. [50] found that in the period of full vegetation of plants (April-July), the Polish primitive horses take up 28.5-34.6 kg of plants a day. Although they inhibit the succession of forest plants, they also enrich the soil bank of meadow plant seeds. Dynowski [51] claims that in natural and semi-natural ecosystems, the Polish primitive horse can have a positive effect on the occurrence of many species of plants, both as regards herbaceous and woody plants. In forest ecosystems, these animals can affect the development and dynamics of the forest. The obtained results confirm this thesis, as it was found that the presence of the horses corresponded to the occurrence of species that were absent from comparative tree stands, e.g. *Acer campestre* L., *Hylocomium splendens* (Hedw.) Schimp. This was also determined by the environment and tree stand conditions, for example, the *Ulmus glabra* Hund. occurred only in the areas with the horse in fertile habitats, and *Crataegus monogyna* Jacq. – only in coniferous forests in the presence of the horse.

Species of bushes and trees, such as *Betula*, *Salix*, *Alnus*, *Sorbus*, found in the ecotone zone of meadows and forests are trampled and frequently used by horses [51]. Horses often use woody plants – willows, mountain ashes, maples, poplars and alder trees [7,46]. The results showed the presence of aspen seedlings only in fertile habitats in the absence of the horse. *Acer platanoides* L. seedlings occurred in areas both with and without the horse, while *A.campestre* L. (both seedlings and shrub layers) and *A. pseudoplatanus* L. – only in areas with the horse. Mountain ash occurred in great numbers, regardless of the presence or absence of the horse and the habitat. The shrub layer of alder trees occurred only in deciduous forests in the presence of the horse. Wounds created on trees as a result of bark damage, biting or breaking can facilitate the penetration of pathogens or insects, resulting in deterioration of wood quality or inhibition of growth [18,40,52]. In the presented research, no occurrence of any symptoms of infectious diseases was found.

The Polish primitive horse, in comparison to other ungulate herbivores, e.g. the red deer, to a significantly lower degree affects the tree stand. The main food base for the horse is meadow and vegetation in the forest, while the diet of the red deer typically consists of about 64% of tree and bush shoots [10, 21, 53]. The Polish primitive horses also use shoots and bark of trees and shrubs as a food supplement, but only in winter [10, 51]. They prefer deciduous trees and shrubs, willingly bite plant shoots of the *Quercus* and *Corylus* genera [54,55]. This is indirectly proven by the results of this research, since the height of the shrub and understory layers in areas with the horse was up to 30% lower than in the areas where the horse was absent. In winter, Polish primitive horses also nibble plants in the herb layer of the forest - mostly the blueberry, the reed grass or rush vegetation, i.e. plants that are of no interest to other animals [10,56]. The results presented in the study show that reed grass (*Calamagrostis arundinacea* L. Roth.) occurred only in poor habitats in the presence of the horse. The European blueberry (*Vaccinium myrtillus* L.) occurred in great numbers regardless of the habitat and the presence of the horse, while the partridgeberry (*Vaccinium vitis-idaea* L.) occurred

both at the presence and the absence of the horse in coniferous forests, but only in the presence of the horse in deciduous forests.

The lack of differences between the values of the Shannon-Wiener index for the herb and undergrowth [below 0.5 m) layer between the tree stands with the presence or absence of the Polish primitive horses observed in this study corresponds to the results of other authors. This suggests that forest vegetation, both in Popielno RS and in Maskulińskie FD, was consumed to a similar degree also by cervids. Cosyns et al. [10], and Dynowski [51] reported that in full vegetation period, meadow and pasture plants are available for the horse diet and more willingly visited than the forest. Dynowski [personal communication] provides that the dung of the Polish primitive horses includes 20 species of plants, none of which occurred in the evaluated forest areas. It should be concluded that the absence of a significant effect of the Polish primitive horse on the forest undergrowth and understory is a natural phenomenon. It results from the food preferences of this mammal, oriented rather towards meadow than forest species. A higher plant diversity evaluated on the basis of the Shannon-Wiener index was found for the shrub and understory layer in the areas inhabited by the Polish primitive horse.

The value of the Shannon-Wiener index obtained for the undergrowth higher than 0.5 m, and understory layer – both in the control areas and in the areas with the Polish primitive horse – were rather low, when compare to Spanish *Pinus radiata* forests, where the grazing of horses took place [57]. According to Magurran [58], the so-called “good” level of the Shannon index ranges from about 3 to 4, while in the pastures in Galicia it was 2.8 and 2.9 [59]. In the presented research, for the areas with the absence of the horse, the value of the Shannon-Wiener index was 0.441 ± 0.034 and for the areas with the horse it was 0.517 ± 0.036 . Stefańska-Krzaczek [60] provides at the same time that the value of the Shannon-Wiener index decreases with the age of the tree stand.

5. Conclusions

The presence of the Polish primitive horse for similar habitat did not affect the biodiversity of the herbs and undergrowth below 0.5 m. A significant effect of the habitat quality on species diversity in the undergrowth higher than 0.5 m, and understory layer was found. The presence of the Polish primitive horse had a significant impact on the increase of species diversity index for the undergrowth higher than 0.5 m, and understory layer. The average height of this layers was by 30% shorter in the forest stands presence of the horse. The average height of herb and undergrowth lower than 0.5 m was 20% higher on coniferous forests, and did not depend on the presence of a Polish horse.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: title, Table S1: title, Video S1: title.

Author Contributions: SB carried out the field measurements and draft the manuscript, EB performed the statistical analysis, ZS conceived the study and draft the manuscript, AZ draft the manuscript, MN performed the maps, JB formulated the idea and conceived the study; all authors wrote parts of the manuscript and provided editorial advice.

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Conflicts of Interest: Authors declare any personal circumstances or interest that may be perceived as inappropriately influencing the representation or interpretation of reported research results.

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