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Spider Diversity in the Fragmented Forest-Steppe Landscape of Northeastern Ukraine: Temporal Changes under the Impact of Human Activity

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Abstract: Semi-natural forests and dry grasslands are highly fragmented and influenced by human activity. Despite the small area, they serve as the refuge for habitat-specific species and enhance agrolandscape biodiversity. We studied spiders in Velykoburlutskyi Steppe Regional Landscape Park (northeastern Ukraine) for 10 years and found 224 species of 26 families; of these, 27 are rare and require protection. The araneofauna of small forests in gullies is poorer than that of the large oakeries and hosts fewer sylvatic species; the dry grassland fauna is rich, has typical steppe traits, and varies depending on topography and grazing history. The ungrazed gully hosted 125 spider species. The richest assemblages (97 species) were at the bottom, and they were similar to those of meadows and forest edges. The most typical steppe assemblages were formed on the ungrazed slope (77 species). The human-induced disturbance had a negative effect on spiders: we found only 63 species at the grazed bottom and 62 on the slope. After abrupt grazing cessation, four spider species appeared and occurred constantly at the bottom, while no species left this habitat. Grazing on the slopes declined gradually, and spider assemblages did not change significantly after the final cessation; moreover, they enriched on the abandoned slope (75 species). The presence of rare species and a variety of spider assemblages confirm the conservation value of the study site and the need to maintain its mosaic pattern.

Keywords: araneofauna; conservation value; long-term dynamics; pasture abandonment; grazed gully; Velykoburlutskyi Steppe

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1. Introduction

Forest-steppe is a climatogenic landscape where the shifting forest and grassland patches are an inherent feature of the ecosystem. Before development of lands, forests and steppes covered vast areas on the flat interfluves and in a network of ravines and gullies, forming a variety of habitats. Because the forest-steppe has been continuously transformed by human activities, the area of natural habitats has decreased dramatically and become incomparably smaller than the surrounding agricultural lands. Now, the remnants of natural areas are threatened by the growing intensification of agriculture and changes in land use (expansion of arable lands, as well as grazing pressure increase and decrease followed by pasture abandonment, afforestation, and deforestation).

In transformed landscapes, biodiversity is confined to fragmented semi-natural habitats [1–3], often being higher in protected areas than outside their limits [4,5]. Fragmentation and insularity hinder species distribution and interexchange, which can affect biodiversity. The habitat-specific species are extremely vulnerable and they quickly disappear with a change in living conditions. Assessment of the biodiversity potential of habitat fragments is crucial in understanding their contribution to maintaining landscape-scale biodiversity [6].

Evaluation of the biodiversity and conservation value of fragmented habitats gives a basis for their protection at regional and national scales.

In the northeast of Ukraine, the portion of arable land is about 69% of the total area, while that of natural lands is only 26% [7]. These lands are highly isolated and embedded in agrolandscape. The grasslands of the area in question have long been used for pasturing livestock and haymaking. In the 1990s, grazing began declining and now has mainly ceased. To preserve part of the forest-steppe landscapes of Eastern Europe in its natural state, a regional landscape park (RLP) called Velykoburlutskyi Steppe was created in 2000 in the northeast of the Kharkiv Region. The park is aimed at enhancing the biodiversity of northeastern Ukraine, protecting rare species and their habitats, and providing complex scientific research on the impact of human activity on steppe biota. The RLP consists of three clusters with a total area of 2043 ha. The focus of investigations was the Steppe Marmot (*Marmota bobak* (Müller, 1776)), now listed in the Red Data Book of Ukraine, and the vegetation of its habitats [7,8]. The data on invertebrates were restricted by periodical short-term observations.

Despite the fact that invertebrates are extremely numerous and are of key importance in ecosystem functioning, their role is still underestimated and they are less frequent subjects of conservation concern than vertebrates [9,10]. This unbalance was revealed decades ago [10] but has not yet been overcome, although the problem is being analyzed to attract the attention of researchers and stakeholders [11–13]. Moreover, the research focuses first on protecting species and their populations, not invertebrate communities in endangered habitats [11]. It is generally accepted that the main condition for invertebrate protection is the restoration of their habitats, which may require both undisturbance and appropriate management [14,15]. In creating a management plan, it is important to take into account a variety of invertebrate responses to the measures applied [2,16].

Spiders are among the most abundant groups of arthropods that have a prominent role in many ecosystems [17,18]. They are distributed worldwide, occur in all habitats and vegetation layers, and consume a great number of insects, including crop pests [19,20]. Since spiders are sensitive to altering environmental conditions, their species composition, abundance, and assemblage structure reflect habitat heterogeneity, global climate changes, and local-scale changes under the impact of various factors [21,22]. Spiders can be used as indicators while assessing areas of conservation concern and the effectiveness of management efforts [23,24]. Although this approach has already been applied in Ukraine [25], the unevenness of arachnological studies [26] makes it difficult to use it widely in the country.

History of Spider Research in the Study Area

Prior to our studies, arachnological research in the area in question was conducted in 1970–1973 on the lands of a former state farm 'Chervona Khvylia' [27]. As a result, 158 species were listed. Later, the collection was lost, and we do not know the exact sampling localities. We analyzed the species list and excluded 48 doubtful and erroneous records based on the species' geographical distribution and/or their habitat preferences in the forest-steppe and steppe zone of Ukraine [26,28–31]. As a result, we found that 110 species occurred in that period in the investigated habitats (agricultural fields, cultivated and natural lands, forest shelterbelts, and a forest in the gully).

After a 40-year break, we resumed spider studies in the Velykoburlutskyi Steppe Regional Landscape Park, located in the lands of the abovementioned state farm. In the first research period (2003 and 2008), we focused on an investigation of spider fauna and species distribution within the park's main habitats (dry grasslands, wetlands, and forests). In 2012–2014, we studied the impact of cattle grazing on steppe arthropod assemblages. In 2015, grazing ceased, and for the next five years, we monitored changes in spider diversity in the steppe plots at various stages of post-grazing succession influenced by uncontrolled fires.

These data were included in the catalogs of spiders of the Kharkiv Region [28,29] and Left-bank Ukraine [26,30,31]. We analyzed the impact of cattle grazing on the ground-dwelling spider and true bug assemblages [32], as well as the impact of steppe fire on autumnal arthropod assemblages [33]. We distinguished rare spider species and included two species in the Red Data Book of the Kharkiv Region [34–37]. Data on the regional spider fauna were used in a comparison with the araneofaunas of the protected steppe areas of Ukraine [38].

Our 10-year research (2003, 2008, 2012–2019) aimed at an (i) inventory of the local spider fauna and species habitat preferences; (ii) assessment of the conservation value and spider diversity of the study site; and (iii) identification of the long-term changes in spider assemblages at the plots corresponding with various topography and grazing management changes.

2. Materials and Methods

2.1. Study Area

The study area lies in the northeast of Ukraine (Kharkiv Region) on the right bank of the Velykyi Burluk River, a tributary of the Siverskyi Donets. The climate is moderate continental; the average annual precipitation is 450–520 mm. According to our observations, the coldest month is January, often with temperatures below −25 °C, and the hottest is July, with heat over +30 °C. In 2012–2019, the winter period began on 25 November (±11.8 days) and lasted about 98 ± 18.3 days (n = 8), and the summer period began on 7 May (±16.4 days), with a duration of 129.75 ± 13.5 days (n = 8).

The area in question has a vast net of variable ravines and gullies and dissected river valleys, and the main soil type is Chernozem [7,39]. Since the treeless flat interfluves have been totally plowed, natural habitats survived only in the gullies and the lands adjacent to rivers. The landscape is a kaleidoscopic mosaic of herb and shrub communities in a comparatively small area of the gully slopes and bottoms. Forb–fescue–feather grass steppe covers the upper and middle parts of the slopes, while the meadow steppes and steppe-like meadows are spread in the bottom parts.

Moreover, the typical landscape elements are brooks, ponds, and small rivers, with floodplain meadows and forests in the gullies. The latter form a specific habitat of the forest-steppe and the north of steppe zone, namely bairak forest, an oakery with a significant proportion of *Acer platanoides*, *A. campestris*, *A. tataricum*, and *Tilia cordata*, sparse understory, and a grass layer dominated by *Aegopodium podagraria* and *Carex pillosa*. Edges are often bordered by steppe shrubs (*Prunus spinosa*, *Swida sanguinea*).

The main research was conducted in the Nesterivka cluster of the RLP Velykoburlutskyi Steppe, covering an area of about 300 ha, including 270 ha of dry grasslands and floodplain meadows. Its land-use history, flora, and vegetation were described in our previous papers [7,39]. From 1991 to the present, we have constantly monitored changes in land use: we assessed annual grazing intensity (number of cattle per hectare), fixed dates of pasture abandonment at various parts of the study site, and the dates and areas of human-induced fires.

2.2. Sample Plots

To study grazing impact on spider assemblages, we chose five monitoring plots depending on topography and grazing history (Table 1). The plots were as follows:

1. Ungrazed gully bottom (≈137 m a.s.l.); chernozem soil. Abandoned in 1993; grazing pressure before the abandonment was weak (1–1.5 cows/ha). Part of the gully neighboring the sample plot is covered with arboreal vegetation (*Pyrus communis*, *Malus domestica*).
2. Ungrazed gully slope (≈155 m a.s.l.); strongly washed chernozem or clayey soil. Abandoned in 1991; grazing pressure before the abandonment was weak.
3. Grazed gully bottom (≈136 m a.s.l.); chernozem soil. Grazing was intensive (4–5 cows/ha) until abandonment in 2015.

4. Grazed gully slope (≈165 m a.s.l.); washed chernozem. Grazing pressure decreased from moderate (2–3 cows/ha) to weak, becoming irregular since 2011. During 2013 and 2014, the grazing area gradually shrank until finally abandoned in 2015.
5. Abandoned gully slope (≈160 m a.s.l.), washed chernozem. Grazing pressure decreased from moderate to weak, becoming irregular since 2005. It was abandoned in 2013.

Table 1. Sample plots in the Velykoburlutskyi Steppe Regional Landscape Park.

Number	Abbreviation	Description	Geographic Coordinates	Vegetation (Community of)	Study Period
			DRY GRASSLANDS		
1	UgB	Ungrazed gully bottom; grazing ceased in 1992. Aspect 330°, inclination 3–4°	49.92499° N; 37.30972° E	*Festuca rupicola*, *Fragaria viridis*, *Chamaecytisus ruthenicus*	April–October 2012–2019
2	UgS	Ungrazed gully slope; grazing ceased in 1990. Aspect 170°, inclination 10–12°	49.92531° N; 37.30829° E	*Galatella villosa*, *Festuca valesiaca* aggr.	April–October 2012–2019
3	GB	Grazed gully bottom; grazing ceased in 2014. Aspect 90°, inclination 2–3°.	49.92850° N; 37.30648° E	*Festuca valesiaca* aggr., *Poa angustifolia*, *Elytrigia repens*	April–October 2012–2019
4	GS	Grazed gully slope; grazing ceased in 2012–2014. Aspect 200°, inclination 17°	49.93078° N; 37.30734° E	*Bromopsis inermis*, *Festuca valesiaca* aggr., *Poa angustifolia*, *Medicago romanica*	April–October 2012–2016
5	AS	Abandoned gully slope; grazing was ceased in 2012. Aspect 120°, inclination 12°	49.93040° N; 37.30956° E	*Stipa capillata*, *Festuca valesiaca* aggr., *Pilosella officinarum*, *Bromopsis inermis*	April–October 2013–2016, and 2018–2019
6	OUgS	Ungrazed gully slope; grazing was ceased in 1990. Aspect 10°, inclination 12°	49.92434° N; 37.30761° E	*Festuca valesiaca* aggr., *Festuca rupicola*	April–July, September–October 2017, 2018
7	OUgS	Ungrazed gully slope; grazing ceased in 2003. Aspect 250°, inclination 8–10°	49.92455° N; 37.30445° E	*Stipa pennata*	April–July 2016
8	OUgS	Ungrazed gully slope; grazing ceased in 2001. Aspect 10°, inclination 12–15°	49.92365° N; 37.30208° E	*Festuca valesiaca* aggr., *Fragaria viridis*	April–June 2017
9	OUgS	Ungrazed gully slope; grazing was ceased in 2003. Aspect 90°, inclination 3–4°	49.92137° N; 37.30346° E	*Festuca rupicola*, *Poa angustifolia*, *Bromopsis inermis*, *Elytrigia repens*, *Agrimonia eupatoria*	April–July 2014
10	OUgS	Ungrazed gully slope; grazing was ceased in 2000. Aspect 210°, inclination 3–4°	49.92307° N; 37.30693° E	*Festuca valesiaca* aggr., *Artemisia marschalliana*	June–July 2003, May–July, and October 2008
11	OUgS	Ungrazed gully slope; grazing was ceased in 2000. Aspect 90°, inclination 2–3°	49.92307° N; 37.31009° E	*Festuca valesiaca* aggr., *Calamagrostis epigeios*, *Carex praecox*	May–October 2012
			FORESTS		
12	FE	Forest edge	49.92282° N; 37.29781° E	*Prunus spinosa*	June–July 2003, May–July, and October 2008
13	BF	Forest under the canopy. Lower third of a slope with dense grass cover	49.92223° N; 37.29713° E	*Quercus robur*, *Acer platanoides*, *Acer campestre*, *Ulmus glabra*, *Corylus avellana*, *Aegopodium podagraria*, *Carex pilosa*	June–July 2003, May–July, and October 2008
14	FE	Forest edge	49.91731° N; 37.30168° E	*Prunus spinosa*, *Ulmus* sp., *Swida sanguinea*, *Acer platanoides*, *Cerasus avium*, *Pyrus communis*	April–July 2018
15	BF	Forest under the canopy. Lower third of a slope with sparse grass cover	49.91704° N; 37.30116° E	*Quercus robur*, *Acer platanoides*, *Acer campestre*, *Cerasus avium*, *Corylus avellana*	April–July 2018
16	FE	Forest edge	49.93089° N; 37.30295° E	*Prunus spinosa*	April–July 2018, 2019
17	BF	Forest under the canopy. Lower third of a slope with sparse grass cover	49.93146° N; 37.30181° E	*Quercus robur*, *Acer platanoides*, *Acer campestre*, *Acer tataricum*, *Tilia cordata*, *Euonymus verrucosa*, *E. europaea*, *Aegopodium podagraria*	April–July 2018, 2019
			MEADOWS		
18	FM	Floodplain meadow	49.92765° N; 37.31251° E	*Festuca pratensis*, *Poa angustifolia*, *Carex* cf. *riparia*	May–July 2014, April–May 2019
19	WB	Bank of a brook	49.92390° N; 37.30610° E	*Phragmites australis*, *Calamagrostis epigeios*	May–July, and October 2008
20	FM	Floodplain meadow	49.92030° N; 37.31527° E	*Festuca rupicola*	May–July, and October 2008
21	WB	Riverbank	49.92027° N; 37.31551° E	*Phragmites australis*, *Carex* cf. *riparia*	June–July 2003, May–July, and October 2008
			OTHER HABITATS		
22	Sn	Dwellings and outbuildings	49.92474° N; 37.31303° E		May–July, and September–October 2014, 2018, and 2019

We also investigated various gully slopes and pulled them together in a group of "other ungrazed slopes". The total area of studied dry grasslands in the Nesterivka cluster is 100 ha. To find out the features of spider habitat distribution in the study site, we investigated two bairak forests and their edges, floodplain meadows, banks of water bodies (bank of a brook and the riverbank), and dwellings and outbuildings in the village. The collecting localities, their coordinates, brief description, and study period are arranged as a table (Table 1), and their positions are shown on the map (Figure 1).

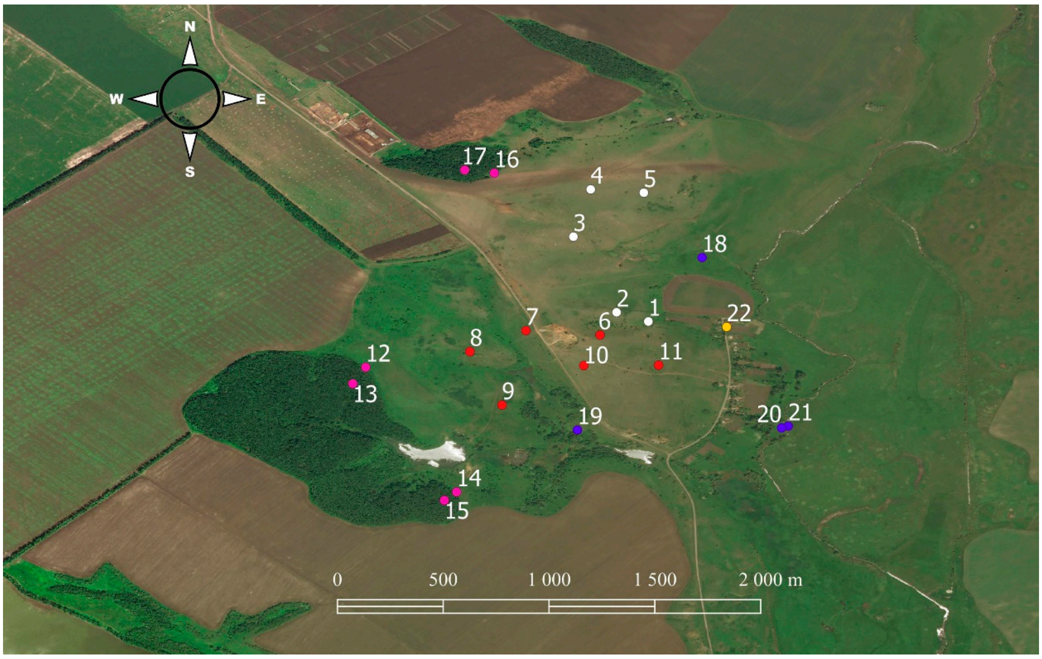

Figure 1. Map of collecting localities. Dry grasslands: 1–5 are monitoring plots on the gully bottoms and slopes, while 6–11 are other gully slopes; forests: 12–17; wetlands: 18–21; farm houses: 22. For the locality description, see Table 1.

2.3. Spider Collection

Spiders were collected with the use of standard methods: pitfall trapping, sweep netting, and by hand. Trap design depended on the research aims. For faunistic studies (2003 and 2008), we set a line transect of 10 traps at a 10 m distance in each study habitat: steppe slope, floodplain meadow, forest edge, and a bairak forest under the canopy. We used 6.5 cm diameter plastic caps half-filled with 4% formalin. The traps were exposed from late May to July and checked approximately once in 20 or 30 days.

The same method was used at five monitoring plots in 2012–2014 when studying grazing impact on ground-dwelling arthropods. The collection period lasted from April to July and from early September to early October. After a grazing cessation and a series of uncontrolled fires in the summer of 2014 and the spring and autumn of 2015, we set additional traps to collect arthropods at the burnt and unburnt plots. The number of traps per monitoring plot ranged from 15 to 24 depending on the configuration of bunt/unburnt areas (for a detailed description, see [33]).

Sweep netting and hand collecting were performed on the dates of setting and checking traps. We made a minimum of 3 samples of 50 sweeps in each plot.

2.4. Data Analysis

A list of spider species was compiled based on all the material collected in the Velykoburlutskyi Steppe RLP during the study period (2003, 2008, 2012–2019). The list is organized as a table of species habitat distributions within the study site (Table S1). Species are listed alphabetically by families and within the families, and the taxonomy follows [40].

Species collected at the five monitoring plots are given in separate columns. We mentioned juvenile individuals only if the adults were absent from our collection. The species richness of spider families was calculated for the dry grassland, forest, wetland habitats, farmhouses, and the entire territory. A dendrogram of faunistic similarity of the spider assemblages was performed in the package Statistica based on the species presence/absence in the study habitats. We used the Complete linkage as a cluster algorithm and Euclidian distance as a similarity measure.

To assess the species/site conservation value, we defined spider species rarity based on their geographical [40,41] and habitat distribution [25,30,31,38,42,43] in the forest-steppe and steppe zones of the East European Plain, with special attention paid to the species associated with threatened habitats listed in the Revised Annex I from Resolution 4 of the Bern Convention [44]. We consider a species regionally rare and requiring protection if it is habitat-specific, has a patchy distribution in semi-natural habitats, and/or exists at the border of its distribution range.

We compared the spider fauna of the study site with the faunas of two protected steppe areas in northeastern and eastern Ukraine: Dvorichanskyi National Nature Park (Kharkiv Region, area of dry grasslands about 1160 ha, study years 2014–2016, and 2019, and ad hoc collection in 2017, 2018) and Striltsivskyi Steppe department of the Luhansk Nature Reserve (Luhansk Region, area of dry grasslands 800 ha, study years—periodically in 1982, 1984, 1986, 2010, May–September 2009 and 2011) [30,31]. Araneofauna of the bairak forest was compared with that of an oakery in the northeast of the Kharkiv Region (Staritsa forestry, 50.264241° N 36.840316° E) [30]. The similarity of the faunas of compared areas was calculated by the Jaccard coefficient.

Comparison of the ground-dwelling spider assemblages of the five monitoring plots was based on the pitfall trapping in each plot/year in April–July, since this time is the most effective for spider collection. Autumnal samples were taken into account when compiling the list. The sampling effort was estimated as the number of traps per exposition period (trap-days). Since all plots were subjected to fires in whole or in parts during 2014–2015, we pulled the samples from each plot's burnt/unburnt parts together. The impact of fire on spider assemblages will be analyzed in a special publication.

We counted the number of spider species and the number of trap-days in each plot/year and analyzed whether sampling efforts impact the number of recorded species. For this purpose, we calculated the average number of trap-days and that of species at each monitoring plot during the study period. Furthermore, we clarified whether the share of species in each year (% out of the average number at the plot) depends on the share of the number of trap-days in the same year (% out of the average number at the plot). The relationship between calculated values was assessed using Spearman's correlation coefficient (rS). The comparison was performed in the R software environment [45]. In general, a correlation between the sampling effort and the number of species per plot/year was absent (rS = 0.081, $p = 0.65$). This indicates that the number of trap-days was sufficient to characterize spider diversity; therefore, we can disregard it when comparing spider species richness between different years. Sweep netting and periodical collection at other plots did not give enough material for quantitative analysis; therefore, we used them only in comparisons of the species composition.

We estimated the diversity of ground-dwelling spider assemblages at the monitoring plots by using the Shannon index of species diversity and evenness calculated in the program PAST [46]. These indices are commonly applied in ecological studies, including spider diversity [47–49], making our data comparable with the data of other researchers.

Species relative abundance was identified by the Tischler rating scale [50]: we considered dominants ($5 \leq n < 10\%$ of the total individuals on the sample plot) and eudominants ($10\% \leq n$) as a dominant complex and analyzed their structure in each plot/year. Graphs of the eight-year dynamics of the four most abundant spider species were built based on a proportion (%) of species in the assemblage.

3. Results

During the study period, 224 spider species of 26 families were collected at the study site (Tables 2 and S1). Two families, Linyphiidae (34 species, 15.2% of the fauna) and Gnaphosidae (33 species, 14.7%), were the most species-rich; the other speciose families were ranked as follows: Lycosidae (27 species, 12.1%), Salticidae (21 species, 9.4%), Theridiidae (21 species, 9.4%), Thomisidae, (17 species, 7.6%), and Araneidae (16 species, 7.1%). The proportion of Gnaphosidae was the highest in dry grasslands and declined dramatically in wetlands. Liniphyiidae had the highest ratio in the forests and wetlands and declined insignificantly (by 1.5–2%) in dry grasslands (Table 2). Salticidae and Theridiidae were the most diverse on the gully slopes, while Thomisidae and Araneidae were the least diverse in these habitats. Spider assemblages of the forest edges were the richest in species number (108). The monitored ungrazed gully and the combined group of ungrazed slopes hosted 96–97 species each, and spider assemblages of the other monitoring steppe plots were poorer than those of the meadows (63–77 vs. 88 species); the lowest number of species (51) was recorded from the water bodies, but this habitat was the least studied.

Table 2. Species richness of the spider families in the main types of habitats of the study site. Number of species (% in the fauna).

Families	Dry Grasslands Slopes of the Gullies	Dry Grasslands Bottoms of the Gullies	Forests	Wetlands	Buildings	Total
Agelenidae	1(0.8)	1(0.9)	2(1.4)	.	2	3(1.3)
Araneidae	7(5.3)	9(8.3)	12(8.7)	11(10.6)	4	16(7.1)
Atypidae	1(0.8)	.	.	.	.	1(0.4)
Cheiracanthiidae	2(1.5)	3(2.8)	5(3.6)	3(2.9)	.	5(2.2)
Clubionidae	1(0.8)	1(0.9)	2(1.4)	5(4.8)	.	6(2.7)
Dictynidae	4/3.1	2(1.8)	2(1.4)	3(2.9)	.	5(2.2)
Dysderidae	.	.	.	.	1	1(0.4)
Eresidae	1(0.8)	1(0.9)	.	.	.	1(0.4)
Gnaphosidae	28(21.4)	22(20.2)	19(13.8)	13(2.5)	.	33(14.7)
Hahniidae	1(0.8)	1(0.9)	1(0.7)	.	.	2(0.9)
Linyphiidae	17(13.0)	14(12.8)	21(15.2)	15(14.4)	.	34(15.2)
Liocranidae	3(2.3)	2(1.8)	4(2.9)	2(1.9)	.	5(2.2)
Lycosidae	13(9.9)	15(13.8)	19(13.8)	20(19.2)	.	27(12.1)
Mimetidae	.	1(0.9)	1(0.7)	.	.	1(0.4)
Miturgidae	1(0.8)	2(1.8)	3(2.2)	2(1.9)	.	3(1.3)
Philidromidae	6(4.6)	5(4.6)	5(3.6)	2(1.9)	1	9(4.0)
Pholcidae	.	.	.	.	1	1(0.4)
Prurolithidae	2(1.5)	2(1.8)	1(0.7)	1(1.0)	.	2(0.9)
Pisauridae	1(0.8)	1(0.9)	1(0.7)	1(1.0)	.	1(0.4)
Salticidae	16(12.2)	9(8.3)	11(8.0)	8(7.7)	.	21(9.4)
Sparassidae	1(0.8)	1(0.9)	1(0.7)	1(1.0)	.	1(0.4)
Tetragnathidae	.	1(0.9)	3(2.2)	5(4.8)		5(2.2)
Theridiidae	14(10.7)	7(6.4)	13(9.4)	3(2.9)	3	21(9.4)
Thomisidae	9(6.9)	9(8.3)	11(8.0)	9(8.7)	.	17(7.6)
Titanoecidae	1(0.8)	.	1(0.7)	.	.	2(0.9)
Uloboridae	1(0.8)	.	.	.	.	1(0.4)
Total	131(100)	109(100)	138(100)	104(100)	12	224(100)

Only five spider species (*Mangora acalypha, Dictyna arundinacea*, *Haplodrassus signifier*, *Xerolycosa miniata*, and *Xysticus cristatus*) were generalists and occurred in all the natural habitats of the study site, and 99 species (45.6% of the fauna) were recorded from one or two habitats only. Most species (86) were found in singletons, which does not give us an opportunity to define their habitat preference. Thirteen species can be considered stenotopic within the study area: *Abacoproeces saltuum*, *Trematocephalus cristatus*, *Alopecosa trabalis*, *Agroeca brunnea*, *Euryopis flavomavulata*, and *Xysticus luctator* occurred only in the bairak forests and on the forest edges; *Linyphia hortensis* was collected in the forests and on the riverbank; *Arctosa leopardus, Pardosa paludicola.*, and *P. pullata* were collected in the floodplain meadows; *Bathyphantes nigrinus* occurred near water bodies; and we observed *Gnaphosa leporina* and *Eresus kollari* in two steppe habitats. If considering dry grassland spiders in general, 46 species were not found in forests and wetlands.

In the dendrogram of faunistic similarity, spider assemblages of the wetlands and bairak forests form a separate cluster, spiders of the forest edges and ungrazed gully bottom make one branch, and a cluster of dry grassland spiders is divided into two parts depending on grazing regime at the plot (Figure 2).

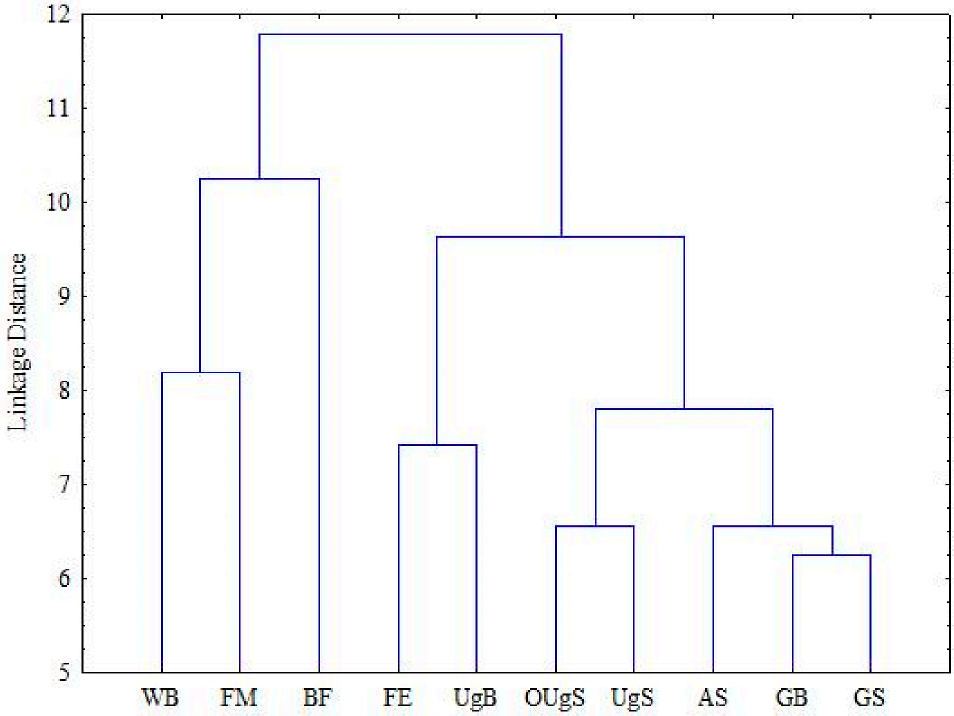

Figure 2. Dendrogram of faunistic similarity of spider assemblages of the study habitats (complete linkage, Euclidean distance). For the abbreviations, see Table 1.

A comparison of spider faunas of the dry grassland habitats of three protected areas, namely Velykoburlutskyi Steppe (VS; 150 species), Dvorichanskyi Park (DP; 143 species), and Striltsivskyi Steppe (SS; 159 species), showed their high similarity: $K_{Jaccard}$ VS/DP = 73.7%, VS/SS = 65.5%, and DP/SS = 67.1%. The similarity of the spider faunas of the bairak forests of VS and the Starytsia oakery was lower ($K_{Jaccard}$ 60.2%). Forest edges in VS were richer in dry grassland species coming from neighboring steppe plots, especially gnaphosids and lycosids, while sylvatic species occurring under the canopy were rare (*Tenuiphantes flavipes, Microneta viaris, Linyphia hortensis*) or absent (*Helophora insignis* (Blackwall, 1841), *Zilla diodia* (Walckenaer, 1802), *Anyphaena accentuata* (Walckenaer, 1802)). The number of Linyphiidae species in VS was twice as low as in Straitsa (21 vs. 42).

We consider 27 spider species (12.1% of the Velykoburlutskyi Steppe araneofauna) as rare and requiring protection. *Altella hungarica* is known from several localities in Hungary, Ukraine, and south European Russia; *Pardosa maisa* and *Agroeca makarovae* are rare in the East European Plain; *Agyneta fuscipalpa, A. saaristoi, Ipa terrenus, Micaria silesiaca, Laseola coracina, Chalcoscirtus nigritus*, *Aelurillus laniger*, and *Argenna patula* are rare in Ukraine, with the latter two species were recorded for the first time from the Kharkiv Region. *Argenna subnigra, Eresus kollari, Civizelotes pygmaeus, Drassyllus vinealis, Gnaphosa dolosa*, G. lugubris, G. licenti, Micaria rossica, Zelotes mundus, Z. segrex, Zora pardalis, Thanatus oblongiusculus, Talavera aperta, T. petrensis, Euryopis quinqueguttata, and *Titanoeca veteranica* are rare and/or locally distributed in the Kharkiv Region. In 2013, *G. lugubris* and

E. kollari were listed in the regional Red Data Book. *Trachyzelotes lyonneti* is classified as rare [25] but needs verification due to the loss of a specimen. All habitats, except water bodies and forests under the canopy, hosted from two to eleven rare species. The richest were the grazed and ungrazed slopes, as well as a group of "other slopes", but no one rare species had one preferable habitat.

Pitfall collecting at the five monitoring plots revealed 106 species. There, 36 species (34.9%) were spread widely, and 58 species (54.7%) were found in one or two plots. The total number of recorded species per plot ranged from 47 to 66 (Figure 3). The poorest habitat for spider species was the grazed slope; however, it was investigated for a shorter period (5 years). It is worth mentioning that in the fifth study year, the accumulated number of recorded species per plot reached an approximately equal level of 47–50 species (except the ungrazed gully with 61 species).

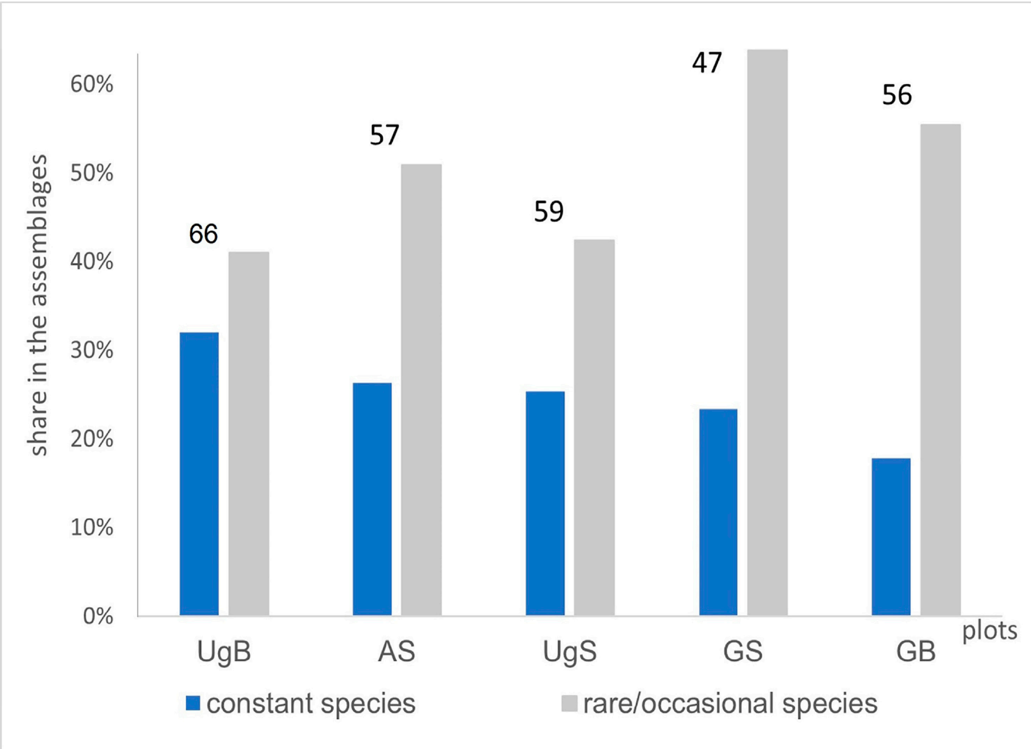

Figure 3. The constant and rare species ratio at the monitoring plots during the study period (2012–2019). The numbers above the bars indicate the total number of species in the habitat. For the abbreviations, see Table 1.

The second-year sampling gave 10 new species (37%) on the ungrazed slope and 19 species (58%) at the ungrazed bottom. Then, the number of accumulated species increased by two to six annually. At the ungrazed bottom, the species richness stopped growing in 2016 and 2017, but then the growth continued (Figure 4). At the grazed gully bottom, the proportion of new species was 48% in the second year and 32% in the third year, and only then it started growing slowly. On the grazed and abandoned slopes, the second-year contribution was 28% and 13%, respectively, with further slow growth. Thus, the process of record accumulation was specific to each plot.

The ratio of species commonly recorded during the entire study period (constant), and rare/occasional species found only in one or two years, varied significantly (Figure 3). A maximum of constant and a minimum of rare species were recorded from the ungrazed bottom. At the grazed plots, we recorded a minimal number of constant species at the bottom and a maximal number on the slope.

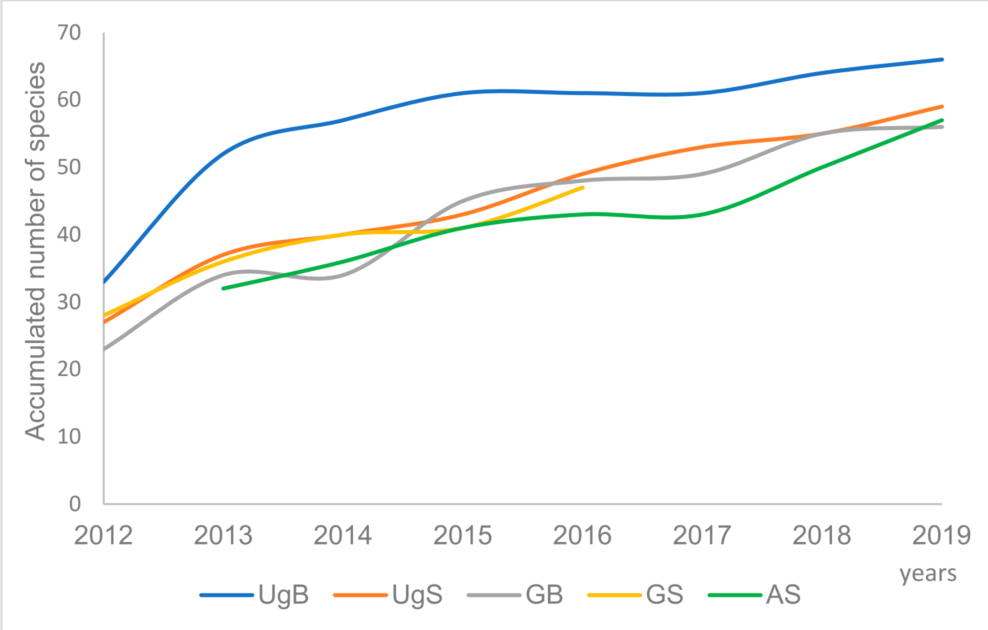

Figure 4. Accumulation of the number of recorded spider species at the monitoring plots during the study period. For the abbreviations, see Table 1.

Sixteen species dominated (i.e., exceeded 5% of the collected individuals) at least in one plot/year and formed the dominant complex (Figures S1–S5). *Alopecosa farinosa* was dominant/eudominant at five monitoring plots, *Throchosa robusta* and *Gnaphosa lugubris* at four plots, and *Alopecosa cuneata* and *Thanatus arenarius* at three plots. The others were periodically dominant at various plots, only *Xerolycosa miniata* was very common in the grazed gully bottom, and *Gnaphosa licenti* and *Berlandina cinerea* were the first- or second-ranked dominants on the ungrazed slope. The peaks and falls of a certain species' relative abundance at various plots did not match in years (Figure S6). Only *Alopecosa farinosa* showed a common tendency in 2012–2014 and 2016–2018, and *Gnaphosa lugubris* peaked in 2015.

All the monitoring plots hosted the specific dominant complexes. The grazed bottom is characterized by the annual dominance (three years in very high proportions) of *Xerolycosa miniata*, while the abandoned slope is characterized by *Gnaphosa lugubris*. On the grazed slope, *G. lugubris* predominated only in some years. The ungrazed bottom had a dominant complex of mesic grasslands (*Alopecosa farinosa*, *A. cuneata*, *A. pulverulenta*, *Haplodrassus signifier*, and a generalist *Trochosa terricola*). The latter three were rare or absent from other studied plots. The ungrazed slope, on the contrary, was dominated by *Berlandina cinerea* and *Gnaphosa licenti*, which are species of open habitats with sparse vegetation and high insolation. Periodically, this dominant complex was supplemented by *Alopecosa cuneata* and *A. farinosa*, the most numerous at the adjacent ungrazed bottom.

Dominant spider complexes of the ungrazed slope were the most balanced, they included five to seven species annually, and the maximum proportion of one species did not exceed 24%. In the other plots, the proportion of main eudominants fluctuated over a wide range, and the number of species in dominant complexes ranged from two to seven. Thus, *Gnaphosa lugubris* reached 42% and 60% on the abandoned and grazed slopes, respectively, the proportion of *Xerolycosa miniata* fluctuated from 20 to 72% in the grazed bottom, and that of *Alopecosa cuneata* from 15 to 46% in the ungrazed one.

Annual changes in the dominant species abundance and the number of rare species were reflected in the dynamics of spider alpha diversity. The minimum and maximum index values in the studied plots did not coincide by years. (Table S2). Thus, at the ungrazed bottom, the Shannon index was the highest in 2013 and the lowest in 2018, while at the grazed one, it was highest and lowest in 2013 and 2015, respectively. There were no significant peaks and falls in spider diversity on the ungrazed slope, while on both grazed and abandoned slopes, a sharp decrease was observed in 2015. On average, spider alpha diversity was the highest on the ungrazed slope and the lowest at the grazed bottom (Figure 5). Although the difference is statistically insignificant, there is an obvious trend of diversity growth with the decrease in grazing pressure. Moreover, the deviation from the mean value was the highest in the grazed plots.

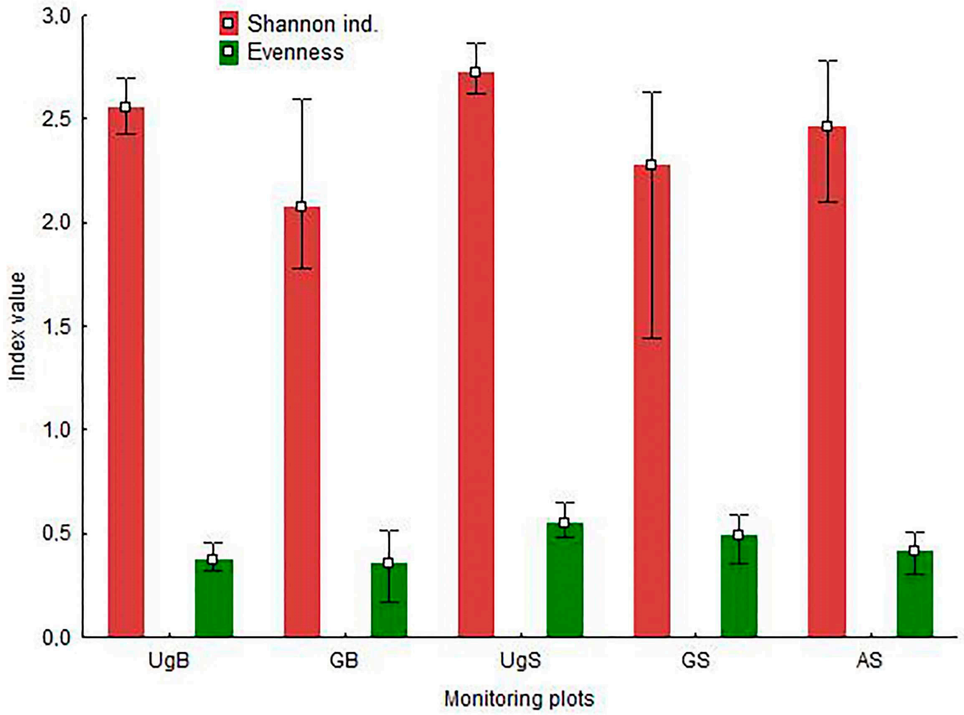

Figure 5. Alpha diversity of the ground-dwelling spider assemblages at the monitoring plots. Whiskers mean the non-outlier range. For the abbreviations, see Table 1.

4. Discussion

The spider fauna of Velykoburlytskyi Steppe RLP is rich and well preserved. It includes approximately half of the species registered in the Kharkiv Region (446), and its spider assemblages have traits typical of the dry grassland spiders [38,43,51]. We recorded 83 species, found in previous research 40 years ago [27], while 27 species were absent from our collection. These occur in the grassland and forest habitats in other localities, with those in the Kharkiv Region never being numerous [25,29].

The studied fauna differed from the other local faunas of the forest and forest-steppe zones [38,42,52] by an approximately equal ratio of Linyphiidae and Gnaphosidae. As a rule, Linyphidae is richer, and its prevalence increases in the localities with forest and wetland habitats, especially in the forest-steppe [42,51,52]. Presumably, the lower Linyphiidae number in the grassland part of the RLP is explained by the long grazing history and shallow litter periodically destroyed by fire. In the bairak forests, the reason for the decrease could be the monotony of lighting and humidity conditions, as well as sparse grass cover. The third dominant family is Lycosidae, which is typical of the forest-steppe zone. In the steppe zone, Lycosidae is less represented than Salticidae [42].

A comparison of the dry grassland spiders in a range of conservation areas of Ukraine shows that Velykoburlutskyi Steppe fauna is a little poorer than those of Stritiltsivskyi Steppe (Luhansk Region) and Buzkyi Hard (Mykolaiv Region)–159 species each—but richer than the faunas of Khomutivskyi Steppe (Donetsk Region)–126 species—and Dvorichanskyi Park (Kharkiv Region)—142 species. Only the araneofauna of Kamyani Mohyly (Donetsk Region) is significantly richer—196 species. This nature reserve is best studied and has elements of the vertical zonation of the Donetsk Ridge. All the above-mentioned conservation areas aim at protecting the habitats listed in Resolution 4 of the Bern Convention—E1.2 Perennial calcareous grasslands and basic steppes. [41]. Our data confirm that even small areas of natural habitats in the agricultural landscape provide shelter for a high variety of species, including stenotopic ones, enhancing biodiversity on both local and landscape scales [1,2,5].

Velykoburlutskyi Steppe has a high conservation value since it hosts the bulk of rare species and typical grassland and forest spider assemblages. The nearest conservation area, Dvorichanskyi Park, located 40 km from VS, is also rich in rare species (30, which is 14.7% of the fauna). Although the parks cover similar habitats (except chalk grasslands in Dvorichanskyi Park), only 14 rare species were common [35] (NP pers. data) This suggests a strong limitation of habitat-specific species distribution due to the fragmentation and insularity of their natural habitats. For this reason, the fragments of natural habitats are of high conservation concern [4,53], and the current task is to create a network of natural areas to increase the level of connectivity and maintenance [1,3,54]. For this purpose, a project of the Eastern Steppe National Nature Park has been developed in the frame of the fundamental scientific and conservation programs of V.N. Karazin Kharkiv National University.

Long-term studies make it possible to discover rare species and estimate biodiversity changes in the study area [55]. Another approach is an investigation of a variety of habitats in a shorter period [18,56,57]. Both were applied in our studies.

Periodic spider collection on various ungrazed slopes revealed 96 species—as many as eight-year sampling at the ungrazed gully bottom (97 species) and more than on the ungrazed slope (77 species). Sporadic short-term sampling on the slopes enriched the spider list by nine species, while the long-term studies of the monitoring plots added from one to three (or zero at the ungrazed bottom) new species to the study site. Thus, the investigation of a mosaic of plots provided a faster accumulation of the species records than the long-term studies at one plot/habitat. This corresponds with the statement that both the amount [6] and variety of suitable habitats [2] enhance species diversity.

The annual accumulation of species has its specific dynamics at each monitoring plot, never reaching a permanent maximum. We observed a high increase in the second study year in the undisturbed plots (ungrazed bottom and slope), and then a slow growth during the entire study period. Interestingly, no new species appeared at the grazed bottom for two years, but then the species accumulation continued. At the disturbed plots (grazed bottom and grazed and abandoned slopes) we did not reveal a common tendency. Presumably, the variety was caused by the disturbance and its uneven changes. Lack of grazing promoted the long-term stability of spider assemblages. The ungrazed bottom was characterized by the highest proportion of constant spider species and the lowest of the occasional ones.

An abrupt abandonment of intensive grazing in the gully bottom in the third sampling year may have contributed to an increase in species richness. Therefore, the species accumulation grew quickly in both the second and third years. Four spider species appeared in the plot in the year of grazing cessation and then were recorded in the following years. Moreover, *Zelotes electus* was part of the dominant complex. No species left the abandoned pasture or decreased in number dramatically after the pasture abandonment. On the grazed slope, where the grazing pressure was slowly declining, we did not find significant changes after the final grazing cessation. The appearance of a species on the plots does not always mean that it is new to the study site. This often happens due to species exchange between the plots.

Based on our eight-year study, we can conclude that the lowest spider species richness on the grazed slope was due to a shorter sampling period, while the highest richness in the ungrazed bottom was caused by other reasons, probably habitat conditions. Without grazing, steppe gullies overgrow with meadow vegetation, namely shrubs and trees, causing the formation of a mesophilic complex of spider species, inherent in the forest edges and meadows [32].

Despite the small areas and close locations, all the monitoring plots host the specific dominant spider complexes, with abundant mesophilous species at the ungrazed bottom and xerophilous species on the ungrazed slope. *Xerolycosa miniata*, the most numerous species of dry grassland pastures, predominated at the grazed bottom. In the forest-steppe and steppe, *Gnaphosa lugubris* occurs only in open grasslands, not in humid habitats, as mentioned for most of Europe [58]. Although existing on the border of its geographic range, this species was abundant in suitable habitats, especially grazed and abandoned slopes. Interestingly, 40 years ago it was also spread widely in the study area [27].

In general, spiders preferred the undisturbed habitats of the study site. Their alpha diversity at ungrazed plots was high and most stable during the study period. The lowest was at grazed gully which was under the most intensive grazing pressure, and further pasture abandonment did not cause a swift diversity increase. We recorded 125 species in the ungrazed gully and 99 species in the grazed one and on the abandoned slope. A reduction in spider species richness was also recorded in acidophilous steppe grasslands [55,59]; however, other researchers reported a few [60] positive effects regarding moderate grazing and spider diversity, recommending it as a conservation method [61,62]. When creating management plans, we should take into consideration the varied, sometimes opposing, responses of various members of the dry grassland biota on the impact of a certain factor [32,33,63]. The best method may be to simulate natural disturbance and create patchwork conditions, which will attract a wide range of species [50,58]. In Velykoburlutskyi Steppe, patchy dry grassland communities have been formed over years due to uneven pasture abandonment and gully topography, which promoted high spider diversity in a relatively small, isolated area.

5. Conclusions

The remnants of natural forest and grassland habitats in the forest-steppe of northeastern Ukraine host rich spider fauna. Despite the small area, they enrich local biodiversity with both rare and typical dry grassland species. Because of insularity, most rare species were specific to each fragment. Spider distribution in the area in question indicates the necessity of preserving each natural site as a refugium of the biodiversity of modern agricultural landscapes. The fauna of bairak forests, especially under the canopy, is poorer and less specific than in the large oakery. High spider diversity in Velykobutlutslyi Steppe Regional Landscape Park is due to the large variety of habitats and uneven pasture abandonment. Moreover, long-term studies revealed a large number of rare and less-abundant species. Sampling in small plots on several steppe slopes provided a faster accumulation of spider species records than a collection in one habitat for several years.

Spider assemblages were more stable and richer in ungrazed gullies. In the grazed and abandoned plots, the assemblages are more dynamic, with a high number of periodically appearing species. Abrupt grazing cessation attracted some new spider species but did not cause the disappearance of those that already existed in the pasture. Meanwhile, long-term abandonment of dry grasslands in relief depression causes vegetation alteration and a bias to mesophilic spider assemblages typical of meadows and forest edges. Spider studies confirmed that in the conservation management of fragmented forest-steppe habitats, it is mandatory to combine undisturbance and traditional pastoralism to maintain high biodiversity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15030351/s1, Table S1: Spider species composition and habitat distribution in the Velykoburlutskyi Steppe Regional Landscape Park (2003, 2008, 2012–2019); Figure S1: Dominant spider complexes in the ungrazed gully bottom (UgB) in the study years, Figure S2: Dominant spider complexes on the ungrazed gully slope (UgS) in the study years, Figure S3: Dominant spider complexes in the grazed gully bottom (GB) in the study years, Figure S4: Dominant spider complexes on the grazed gully slope (GS) in the study years, Figure S5: Dominant spider complexes on the abandoned gully slope (AS) in the study years, Figure S6: Eight-year dynamics of the most numerous spider species at the monitoring plots.

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