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Impact of Secondary Succession in the Xerothermic Grassland on the Population of the Eastern Pasque Flower (*Pulsatilla patens*)—Preliminary Studies

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Abstract: We studied the impact of secondary succession in xerothermic grasslands on a population of *Pulsatilla patens*, a species of European Community interest. We established two permanent plots with a high number of individuals of *P. patens* in a xerothermic grassland in Southern Poland. We compared two areas, the first in open grassland (plot A), and the second with overgrowing vegetation (plot B). We assessed the population structure as well as the individual traits of the species. The total abundance of *P. patens* in the open xerothermic grassland was five times higher than in the overgrowing xerothermic grassland. A randomly clustering distribution was noted only in plot A; in plot B a random type of distribution occurred. The density structure of the rosettes was higher in plot A. The mean number of leaves in rosettes of *P. patens* as well as dimensions of intermediate stems and leaves of the species is strongly correlated with habitat conditions. The shadowing caused by shrubs and trees and high weeds observed in the overgrowing xerothermic grassland negatively impacted on the number of individuals, distribution, structure and morphology of *P. patens*.

Keywords: population structure; rare species; semi-natural community; floristic composition; conservation; land use

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1. Introduction

Light is a significant factor in the formation of varied plant communities [1,2], as well as playing a fundamental role for different plants species [3,4]. Light provides the energy for the production of organic matter and is responsible for growth effects [5], e.g., seed germination or organ elongation. One of the major factors affecting plant growth is light intensity [6]. The ecological effects of changes in light intensity are most clearly visible in non-forest semi-natural plant communities, i.e., meadows or grasslands [7]. In these communities, changes in access to light for herb species are correlated with secondary succession [8,9]. After the cessation of usage (mowing or grazing), the overgrowing secondary succession species like *Rhamnus cathartica* and *Pinus sylvestris* intensify the shadowing [10,11].

This phenomenon was observed in the xerothermic grassland communities that are characteristic of the *Festuco-Brometea* class. These communities have a high conservation value and they are included in the European Natura 2000 network [12]. In xerothermic grasslands, the amount of light significantly affects the species richness and changes in plant cover. The transformations of xerothermic grasslands are caused mainly by changes in the ways and the intensity of their use, especially the cessation of grazing [13]. Across Europe these communities have been endangered mainly by secondary succession [14], which causes, firstly, overgrowth of gaps, next, the appearance of high hardy perennial plants, and lastly, saplings of shrubs and trees. The overgrowing grassland species are forced to adapt to the changing quality and quantity of light.

The Eastern Pasque Flower is one of the heliophilous species which occurs in xerothermic grasslands characteristic of the *Festuco-Brometea* class [15,16]. *Pulsatilla patens* (L.) Mill. ssp. *patens* (exemplary synonyms: *Pulsatilla patens* (L.) Mill., *Anemone patens* L.) is a lowland species of boreo–meridional–continental and circumpolar distribution. Its range covers the sub-polar areas of Europe, Asia and North America [17,18]. In Europe, it occurs in the central and eastern parts. The western border runs across Germany and the Czech Republic [19]. In Poland, the localities of the species are concentrated in the north-eastern part, and its frequency decreases towards the west and south [20].

In many European countries it is a rare and threatened plant species. It has been included in Appendix I of the Bern Convention [21] and in Annexes II and IV to the Habitat Directive of EU [12]. It is also listed in the IUCN List of Threatened Species—DD category [22].

Within its geographical range, *P. patens* appears in different habitats and types of plant communities, but the species prefers habitats with good access to light—mainly open, dry coniferous forests and different types of grasslands [12,23]. Additionally, in forests, the most favourable conditions *P. patens* finds are disturbed areas, which allow for better exposure, e.g., gaps in stands, forest edges, near forest paths and clearances. Moreover, in its entire geographical range, many localities are found in well-exposed anthropogenic sites, i.e., pastures, at the edges of yard areas, near roadsides, on forest fringes and clearances in forests, and also on the edges of gravel pits and on almost bare rock pavements (primarily limestone pavements) [12].

Still, the constant disappearance of localities of the Eastern Pasque Flower is observed, as well the diminishing size of its populations [24,25], hence species conservation is of special concern. Due to this fact, it is very important to become acquainted with every ecological factor which can impact its decreasing populations.

Among the major factors threatening the species are the destruction and fragmentation of habitats, continuously decreasing forest areas, eutrophication of habitat and also cessation of grazing [23,26,27]. Sometimes the reasons for the decline are unknown and named as a 'biocoenotic evolution'—but we still do not know which particular factor impacts this process and what are the symptoms of the disappearance of the species.

Considering this insufficient state of knowledge, the present investigations—aiming at an assessment of the impact of secondary succession on the population of this threatened species in a new, abundant location in Poland—were undertaken.

Our hypothesis is that secondary succession in the xerothermic grassland (connected with a reduction in the intensity of light) is one of the main factors that negatively impacts on the number of individuals, distribution, age, structure and morphology of *Pulsatilla patens*.

The main goal of the study was to show the impact of the habitat conditions (coverage, light intensity, species composition and selected soil characteristics) on the population and individual traits of the Eastern Pasque Flower occurring in permanent plots established within two different successional stages (open and overgrowing) in the xerothermic grassland.

2. Materials and Methods

2.1. Study Area

The study area is located on the Chęciny Hills, a region situated in Southern Poland (Central Europe). The climate of the region is temperate continental; the mean annual air temperature is 7.4 °C (the average temperature in January is 2.9 °C; in July, 17.2 °C). The annual number of days with snow cover varies from 60 to 84. The mean annual precipitation is 600 mm. This area is formed of Middle Devonian limestones and dolomites or Upper Jurassic limestones that cover the Lower Cambrian slates. The prevailing soil type is rendzina (shallow alkaline or neutral soils). The agricultural landscape predominates, however, in some parts of the area the mining landscape is also clearly marked. Semi-natural xerothermic grasslands of the *Festuco-Brometea* class have developed on the highest parts of deforested hills (mainly on the southern and south-western slopes) as a result of human agro-pastoral activity. After abandoning the traditional management practices, large areas of grasslands are overgrown by thermophilous shrubs communities, or they have been reforested with *Pinus sylvestris*. Small patches of xerothermic grasslands have been preserved in the vicinity of carbonate rock quarries [15].

Pulsatilla patens grows in a xerothermic grassland between two active limestone and dolomite quarries. This phytocoenosis covers about 0.2 ha and is surrounded by a pine forest. Halfway, the grassland has not been managed during at least 30 years (owner information), and it is overgrown with *Pinus sylvestris*, *Rhamnus cathartica* and *Frangula alnus*.

2.2. Field Sampling

Fieldwork was carried out in the growing season of the year 2020 in the xerothermic grassland (50°47′11″ N, 20°33′51″ E) where *Pulsatilla patens* ssp. *patens* occurs. In this habitat, two permanent study plots (with the dimensions 5 m × 5 m) were established in two different structures of this community: open xerothermic grassland (plot A) and overgrowing xerothermic grassland (plot B). Both plots were located next to each other (at a distance of 10 m), occupy the same geological background, and had the same exposure (south) and slope (15°).

In each plot, the following parameters about the population structure of *P. patens* were surveyed: (i) total abundance—number of individuals (leaf rosettes), (ii) distribution and density and (iii) age structure (share of juvenile, vegetative and generative individuals).

Then, we measured the following traits for every individual rosette: (i) the number of leaves, (ii) the height of the lowest and the highest leaf, (iii) the width of the largest and the smallest leaf blade and (iv) the length of the greatest and the lowest lobe of the leaf.

To determine the floristic composition, the structure and plant cover of the grassland in every plot, phytosociological relevés using the Braun-Blanquet method [28] were made (one in each permanent plot). In each relevé, the cover-abundance of each species was scored using the Braun-Blanquet scale (+ < 5%, 1 = 5%, 2 = 5–25%, 3 = 25–50%, 4 = 50–75% and 5 = 75–100%). In addition, the height of the neighbouring plants was studied in each plot on the basis of the 10 lowest and 10 highest stems [29].

Additionally, in the two study plots, the light conditions (illuminance) were measured in the centre of each plot using a hand-held light meter (Digital Lux Meter, Model: GM 1020L-50, Białystok, Poland), in lux (lx)—SI unit of illumination equal to a luminous flux of 1 lumen per square metre, where 1000 lx = 1 kilolux (klx). During the measurement, the light meter sensor was aligned directly upwards, detecting the amount of light reaching the sensor from the entire sky hemisphere [30]. Two locations were within a distance of 10 m, so the time between the light measurement did not exceed 5 minutes [31]. The measurements were done every week for four weeks. Three repetitive replicates were taken for each plot. In total, we collected 24 measurements for both plots.

Moreover, within the border of each plot, three substrate samples (mineral material) were taken from a depth of 2–30 cm (in the beginning, the middle and the end of each plot) using a soil auger (three soils for one plot for a total of six soil samples), in order to analyse the basic soil characteristics.

2.3. Data Analysis

Chemical analyses were made from the collected soil samples, using methods generally accepted in the field of soil science. We determined the following parameters: the pH, KCl and Ca (calcium), Mg (magnesium), K (potassium) and P (phosphorus) availability content.

The non-parametric Mann–Whitney U test was applied to check whether there were significant differences between two plots in terms of: i) studied individual traits *of Pulsatilla patens* ssp. *patens*, ii) height of stems of the highest and the lowest species growing in the vicinity of *Pulsatilla patens* ssp. *patens,* iii) intensity of light and iv) soil characteristics.

The chi-square test was applied to establish whether significant differences existed among the two study plots in terms of some parameters of the structure of the populations.

All the results of the conducted analyses were considered as statistically significant at $p \leq 0.05$.

The nomenclature of the species was used according Mirek et al. [32], and syntaxa names according to Mucina et al. [33]. The analyses were carried out using Statistica 6.1 [34].

3. Results

3.1. The Habitat Conditions

The chemical analyses of soil samples showed that there were no differences between both plots in terms of the soil properties. The plots are located on the same soil type and the soil properties showed no difference between the two plots (Table 1). This suggests that the differences between the population structure and individual traits are connected with other factors (cf. Methods section).

Table 1. Mean (range) of some habitat conditions (height of plants, light, properties of soil) in two study plots: open xerothermic grassland (A) and overgrowing xerothermic grassland (B).

	Conditions	A	B	The Mann–Whitney U Test Value	p Value
plants	The maximum height of the neighbouring plants (cm)	53.4 (34–90)	98.9 (78–117)	U = −3.55	$p \leq 0.001$
	The minimum height of the neighbouring plants (cm)	8.0 (4–15)	47.6 (26–60)	U = −3.77	$p \leq 0.001$
light	The light (klx)	16.97 (15.12–17.45)	0.44 (0.32–0.55)	U = 2.88	$p \leq 0.001$
soil	pH_{KCl}	7.6 (7.3–7.7)	7.4 (7.2–7.5)	U = 0.48	$p = 0.63$
	Ca (mg/l)	4135 (4125–4142)	4125 (4121–4126)	U = 0.53	$p = 0.71$
	Mg (mg/100 g)	1337 (1332–1338)	1338 (1331–1340)	U = −1.28	$p = 0.21$
	K (mg/100 g)	57.1 (55.1–57.4)	54.7 (54.1–54.8)	U = 0.16	$p = 0.87$
	P (mg/10 0g)	17.9 (16.2–18.0)	16.1 (15.9–17.0)	U = −0.16	$p = 0.87$

The two studied plots were located in two different structures of xerothermic grassland. The statistical analysis (the Mann–Whitney U test) confirmed that the mean light conditions differed significantly between the study plots (U = 2.88, $p \leq 0.001$). In the open xerothermic grassland (plot A), the light had almost 100 times higher value than in plot B (Table 1). Different light conditions may have influenced changes in the structure of the xerothermic grassland, i.e., percentage cover, floristic composition, as well as impacted the structure of the herbaceous plants (including *Pulsatilla patens*).

The analysis of the floristic composition of the communities indicated that it is xerothermic grassland of the *Festuco-Brometea* class (Table 2). However, the structure of the phytocoenosis differs in the two plots. Plot A showed a single herbaceous layer (Table 2, relevé 1) with 34 plant species. Some gaps in the cover (85%) are typical of xerothermic grasslands. Sixteen thermophilic species characteristics of the *Festuco-Brometea* and *Trifolio medii-Geranietea sanguinei* classes were noted in the investigated patch (Table 2, relevé 1). *Pulsatilla paten*s occupied a 25% area of the whole plot.

Plot B showed a different structure (Table 2, relevé 2). The phytocoenosis is structured in a three-layer community and follows an evident secondary succession process. The tree layer was built of *Pinus sylvestris*. The shrub layer occupied ca. 65% of the whole plot. In this layer, *Rhamnus cathartica* prevailed. In the herb layer, 32 species were noted (including 17 thermophilic species characteristics of the *Festuco-Brometea* and *Trifolio medii-Geranietea sanguinei* classes—Table 2, relevé 2). The total coverage of the herb layer was 100% and no gaps were observed. In this plot, *Pulsatilla patens* had the lowest percentage cover of all plots (ca. 5%).

Table 2. Floristic composition of the two studied plots of *Pulsatilla patens*: open xerothermic grassland (relevé 1) and overgrowing xerothermic grassland (relevé 2). To show the percentage cover of species, the Braun-Blanquet scale is used (+ < 5%, 1 = 5%, 2 = 5–25%, 3 = 25–50%; Ch—species characteristic for respective syntaxa, All.—alliance, Ass.—association, Cl.—class, O.—order [28].

No. of Relevé		1	2
Date (d/m/y)	-	5 June 2020	5 June 2020
Area of relevé [m^2]		25	25
Coverage of tree layer a [%]		-	25
Coverage of shrub layer b1 [%]		-	25
Coverage of shrub layer b2 [%]		2	40
Coverage of herb layer c [%]		85	100
No. of species in relevé		38	41
Trees and shrubs:			
ChCl. *Vaccinio-Piceetea* Br.-Bl. in Br.-Bl. et al. 1939:			
Pinus sylvestris a		.	2
Pinus sylvestris b1		.	2
Pinus sylvestris b2		+	+
Pinus sylvestris c		+	.
ChCl. *Crataego-Prunetea* Tx. 1962:			
Rhamnus cathartica b2		.	2
Cornus sanguinea b2		.	1
Cornus sanguinea c		.	+
Prunus spinosa b2		.	+
Crataegus monogyna b2		.	+
Rosa rubiginosa c		+	.
Others:			
Frangula alnus b2		.	2
Frangula alnus c		.	+
Betula pendula b1		.	+
Juniperus communis b2		.	+
Juniperus communis c		+	.
Quercus robur c		+	.
Padus avium b2		.	+
Herbs:			
ChAll. *Cirsio-Brachypodion pinnati* Hadač et Klika in Klika et Hadač 1944:			
Asperula tinctoria c		+	1
Seseli annuum c		+	+
Viola rupestris c		+	.
ChO. *Festucetalia valesiacae* Soó 1947:			
Thymus marschallianus c		+	+
ChCl. *Festuco-Brometea* Br.-Bl. et Tx. ex Soó 1947:			
Brachypodium pinnatum c		3	3
Filipendula vulgaris c		+	2
Helianthemum nummularium subsp. *obscurum* c		1	+
Euphorbia cyparissias c		+	+
Veronica spicata c		+	+
Carlina vulgaris c		.	+
Plantago media c		+	+
Carex caryophyllea c		.	+
ChCl. *Trifolio medii-Geranietea sanguinei* T. Müller 1962:			
Anemone sylvestris c		2	1
Peucedanum oreoselinum c		1	2
Viola hirta c		+	1
Anthericum ramosum c		1	2
Polygonatum odoratum c		.	1
Coronilla varia c		+	+
Galium verum c		+	.
ChCl. *Molinio-Arrhenatheretea* Tx. 1937:			

Table 2. *Cont.*

No. of Relevé	1	2
Galium boreale c	2	2
Knautia arvensis c	.	1
Taraxacum officinale c	+	.
Galium mollugo	.	+
Anthyllis vulneraria c	+	.
Trifolim montanum c	+	.
Plantago lanceolata c	+	.
Centaurea jacea c	.	+
Others:		
Pulsatilla patens c	**2**	**1**
Chamaecytisus ruthenicus c	2	2
Festuca trachyphylla c	1	1
Sanguisorba minor c	1	+
Pimpinella saxifraga c	+	+
Vicia angustifolia c	+	+
Briza media c	+	+
Prunella grandiflora c	1	.
Poa angustifolia c	+	1
Carex montana c	+	.
Polygala comosa c	+	.
Carlina acaulis c	+	.
Hieracium pilosella c	+	.
Viola collina c	.	+
Vincetoxicum hirundinaria c	.	+
Primula veris c	.	+

The dot - '.' - means that the taxon not occurs in the relevé.

The differences between the two studied plots were also noted in the mean height (range) of stems of the herbaceous plants (Table 1). The mean height of the stems of the highest herbaceous plants on plot A had a medium value—53.4 cm. On plot B, the value was the highest and amounted to 98.9 cm. Additionally, the mean height of the stems of the lowest herbaceous plants was different between the plots. The statistical analysis confirmed that the height of the herbaceous plants differed significantly between the study plots in the case of the tallest plant species, as well as the lowest ones (Table 1).

3.2. The Population Structure

The total abundance of individuals (leaf rosettes) in the open xerothermic grassland (plot A, 40) is five times higher than in the overgrowing xerothermic grassland (plot B, 8). The distribution of leaf rosettes in plot A is randomly clustering and in plot B it is a random type of distribution (Figure 1).

Additionally, the density structure of the rosettes of *Pulsatilla patens* differed significantly between the study plots ($\chi^2 = 12.2$, $p < 0.01$, df = 3). Leaf rosettes showed from 6 to 10 leaves in plot A, whereas only from 1 to 5 leaves in plot B. Moreover, only in the open xerothermic grassland the species occurred in every four of the studied ranges (Figure 1).

The difference between the plots occurs also on the basis of the age structure of the *P. patens*. Juvenile individuals were only noted in plot A. The share of the juvenile, vegetative and generative individuals between A and B plot differed significantly ($\chi^2 = 12.2$, $p < 0.01$, df = 3) (Figure 1).

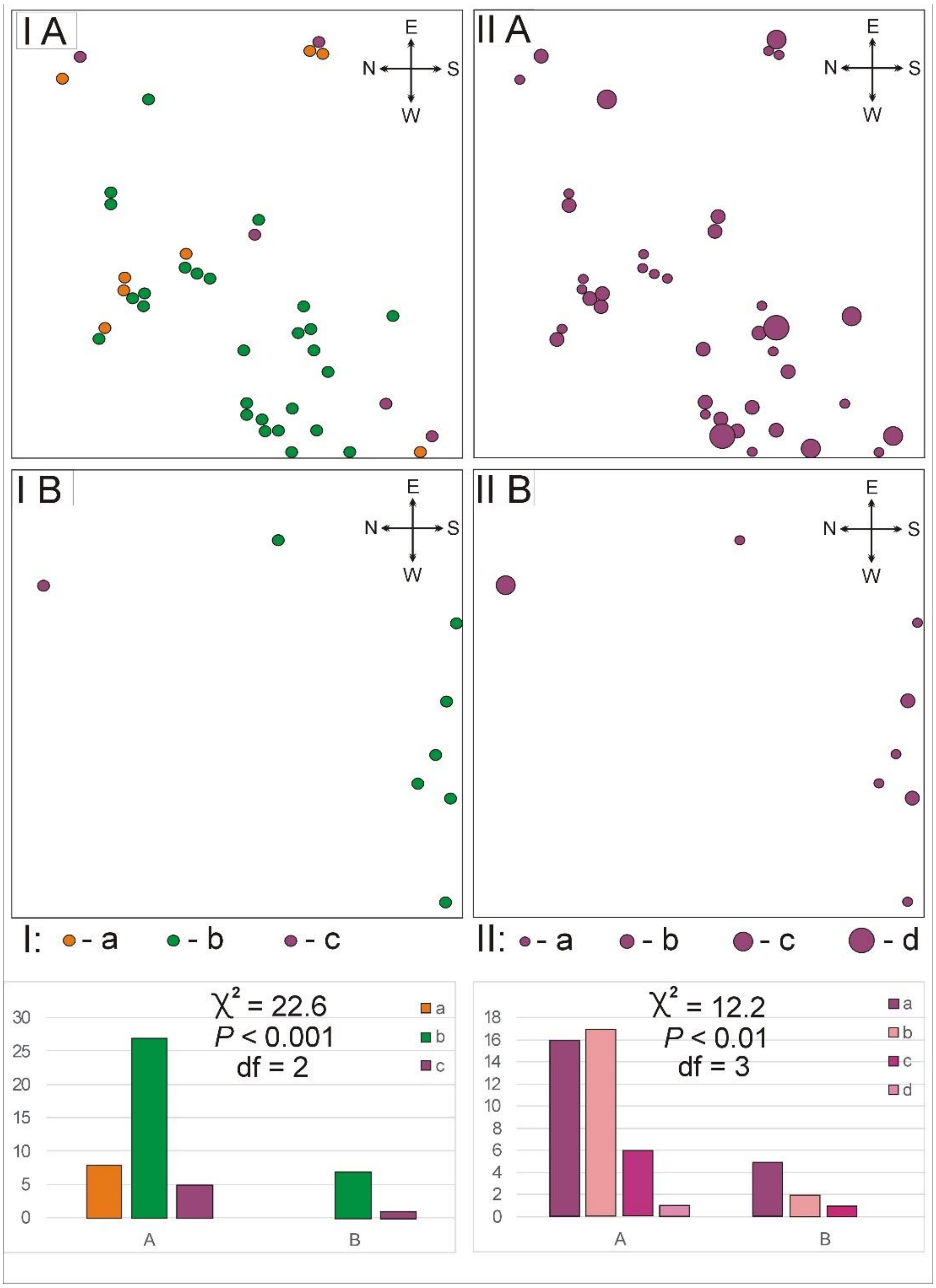

Figure 1. Some parameters of the structure of population of *Pulsatilla patens* in two study plots: (**A**) open xerothermic grassland and (**B**) overgrowing xerothermic grassland. I—distribution and age structure of individuals (a—juvenile, b—vegetative, c—generative); II—distribution and density of leaf rosettes (number of leaves in rosettes: a—1–5, b—6–10, c—11–15, d—16–20).

3.3. The Traits of Individuals

The mean number of leaves in the rosettes was higher in the open xerothermic grassland (plot A), than in the overgrowing xerothermic grassland (plot B), but the difference was not statistically significant (Table 3). The investigations of leaf dimensions showed significant differences between the two plots occurring in the differing structure of xerothermic grassland and they showed that a shadowing caused by a process of secondary succession brought increased height and width to the leaves (Table 3).

Table 3. Mean (range) of traits of leaves of *Pulsatilla patens* in permanent plots established in the open xerothermic grassland (A) and overgrowing xerothermic grassland (B).

Traits	A	B	The Mann–Whitney U Test Value	*p* Value
The number of leaves in rosettes	6.6 (1–18)	5.7 (2–14)	U = 141.0	$p = 0.59$
The height of the greatest leaf in rosette	11.2 (4.5–17)	18.1 (12.5–23)	U = 22.0	$p = 0.0001$
The height of the lowest leaf in rosette	6.9 (3–14)	12.9 (7.5–18.5)	U = 28.5	$p= 0.0003$
The width of the largest leaf blade in rosette	6.2 (2–9.8)	8.1 (6.5–10)	U = 50.5	$p = 0.002$
The width of the least leaf blade in rosette	3.9 (1.5–6.2)	5.4 (3.5–8)	U = 89.5	$p = 0.051$
The length of the greatest lobe of the leaf in rosette	3.4 (1–5.5)	4.3 (3.5–5.5)	U = 53.5	$p = 0.003$
The length of the lowest lobe of the leaf in rosette	2.3 (0.5–3.5)	3.4 (2–5)	U = 72.5	$p= 0.01$

4. Discussion

Xerothermic grasslands are among the ecosystems currently undergoing the most profound transformation [35,36]. These phytocoenoses are threatened by advancing succession in many countries [36,37]. One of the results of succession is the disappearance of heliophilous species and the formation of shrubs and forest species more adapted to poor light conditions [38,39]. The overgrowing xerothermic grassland on plot B, where the lack of agricultural use was observed for more than 30 years, was transformed into a three-layer association dominated by tall herbaceous plants with shrubs and trees (Table 2). The emergence of shrub and trees resulted in shading, which reduced the amount of available light (Table 1), and at the same time impacted the herb layer.

In this grassland, the species characteristic of the *Festuco-Brometea* class still occur, but they are over two times higher compared to the open xerothermic grassland on plot A (Table 1). Additionally, the herb layer is more compacted compared to the herb layer from plot A. In plot B, there were no gaps which contributed to the recruitment and recovery of numerous grassland species because of better light conditions [40,41]. Lack of gaps and more compacted herb layer influences the structure of *Pulsatilla patens*—on overgrowing grassland, no juvenile individuals occurred; the total abundance of species was 5 times lower, compared with open grassland; in distribution type, no clusters were noted, and the leaf rosettes were smaller than in the open xerothermic grassland (Figure 1).

The reduced access of light also impacted the individual traits of *Pulsatilla patens*. The presence of significantly longer and wider leaves of *Pulsatilla patens* in the overgrowing xerothermic grassland is presumably caused by the greater height of the neighbouring plants (Table 1). Such a phenomenon has previously been observed by many authors—in different semi-natural phytocoenoses and with different species, e.g., *Iris sibirica*, *Dianthus superbus* or *Gladiolus imbricatus* [29,42,43]. All of these species show a similar scenario, allowing plants to outperform their neighbours and contributing to more effective light interception, as well as possibly improving flower visibility for pollinators and seed dispersal [29].

Pulsatilla patens in the open xerothermic grassland (plot A) had the most typical structure—distribution, age structure and density [44–46]—with the medium values of the investigated leaf and stem dimensions (Figure 1, Table 3).

The chemical analyses of soil samples showed that there were no statistical differences between both plots in terms of the soil properties (Table 1). However, it should be pointed out that in the overgrowing xerothermic grassland, a slightly lower pH was observed, which is probably caused by the presence of *Pinus sylvestris*, whose needles acidify the topsoils [47] and it may have influenced some characteristics of specimens in the population [48]. According to some scientists, the degree of soil acidification is the major variable determining the size of populations and size of the individuals [48,49].

The dominance of vegetative individuals of the Eastern Pasque Flower observed in our plots is similar to other localities from different areas where the vegetative individuals prevailed [50]. *P. patens* has a long life cycle that may last for several decades, which leads to the formation of compact clumps [46]. The great number of leaves in rosettes and the width of the leaf blades in clumps of our study population suggests a long cycle of life of species in the xerothermic grassland and that we have to deal with an old population that needs protection.

5. Conclusions

1. The total abundance of *Pulsatilla patens* in the open xerothermic grassland is five times higher than in the overgrowing xerothermic grassland, (40:8 leaf rosettes, respectively).
2. A randomly clustering distribution of *P. patens* was noted only in the open xerothermic grassland (in the overgrowing xerothermic grassland a random type of distribution occurred).
3. The density structure of the rosettes of *P. patens* differed significantly between the study plots. Leaf rosettes showed from 6 to 10 leaves in the open xerothermic grassland, whereas only from 1 to 5 leaves in the overgrowing xerothermic grassland.
4. The difference on the basis of age structure of the *P. patens* shows that only in the open xerothermic grassland juvenile individuals were noted.
5. The mean number of leaves in the rosettes was higher in the open xerothermic grassland, but the leaf dimensions were higher in the overgrowing xerothermic grassland (a shadowing caused by a process of secondary succession increased the height and width of the leaves).

The long-term changes observed in an overgrowing xerothermic grassland (shadowing caused by shrubs and trees and high weeds, increased of percentage cover and the height of plants) impacted the structure and individual traits of the *Pulsatilla patens*.

Within the range of *P. patens*, abandoned grazing has caused overgrowth in several habitats and caused the species to be in alarming decline for decades, and now it is critically endangered. We hope that the results of our research—impact of the different successional stages of xerothermic grassland on the structure and individual traits of population of the Eastern Pasque Flower—can help us find a way to better protect the species.

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