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Relic Vergilius Oak (*Quercus virgiliana* Ten.) Trees Could Preserve Microhabitats of Pannonian Forest–Steppe Vegetation

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Abstract: In the framework of an ongoing gene conservation programme in the Tolna Hills Region, Hungary, a total of 41 site plots were selected on agricultural land that had recently been used as grassland, meadow or vineyard. Aims of our study were (i) to test the taxonomic status of relic oak trees growing out of forested lands; (ii) to verify the origin of relic trees whether they regenerated by sprouts and suckers or by seeds in order to estimate their age and origin (nativeness); (iii) to test their microhabitats to see if forest-specific plant and fungal species were presented; (iv) and to test species to see if their presence linked to any forest vegetation in the past. Furthermore, the land-use type did not show a significant effect on the abundance of woody, dicot herbaceous, or monocot herbaceous plants recorded on sites based on maps from 1941 or earlier. The follow-up univariate ANOVA revealed a significant direct effect of recent land-use type (of 2022) on monocot herbaceous plants ($F(3,33) = 5.21$, $p < 0.01$). Additionally, a significant but weaker effect was observed on woody plants ($F(3,33) = 3.22$, $p < 0.05$). The overall past effect of land-use type showed a significantly high positive correlation between the abundance of woody plants and the number of times the maps showed forest or forest boundary land-use types ($R = 0.46$, $p < 0.01$). The plots have likely preserved and sustained the microhabitats of the native forest vegetation that was once distributed in the region. The site plots of relic oak trees, considered as flagstone habitats, create opportunities for gene flow, not only for the species with dispersal or discontinuous distribution, but also for the Vergilius oak populations. Therefore, relic trees and their microhabitats might have an important role in the mating system of various species and might also be valuable resources for genetic conservation programmes.

Keywords: climate change; genetic resources; grasslands; microhabitats preservation; oak hybrids; phenotypes; protected plants; *Quercus virgiliana*; wood-pastures

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1. Introduction

Oaks (*Quercus*) are the most important woody genus in the Pannonian Basin. The majority of natural forest associations in this geographic region consist of various oak taxa that have evolved to thrive in a diverse array of ecological conditions. The central part of the Pannonian Basin lies at the border between the deciduous forest vegetation zone and Eurasian steppe zone. It was hypothesized that the specific geographic and environmental features of that area would have been significant adaptive factors for oaks, particularly during the postglacial period. Genetic studies have reported a high level of genetic diversity among oak populations in the Pannonian Basin and Balkan Peninsula [1–3]. This geographic region served as a refugial area for oak taxa during the glacials [4], which then colonized various sites during the postglacials [1,4,5].

The white oak species are distributed throughout Central Europe, including *Quercus robur* s.l., *Quercus petraea* s.l., and *Quercus pubescens* s.l. Additionally, *Quercus frainetto* is found in the eastern part of the Mediterranean region, specifically in the Italian and Balkan peninsulas and the Pannonian Basin. The *Quercus virgiliana*, also known as the oak of Virgil, is included in the EUFORGEN genetic conservation programmes, which highlight its remarkable capacity to adapt to arid conditions [http://www.euforgen.org/species/quercus-virgiliana/ (accessed on 3 July 2024)]. Nevertheless, due to its propensity to hybridise with other taxa within the *Quercus* section, it is frequently not recognised as a distinct species and is instead classified within *Quercus pubescens* (downy oak) sensu lato. The taxon is indigenous to Italy, the Balkan Peninsula and the Ponto-Mediterranean region, including Hungary [6].

The taxon is well adapted to habitats with Mediterranean and continental climates, such as summer and winter droughts, hot summers and medium-cold winters, and poor soil conditions [7,8]. The Vergilius oak grows on shallow to medium-deep neutral sandy or loess soils, but not on acidophilic or rocky soils. These sites are frequently utilised for agriculture purposes, mostly as ploughland, viticulture, fruit orchards or even grasslands. The distribution of these forest types has been radically reduced, largely due to the long-term conversion of land to agricultural use [9]. According to historical sources, the acorns of Vergilius oak are sweet and tasty and were locally consumed as food [http://www.euforgen.org/species/quercus-virgiliana/ (accessed on 3 July 2024)]. The taxon has recently been reported in many European regions [10–12] reflecting its specific ecological importance within the region, particularly in the context of climate change expectations. Considering these aforementioned aspects, a dynamic gene conservation programme was initiated a few years ago with the aim of identifying Vergilius oak trees and listing their in situ genetic resources, both within and outside of woodlands of the Tolna Hills region.

The Vergilius oak is a species that naturally grows in the forest–steppe vegetation zone (*Aceri tatarico–Quercetum pubescenti-roboris*) and mixed oak forest zones (*Orno-Quercetum*, *Quercetum petraeae-cerris*, *Tilio tomentosae-Quercetum petraeae-cerris*). The European Nature Information System (EUNIS) provides a database that lists species, habitats and protected sites. The EUNIS database classifies habitat types [https://eunis.eea.europa.eu/ (accessed on 3 July 2024)] and describes oak forest habitats, including mixed oak forests, as a type of Euro-Siberian steppe oak woods. The Pannonic loess steppe oak woods (Code G1.7A1211) are classified as a type of Pannonic turkey oak-sessile oak woods (Code G1.7A112) which are located at the western range of Euro-Siberian steppe oak woods (Code G1.7A1). These forests once extended throughout the entire Pannonian Basin, from Hungary and southern Slovakia to the Srijem area of north-eastern Croatia, as well as northern Serbia. However, they have now been reduced to only a few dozen remaining stands.

In many cases, the white oak species with large distributional areas have been described sensu lato, including conglomerates of taxa with specific distributional areas or ecological niches. For example, *Quercus dalechampii* is a sessile oak taxon that is distributed in the south-eastern part of Europe but is often considered as *Quercus petraea* sensu lato [13,14]. Dalechamp oak has been specifically adapted to xeric continental and sub-Mediterranean ecological conditions [13,15–17]. The importance of forest genetic resources has also been recognized by the European governments since the 1990s. In order to implement the recommendations, set forth in the Strasbourg Resolution 2 of the first Ministerial Conference on the Protection of Forests, many European governments established EUFORGEN, a Pan-European professional platform. EUFORGEN has successfully coordinated national policies and activities for forest genetic resources at an international level [18]. The natural populations of trees are valuable genetic resources not only for the forestry sector but also in general, in order to protect natural ecosystems in Europe. Many species-specific technical guidelines have been published by EUFORGEN, which serve to summarize the most relevant scientific knowledge on genetic resources, including various oak species [6,14,19,20]. All the oak conservation guidelines emphasize the importance of conserving natural populations in situ, as well as maintaining up-to-date inventories of genetic resources.

Due to the long-term impact of EUFORGEN activities, many gene conservation programmes, both in situ and ex situ approaches, have been initiated and are currently being implemented across Europe, including Hungary. The in situ mapping of autochthonous oak populations has been conducted for about 5 years, primarily in forested lands within mixed oak forests and forest-steppe vegetation zones. The programmes aimed to create specific, geo-referenced inventories of the oak phenotypes that are adapted to arid site conditions, especially in the context of climate change. According to traditional selection procedures, the phenotypes have been registered as plus trees in forestry databases, having records of their taxonomic status, geo-reference data, phenological and morphological traits, and a brief description of habitats. In addition to their preservation in situ, many plus trees will also be utilized as seed source for the establishment of ex situ conservation stands or basic materials for the production of forest reproductive material. In addition, non-forested lands, such as grasslands, former fruit orchards and vineyards were also visited in order to find and describe old oak trees that were supposed to be autochthonous.

In the past, various types of grassland, such as grazing lands, meadows, and pastures, were widely distributed across Europe with wood-pastures. These grasslands occurred in various vegetation zones, mostly in steppe, forest–steppe and mixed oak forest climatic zones. They often co-occurred with tree and shrub species [21–23]. Trees were utilized for a variety of purposes in wood-pastures and grasslands. The trees provided shade and shelter for the cattle. Additionally, various species, such as oaks, chestnuts, apples, pears, and rowan trees offered food and feed for both humans and animals. Tall and large trees were employed as boundary markers, both on flat and hilly regions. Landmarks were usually made of stone and embedded in the ground, but they were not visible from long distances. Therefore, single trees, or short rows of trees were permitted to grow, making the boundaries of land properties visible. If such a ‘boundary marker tree’ was accidentally or intentionally felled, it was often regenerated vegetatively by root or stump suckers, and the offspring tree took over the marker function. Due to their vegetative reproduction, these relic trees may partially conserve a piece of the genetic pattern of natural populations that were distributed in the past. Historical maps of various regions often illustrated such trees to indicate the boundaries or strategic points [https://maps.arcanum.com/hu/map/cadastral/ (accessed on 3 July 2024)].

Climate change is a challenging environmental issue for the world’s fauna and flora. The climatic parameters in Central Europe have exhibited a pattern of change consistent with global trends, with an increase in temperature and a growing prevalence of xerothermic influences. This phenomenon is characterised by a reduction in precipitation, a decline in air humidity and, therefore, a lowering of groundwater levels. The changing climate has already had a significant impact on European forests, particularly those on the xeric limit, such as those in the Pannonian Basin [24,25]. Based on moderate climatic model predictions, approximately 60% of the Pannonian Basin will be in the steppe vegetation zone by 2050, and the distribution of beech (*Fagus sylvatica*) and sessile oak (*Quercus petraea*) in Hungary will be drastically reduced [26]. Therefore, plant species that are adapted to arid ecological conditions, both woody and non-woody, would be more significant in the Pannonian region. The importance of pubescent oaks (*Quercus pubescens* sensu lato) is likely to increase, and they will be used on the majority of forest sites where *Quercus petraea* has recently been distributed. As a result, the genetic resources of pubescent oaks will be more intensively utilized for forests on xeric limits [6], including in Hungary. Since loess and sandy soils are prevalent in Hungary, the majority of the land areas are suitable for Vergilius oak. This species is well adapted to such soil conditions [7,8,27], and its distribution is likely to increase in the future.

Our study was carried out in the framework of a genetic conservation programme for *Quercus virgiliana* populations in the Tolna region of Hungary. The programme aims to select, describe, map and inventory plus trees for further in situ and ex situ conservation activities. The selection process primarily focused on forest stands, but agricultural lands (land used by pastures, meadows, fruit orchards, vineyards) were also visited. Initial observations (2019) revealed that the examined habitats resembled forest or forest–steppe habitats, associated with plant and fungal species that are typically found in forest plant associations. Hypothetically, the old relic trees formed micro forest habitats on grasslands, preserving small patches of primary natural forest vegetation [21,28–30].

Aims of our study were (i) to test the taxonomic status of relic oak trees growing out of forested lands; (ii) to verify the origin of relic trees whether they regenerated by sprouts and suckers or by seeds in order to estimate their age and origin (nativeness); (iii) to test the microhabitats to see if forest-specific plant and fungal species were present; (iv) and to test species to see if their presence linked to any forest vegetation in the past.

2. Materials and Methods

2.1. Methods of Selection and Description

The geographic area of the conservation programme is located in the southern Transdanubian region, in close proximity to the Hungarian Great Plain and the Transdanubian Hills. The region is geographically very diverse, consisting of smaller plains, eroded loess valleys and hills with altitudes ranging from 100 to 300 m. The soils are predominantly developed on loess bedrock, but calcareous sandy soils are also common. In general, trees were found and selected based on the information provided by local foresters and farmers. However, as the initial phase of the non-forested land selection process, a systematic scan of Forest Maps [https://erdoterkep.nebih.gov.hu/ (accessed on 3 July 2024)] was also conducted. Sites with xeric ecological conditions, such as eroded soils, southern exposures, ridges, or hilltops, were preferred. Then, the candidate trees were visited and inspected, using botanical descriptors and traits listed in Supplementary Table S1. Trees belonging to the Turkey oak species (*Quercus cerris*), which were often artificially distributed in the past and common in the region, were excluded from further selection. However, all trees exhibiting characteristics of the white oak species (Section *Quercus*) were included.

The list of descriptors was compiled from common morphological traits and characters published by several authors [7,8,15,27]. The trees were visited frequently between 2019 and 2022, focusing on growing seasons (spring, summer, and fall), in order to record seasonal traits on buds, leaves, and male and female inflorescences and acorns. The hairiness was also recorded as a seasonal character (Figure 1).

Figure 1. The morphological characteristics of the Vergilius oak observed on the leaves and female peduncles during the blossoming stage. The leaves were hairy, both beneath and above; moreover, a dense hairiness on twigs, female peduncles and primodoral cupules was also observed. (Photo: Horváth, C.).

In 2019–2020, the trees were selected and listed, and they were described in 2020 and 2021. The selected relic trees were marked, geo-referenced and recorded. The position in the landscape, whether it was a solitary tree, part of a row, or a group of trees, was also recorded. In cases where a group of trees was found, the largest tree, which was likely the oldest, was selected and recorded.

Since most of the trees were belted by a specific wooded micro habitat with shrubs and trees, the surveys were repeated during the growing seasons of 2020 and 2021–2022. The seasonal surveys aimed to record vascular (woody and herbivorous) plants and macrofungi classified as character species of any forest association.

To test our hypotheses, observations were conducted to describe vascular plants and macrofungi growing under, next to, or even on the trees. Species characterized specifically for forest habitats were also recorded and listed during the growing seasons of 2020–2022, following the plus tree description. Species characterized for grassland habitats were excluded from the inventory. Macrofungi species were classified based on their functional spectrum [31] and substrate specificity [32], as previously employed in fungal studies [33–35].

The trees were mapped using geo-references (subdecimal coordinates) which were measured in accordance with the forestry plus tree selection (Table S2). The Forest Map software ('Erdőtérkép' v 1.0.) [https://erdoterkep.nebih.gov.hu/ (accessed on 3 July 2024)] was used to record the geo-references and map the trees' coordinates.

Information about soil types was also recorded when data from soil databases or soil analyses were available. However, in all cases, eroded Ramann brown forest soil or chernozem brown forest soil types were recorded, as well as which types are the most characteristic ones in xeric forest vegetation zones.

Supposedly, the microhabitats of relic oak trees, which were formerly located within or next to a forest stand, may have dynamic and living connections to the forest ecosystems, at least preserving a piece of biodiversity of former natural forest as refugial microsites for woody and herbaceous plant species. As most of the trees were vegetatively regenerated and estimated to be over 200 years old, we used and analysed historic maps and cadastral databases to analyse the land-use changes in the long term (Figure 2). The following sources were consulted in order to gain insight into the historical evolution of land use in Hungary:

First Military Land-Survey of Hungarian Kingdom, 1782–1785 [https://maps.arcanum.com/hu/map/firstsurvey-hungary/?layers=147&bbox=2070390.0687322675,6004328.278894642,2173503.61988897,6041629.548697809 (accessed on 3 July 2024)]
Second Military Land-Survey of Hungarian Kingdom, 1819–1869 [https://maps.arcanum.com/hu/map/secondsurvey-hungary/?layers=5&bbox=2114912.2490512794,6021329.6822395325,2127801.442945867,6025992.340964928 (accessed on 3 July 2024)]
Military Land-Survey of Hungary, 1941 [https://maps.arcanum.com/hu/map/hungary1941/?layers=29&bbox=2110378.5778111913,6019224.854830895,2136156.965600367,6028550.172281686 (accessed on 3 July 2024)]
Forestry Map of Hungary, 2022 [https://erdoterkep.nebih.gov.hu/ (accessed on 3 July 2024)]

In order to verify our hypothesis, we measured the minimum distances of the trees from the forested area (woodland) on both historic and recent maps listed above. The distances and the number of forest plants found on microhabitats were correlated in order to identify linkages to forest plant associations in the past.

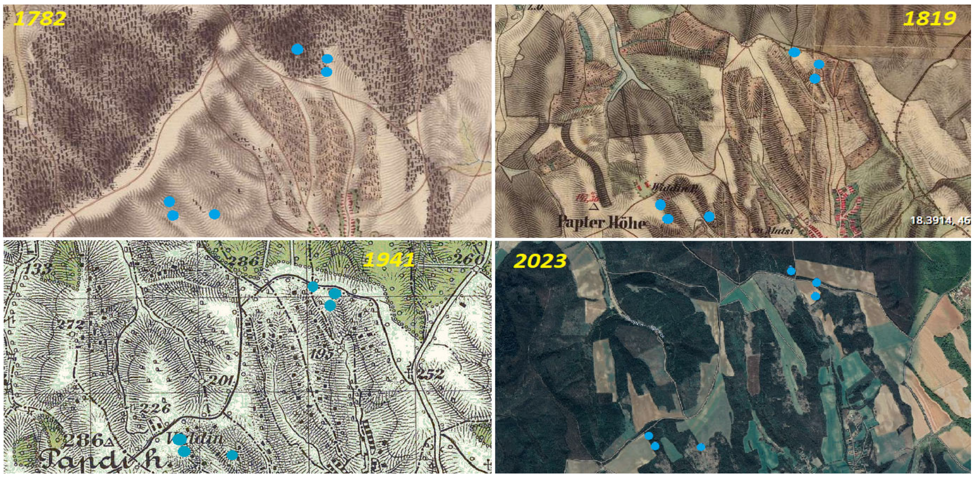

Figure 2. The locations of the microhabitats of trees No. 8, 9, 10, 11, 16 and 17 are indicated by blue dots on historic (1782, 1819, 1941) and recent (2022) maps, used to measure minimum distances of microhabitats from forested lands.

2.2. *Statistical Methods*

Firstly, using Pearson's correlation coefficients, we tested whether the distance from the forest has a significant association with the species richness of woody, dicot herbaceous, or monocot herbaceous plants, or with their ratio.

A one-way multivariate analysis of variance (MANOVA) model with dependent variables woody, dicot herbaceous, and monocot herbaceous plant abundance was used to test the effect of recent land-use type and that of the past (2022, 1941, 1819 and 1782). The overall result was tested based on the unexplained variance rate that was expressed by Wilk's lambda.

The normality of the model residuals was confirmed by the absolute values of their skewness and kurtosis, which were both below 2 and 4, respectively. Since the homogeneity of variances was slightly violated, a robust follow-up one-way analysis of variance was performed with Bonferroni's correction in case of significant MANOVA results, to avoid the familywise error rate inflation. Finally, Games–Howell's post hoc test was applied to separate the homogeneous groups.

The statistical analysis was conducted using IBM SPSS (v27, Armonk, NY, USA, 2020).

3. Results

3.1. *Taxonomic Evaluation of Relic Trees*

The surveys were conducted using first satellite maps and then on-site inspections. A total of six municipalities in the Hőgyész region were found to contain a number of old oak trees. The geographic locations of the trees were recorded and mapped (Figure 3). A sectional overview of the locations of the trees are given in Supplementary Materials, Figure S1a–d. The coordinates of trees are given in Table S2.

In all cases, the morphological traits (branches, leaves, inflorescences, acorns and cups, etc.) were separately evaluated and, finally, the tree was classified based on a summary of the combination of the traits. All the trees exhibited combinative characters and were classified as a hybrid or even introgressed form of oak taxa (Table S1). The trees were classified systematically based on their dominant taxonomic characteristics. For example, if the descriptors were dominantly representative of Vergilius oak and less dominantly for Dalechamp oak, the tree would be classified as *Quercus virgiliana* × *dalechampii* or partly *Quercus virgiliana* × *Quercus petraea*. All the trees exhibited characteristics typically associated with *Quercus virgiliana*, such as hairiness on twigs and buds, on leaves beneath, on long peduncles of female inflorescence including cupules, or even a specific pattern of bark fissures on the trunk (Figure 4). The identified pattern of bark fissures was found on all trees, either partly or predominantly on the trunk, up to a height of 1–1.5 m. This feature was found to be significantly associated with hairiness on both vegetative and generative organs, which was recorded on all parts of all the trees and proved to be a useful descriptor throughout all seasons.

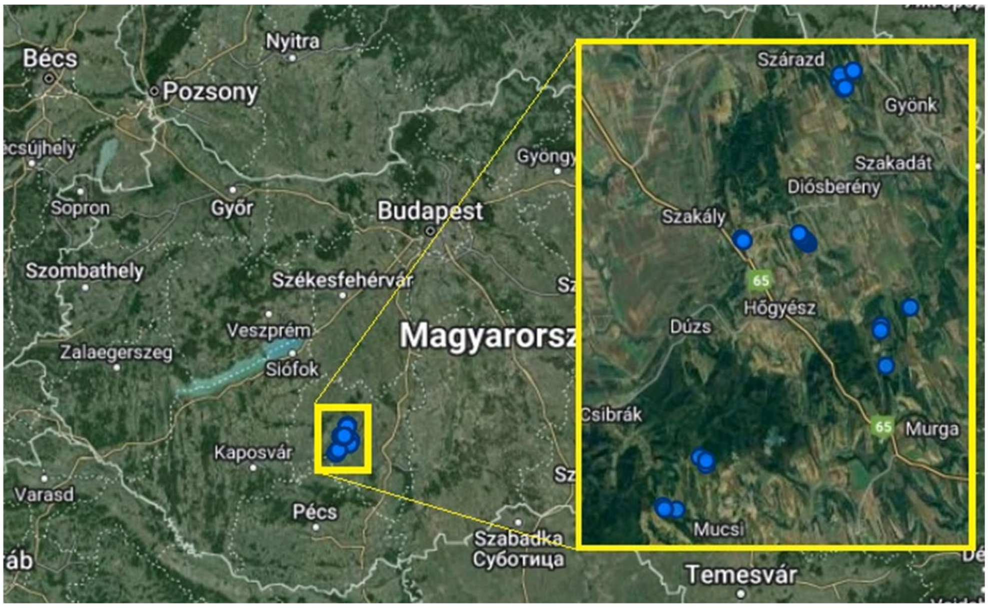

Figure 3. Relic oak trees selected for gene conservation purposes in the Hőgyész region presenting an overview map of the surveyed trees (blue dots Tolna county, Hungary). More detailed maps and coordinates are given in Supplementary Materials (Figure S1a–d, Table S2). The coordinates of trees are given in Table S2.

Figure 4. The relic hybrid oak tree on site plot No. 6 (Kalaznó) exhibits a rugged bark texture. (Photo: Horváth, Cs.).

The selected trees and their characteristics, detailed in Section 2.1., were recorded (Table S1). In cases where a group of trees was found, the largest tree, which was likely the oldest, was marked, geo-referenced and recorded. Trees positioned in solitary or in a line may have served a landmark function in the past to visualize the boundaries of municipalities or land properties. Old landmark stones were also discovered and documented in the vicinity of trees No. 3 and 8. to support this hypothesis (Figure 5). However, trees No. 3 and No. 8 are situated on the recent boundaries of municipalities as well (Szakadát–Gyönk and Dúzs–Mucsi, respectively).

Figure 5. An ancient landmark stone situated along the row of relic trees which have served as markers on the boundary line of the municipalities of Dúzs and Mucsi (Site plot 8). (Photo: Bordács, S.).

3.2. Biocoenology of Microhabitats

The surveys were carried out during the growing seasons of 2020 and 2021–2022, repeatedly. The objective of the seasonal surveys was to record vascular plants (woody and herbivorous) and macrofungi that are classified as character species of any forest association. The site plots are listed in Table 1, including information about the trees and land use history of site plots. Trees were characterized according to the taxonomic descriptors listed in Table S1.

Table 1. The list of trees selected and recorded. In the case of groups of trees, the largest tree was described, and additional trees were noted according to the following categories: forest (f, dark green), forest boundary (fb, light green), arable land (al, brown), pastural (pa, dark yellow), vineyard (vi, olive), hay meadow (hm, light yellow).

Site Plot	Locality	Plant Origin	Taxonomic Status Shortly	Position	1782	1819	1941	2022
1	Dúzs	seedling	virg × dal × pet	4 additional younger tree(s)	f	al	pa	hm
2	Gyönk	root-shoot	dal × virg	1 additional sapling	f	fb	fb	fb
3	Gyönk	root-shoot	virg × dal × pet	1 additional tree	fb	fb	pa	fb
4	Kalaznó	stump shoot	virg × dal	6 additional tree(s)	pa	fb	pa	fb
5	Kalaznó	root-shoot	virg × dal × pet	2 additional saplings	pa	pa	pa	hm

Table 1. *Cont.*

Site Plot	Locality	Plant Origin	Taxonomic Status Shortly	Position	1782	1819	1941	2022
6	Kalaznó	root-shoot	virg × dal	solitaire tree	pa	al	al	hm
7	Kalaznó	seedling	dal × virg	solitaire tree	fb	pa	pa	f
8	Mucsi	stump shoot	virg × dal	12 additional tree(s)	pa	al	al	hm
9	Gyönk	stump shoot	virg × dal	solitaire tree	f	fb	fb	hm
10	Mucsi	stump shoot	virg × dal	solitaire tree	pa	al	al	fb
11	Mucsi	stump shoot	virg × dal	solitaire tree	pa	al	al	fb
14	Gyönk	seedling	dal × virg	border of a new forest	f	fb	fb	hm
15	Gyönk	seedling	virg × dal	border of a new forest	f	fb	fb	hm
16	Dúzs	seedling	dal × virg × pub	solitaire tree	fb	al	al	hm
17	Dúzs	seedling	pet × virg × dal	solitaire tree	fb	al	al	hm
18	Szakály	seedling	dal × virg × pet	solitaire tree	vi	vi	al	f
19	Szakály	stupm shoot	dal × virg	solitaire tree	vi	vi	al	f
20	Szakály	root-shoot	virg × dal	solitaire tree	vi	vi	al	f
21	Gyönk	stump-shoot	virg × dal	solitaire tree	f	fb	pa	fb
22	Gyönk	seedling	pub × virg	solitaire tree	f	fb	pa	fb
23	Gyönk	seedling	virg × dal	solitaire tree	f	pa	pa	hm
24	Diósberény	root-shoot	virg × pet	solitaire tree	pa	pa	fb	pa
25	Diósberény	root-shoot	virg × pub	solitaire tree	pa	pa	fb	pa
26	Diósberény	seedling	virg × pet × dal	solitaire tree	pa	pa	fb	pa
27	Diósberény	seedling	virg × pet × dal	solitaire tree	pa	pa	fb	pa
28	Diósberény	stump-shoot	virg × pet × dal	solitaire tree	pa	pa	fb	pa
29	Diósberény	root-shoot	virg × dal × pet	solitaire tree	pa	fb	fb	pa
30	Diósberény	root-shoot	pet × virg × dal	solitaire tree	pa	fb	fb	pa
31	Diósberény	root-shoot	virg × pet × dal	solitaire tree	pa	pa	fb	pa
32	Diósberény	root-shoot	virg × dal	solitaire tree	pa	pa	fb	pa
33	Diósberény	seedling	dal × virg	solitaire tree	pa	pa	fb	pa
34	Diósberény	seedling	pet × virg × dal	1 additional tree	pa	pa	fb	pa
35	Diósberény	roor-shoot	virg	1 additional tree	pa	fb	fb	pa
36	Diósberény	stump-shoot	virg	solitaire tree	pa	fb	fb	pa
37	Diósberény	seedling	virg × pet × dal	solitaire tree	pa	fb	fb	pa
38	Diósberény	root-shoot	virg × dal	solitaire tree	pa	fb	fb	pa
39	Diósberény	stump-shoot	virg	solitaire tree	pa	fb	fb	pa
40	Diósberény	root-shoot	virg × dal	solitaire tree	pa	pa	fb	pa
41	Diósberény	stump-shooot	virg × dal × pet	solitaire tree	pa	pa	fb	pa

Table 2 lists trees and shrubs, while herbaceous plants are listed in Table 3 (*Rosopsida*) and Table 4 (*Liliopsida*). Additionally, Table 5 includes the macrofungi species recorded in 11 habitats.

Table 2. The list of shrub and tree species recorded on site plots. Species protected by law are underlined. Abbreviations: F = species classified for deciduous forests, WS = species classified for wood–steppe association.

Species	Character Species	1	2	3	4	5	6	7	8	9	10	11	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
Acer campestre	F	x	x	x			x		x				x	x	x				x			x			x	x	x	x	x	x	x		x	x	x	x	x	x		
Acer tataricum	F																																					x		
Berberis vulgare	F	x	x																	x																				
Carpinus betulus	F			x		x							x	x																										
Clematis vitalba	F							x		x						x			x	x	x	x						x	x	x	x	x		x	x				x	
Colutea arborescens	F									x								x																						
Cornus sanguinea	F					x			x	x					x	x																						x		
Corylus avellana	F															x																								
Crataegus monogyna	F/WS	x	x	x		x			x	x			x	x	x	x	x	x				x	x	x	x	x	x		x			x	x	x	x	x	x	x	x	x
Chamaecystus hirsutus	F					x								x																										
Euonymus europaeus	F			x																	x	x					x	x	x	x	x						x		x	x
Frangula alnus	F																					x																		
Fraxinus ornus	F									x			x	x		x											x	x	x	x	x	x	x	x	x	x	x	x		
Ligustrum vulgare	F	x	x	x	x	x	x		x				x	x	x	x	x	x	x	x	x	x						x	x	x	x	x	x			x	x	x	x	
Prunus spinosa	F/WS						x								x	x	x			x	x		x				x		x	x				x	x			x		
Quercus cerris	F	x	x																																					
Rosa canina	F	x	x	x		x			x														x					x								x	x		x	
Rosa gallica	F									x																														
Rubus caesius	F													x																										
Ruscus aculeatus	F								x																															
Ulmus minor	F	x	x		x												x			x	x	x	x		x	x	x	x	x			x	x			x		x		
Viburnum lantana	F					x			x	x																				x		x							x	

Table 3. The list of dicotyledonous herbaceous species (*Rosopsida*) recorded on site plots. Species protected by law are underlined. Abbreviations: F = species classified for deciduous forests, WS = species characterized for wood–steppe associations.

Species Character	Species	1	2	3	4	5	6	7	8	9	10	11	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
Aegonychon purpurea-coeruleum	WS							x	x																															
Ajuga reptans	F	x	x																																					
Anthriscus cerefolium	F			x	x		x	x																																
Anthriscus sylvestris	F			x	x															x	x																			
Galium odoratum	F								x																															
Astragalus glycyphyllos	F								x																															
Campanula persicifolia	F			x																												x								

Table 3. *Cont.*

Species Character	Species	1	2	3	4	5	6	7	8	9	10	11	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
Corydalis solida	WS			x																																				
Digitalis grandiflora	F																												x		x									
Euphorbia amygdaloides	F																											x						x	x					
Galium schultesii	WS			x																																				
Galium sylvaticum	F								x																															
Geum urbanum	WS			x	x								x	x			x					x															x			
Glechoma hederacea	F								x																															
Helleborus odorus	F								x														x	x	x	x	x	x			x		x	x	x				x	x
Hypericum perforatum	F/WS																															x				x				
Knautia drymeia	F					x																																		
Vinca herbacea	WS					x	x																																	
Viola silvestris	F	x	x					x					x	x		x				x		x					x	x	x	x	x	x	x	x	x	x	x	x	x	

Table 4. The list of monocotyledonous forest character species (*Liliopsida*) recorded on site plots. Species protected by law are underlined. Abbreviations: F = species classified for deciduous forests, WS = species classified for wood–steppe associations.

Species	Character Species	1	2	3	4	5	6	7	8	9	10	11	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
Arum maculatum	F	x		x									x	x																										
Brachipodium sylvaticum	F	x	x										x	x	x		x			x		x	x		x	x	x	x	x		x		x	x	x			x		
Bromus sterilis	F/WS				x										x			x			x			x				x					x						x	x
Calamagrostris epigeios	F																																			x				
Carex humilis	WS	x	x		x																																			
Carex michellii	WS	x	x			x																																		
Cephalantera damasonium	F								x	x						x																								
Cephalantera longifolia	F									x																														
Dactylis polygama	WS	x	x		x												x	x						x			x	x			x	x					x		x	
Iris variegata	WS					x																																		
Limodorum abortivum	F								x																															
Melica uniflora	F	x	x																																					
Orchis purpurea	F					x			x																															
Poa nemoralis	F	x	x						x																															
Polygonatum latifolium	F			x	x																																			
Scilla vindobonensis	F					x																																		

Table 5. The list of fungal species recorded on 11 site plots. Abbreviations: Pn = necrotrophic parasite; Sh = xylophagous saprotroph.

Species	Functional Spectrum	Substrate Specificity	1	2	3	4	5	6	7	8	9	10	11
Aleurodiscus disciformis	Sh	only *Quercus* spp.							x				
Antrodia albida	Sh	polyphagous				x							
Auricularia mesenterica	Sh	polyphagous	x	x					x				
Exidia nigricans	Sh	polyphagous			x								
Fuscoporia torulosa	Pn	polyphagous	x										
Hypholoma fasciculare	Sh	polyphagous								x			
Peniophora quercina	Sh	dominantly *Quercus* spp.								x			
Perenniporia fraxinea	Pn	polyphagous	x										
Polyporus arcularius	Sh	polyphagous					x						
Stereum hirsutum	Sh	polyphagous	x				x						
Vuilleminia comedens	Sh	dominantly *Quercus* spp.								x			

Our seasonal observations provided initial data on the diverse flora elements found in association with the trees. A high number of vascular plants were found to be growing alongside the relic trees. Only the forest character species were recorded for further analyses, with the species of steppe vegetation being excluded. The grassland in the areas, where the trees were growing, could have been preserved and maintained through regular grazing and/or mowing, which would have helped to maintain the herbaceous plants of steppe vegetation. However, it is probable that plant species typically found in forested areas were displaced from the grasslands and may have found refuge in the relic oak trees. In conclusion, all the trees were found to be associated with vascular plants that are exclusive to forest habitats. Trees have formed and preserved microhabitats, providing small niches for various forest species in grassland areas. Four forest-linked species of the *Orchidaceae* family, namely *Orchis purpurea*, *Limodorum abortivum*, *Cephalantera longifolia* and *Cephalantera damasonium*, were also found on microhabitats (Site plots No. 5, 8, 9). These taxa are significantly bound to forest habitats due to their specific mycorrhizae.

The site plot No. 8 (one of the largest out of 41 sites) was a line of trees that covered a boundary line between two municipalities. At this location, there was a landmark stone (Figure 6) as well, which had been used to mark the boundaries between municipalities for centuries. An orchid species *Limodorum abortivum* was found on this site. Although it is a relatively common species in the forests, it is not typically found in such a location. The violet limodore (*Limodorum abortivum*) (Figure S2) is specifically associated with *Russula* spp., a genus typically found in forest habitats. The violet limodore is a semi-parasitic plant that grows without any chloroplasts during the springtime and parasitizes the mycelium of *Russula* species. Following the completion of its blossom period, the plant develops leaves with chloroplasts and establishes a mutualistic mycorrhiza partnership with *Russula* species (see Figure S2).

The site plot No. 8 has a relatively large surface area and a high diversity of woody species, which may have created a forest micro-site. This could explain the presence of *Limodorum abortivum*. In 2020 and 2021, respectively, two and three plants of this species were found on site No. 8. Although no specific *Russula* species has been recorded, it is probable that the genus *Russula* has been present due to the active occurrence of *Limodorum abortivum*. In both years, there were prolonged periods of drought during the growing season. This can be taken to explain the low number of fungal species' sporophores found on site plots. A similar set of results and conclusions was reported in a previous study [36].

In addition to orchid plants, several rear and protected plant species were also recorded. Two plots yielded *Liliopsida* species (Table 4, *Iris variegata*, *Scilla vindobonensis*, *Ruscus aculeatus*) were found on two plots and *Rosopsida* species (Table 3, *Helleborus dumetorum* × *odorus*, *Vinca herbacea*, *Chamaecytisus hirsutus*) were found on sixteen plots. Some sub-Mediterranean flora elements are native to the mixed oak forests of the region, such as *Ruscus aculeatus* and *Helleborus dumetorum* × *odorus*, which could be considered natural occurrences. The Tolna region is subjected to a sub-Mediterranean climate, which has resulted in the establishment of numerous sub-Mediterranean plant species within this area. In conclusion, the ancient relic trees may play a pivotal role in maintaining the biological diversity of natural ecosystems. Several studies have documented a high diversity of species in wood-pastures [23,29,37] and emphasized their importance in biological conservation.

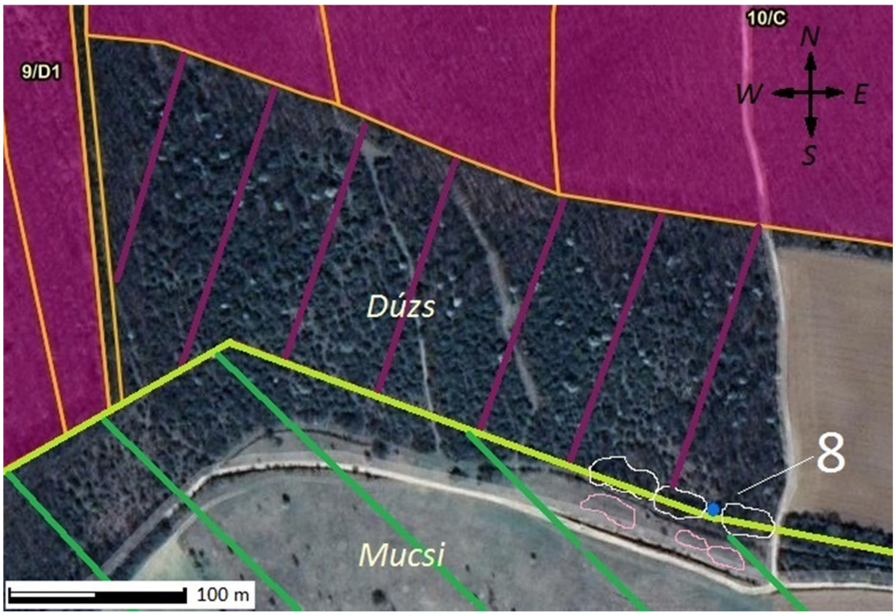

Figure 6. Changes in cadastral records near site plot No. 8. The relic trees (tree No. 8 associated with 12 trees (coloured in white)) have been growing along the boundary line (coloured in yellow) between the municipalities Dúzs and Mucsi. The pink coloured areas (Dúzs) have been registered as forest since the mid 19th century. The area striped in pink (Dúzs) was registered as forest in the 19th century but has recently been registered as grassland, and subsequently been covered by a secondary forest. The areas striped in green (Mucsi) were previously registered as arable land in the 19th century but have more recently been re-classified as grassland. The patches of young root suckers (coloured in rose) were found southward from the line of relic trees likely due to the frequent mowing of the grass.

Observations and records of macrofungi were made in the years 2020–2021. The fungal species did not thrive during this period due to the extremely dry seasons of these years. Some of the species are saprotrophic and were found on dead wood materials that had fallen under the trees or on dying or dead branches. Other species, such as *Fuscoporia torulosa* and *Perenniporia fraxinea*, are parasitic. One species, *Aleurodiscus disciformis*, was species-specific to *Quercus* spp. and two fungal species, *Peniophora quercina* and *Vuilleminia comedens*, which were growing dominantly on woody material of *Quercus* spp. No mycorrhizal macrofungi were found during the investigation, which may be due to the unsuitably dry weather conditions for fruit body production.

3.3. Natural Succession and Landscape Dynamic

Beside orchids, several species of oak woods were also found in micro habitats, such as *Carex michelii*, *Carex humilis*, *Iris variegata*, *Polygonatum latifolium*, *Galium schultesii*, *Buglossoides purpureacoerulea*, *Vinca herbacea*, *Helleborus dumetorum* × *odorus*, *Ligustrum vulgare*, *Crataegus monogyna*, *Chamaecytisus hirsutus*, *Rosa gallica*, *Prunus spinosa* and *Berberis vulgaris*, etc. The majority of trees are vegetatively reproduced through coppicing, and their parent trees (ortet) must have been at least 200 years, i.e., older than the estimated age of ramet trees, based on the size of the trees. Consequently, the trees and their micro habitats have been present on the grasslands for centuries. In some cases, the natural regeneration of oak trees was also observed. A middle-aged Vergilius oak sapling tree, approximately 25 years old, was found and recorded about 80 m away from tree No. 2 (see Figure S3).

This section of the grassland was partially covered by smaller shrubs and seedlings of tree species, specifically *Acer campestre* and *Quercus cerris*. The sapling was surrounded by woody plants. Two Vergilius oak saplings were also discovered next to tree No. 5, growing on the bank of a former boundary line. Although there is no genetic evidence, the saplings were morphologically similar (see descriptors) to the parent oak trees growing nearby and their estimated age was 20 to 25 years, respectively. In both cases, grasslands were frequently used until about 25–30 years ago, when the land use changed due to the radical shifts in society and the economy during the post-soviet era [38–40]. By the end of the 20th century, the regular use of the grasslands in Hungary had ceased and they were typically permitted to revert to bushwood due to the absence of mowing and grazing. The bush woodlands comprise a variety of shrub and tree species, including invasive species such as *Robinia pseudoacaia* and *Ailanthus altissima* (see Figure S4).

The estimated age and the distance from the parent trees support the theory of natural migration and succession of woody species on grassland when the intensive use of grasses is discontinued [41]. Another potential mechanism for natural migration is vegetative expansion. Several patches of root suckers (vegetative plants) were found on the grassland near site plot No. 8 where the grass had been regularly mown. A row of old trees delineated the boundary, with young plants growing alongside them. The patches consisted of a mixture of root suckers derived from turkey oak trees, with an average distance from the parent trees ranging from 10 to 30 metres (see Figure 6, map of Dúzs-Mucsi).

3.4. Effects of Land Use Dynamic

In order to test our hypotheses, we analysed historical maps and recent databases for land-use type. This allowed us to assess the supposed role and ecological effects of the microhabitats at each site plot. Figure 6 shows a typical case of changing land-use type over centuries. Here, the satellite map could be optimally adjusted by historical maps due to the exact geo-references of a landmark stone found on site plot No. 8.

Therefore, we measured the minimum distances from the recent or historical forested lands for each site plot and recorded the type of land-use according to the date of the historical maps. The Pearson's correlation coefficients did not show a significant correlation between the distance from the forest and the species richness of woody, dicot herbaceous, or monocot herbaceous plants, nor their ratio ($p > 0.05$). In other words, the species richness of the site plots was probably not derived from whether the recent forested lands were relatively close or far.

The overall MANOVA result was significant when testing the effect of recent (2022) land-use type (Wilk's lambda = 0.51, $p < 0.05$), although it was insignificant based on the land-use map of 1941 (Wilk's lambda = 0.74, $p = 0.13$) or earlier. The follow-up univariate ANOVA revealed a significant direct effect of land-use type (of 2022) on monocot herbaceous plants ($F(3,33) = 5.21$, $p < 0.01$). There was also a significant but weaker effect on woody plants ($F(3,33) = 3.22$, $p < 0.05$), but no significant effect was found on dicot herbaceous plants ($F(3,33) = 1.08$, $p = 0.37$). The post hoc test results according to Games–Howell are shown in Table 6.

Although in the past, the land-use type did not show a significant effect on the abundance of woody, dicot herbaceous, or monocot herbaceous plants, we searched for a possible pattern that highlights the overall past effect of land-use type. We discovered a significantly high positive correlation between the abundance of woody plants and the number of times the maps showed forest or forest boundary land-use types ($R = 0.46$, $p < 0.01$). It was found that the longer a site plot had been forested or connected to a forest (forest boundary), the greater number of woody species present on the site.

Table 6. Means ± standard deviations of species abundance with the number of species and land-use types of 2022. Different letters denote significantly different groups (Games–Howell's, $p < 0.05$).

Species Types	**Land-Use Types**	**Mean ± Standard Deviation**	**N**
forest woody plant	forest	2.75 ± 1.26 a	4
	boundary line of forested land	6.20 ± 1.10 b	5
	pasture	5.17 ± 1.95 ab	18
	hay meadow	5.60 ± 1.84 b	10
forest dicot herbs	forest	1.00 ± 1.41 a	4
	boundary line of forested land	1.80 ± 0.45 a	5
	pasture	1.89 ± 0.83 a	18
	hay meadow	2.40 ± 2.12 a	10
forest monocot herbs	forest	1.00 ± 1.15 a	4
	boundary line of forested land	3.80 ± 2.77 b	5
	pasture	1.33 ± 0.69 a	18
	hay meadow	2.00 ± 1.25 ab	10

4. Discussion

The objective of the gene conservation programme was to locate and describe ancient Vergilius oak trees both within and outside of forests in the Tolna region. Trees found outside of forests, primarily in grassland areas, were identified as hybrid or introgressed phenotypes of *Quercus virgiliana*, *Quercus dalechampii*, and partly *Quercus petraea*, based on a complex of morphological traits listed in Table S1. Hairiness was observed on all organs of all trees, including twigs and branches, buds, leaves beneath, inflorescences, peduncles, and cupules of acorns. Morphometric hybrid index analyses indicate that many of the oaks identified in this study are likely to be of hybrid origin. This may be a mixture of later generational introgressants between and among pairs and trios of parental species. To confirm our hypotheses of admixture within populations, further genetic data would be required. Therefore, we considered those trees as Vergilius oak s.l., and trees with dominantly Dalechamp/Sessile oak phenotype were considered as Sessile oak s.l.

Natural hybridisation and introgression occur frequently in the *Quercus* genus and may play a major driver of their ecological adaptation. Despite their intensive hybridisation observed in the *Quercus* genus, reproductive barriers between oak taxa may be fixed in the oak's genome [42,43]. The genome of these species contains well-differentiated structures resulting from long-term isolation during the pre- and interglacial periods, which were subsequently affected by an extensive and continuous secondary contact during the postglacial period, and more recently [43,44]. The introgression has allowed the oak taxa to adapt well to various ecological sites and diversify their genomic structure. According to a recent study, the genetic clines detected by SNPs markers along climatic or phenotypic gradients provide further evidence for the probable role of introgression in the adaptive divergence of oak populations [42].

In conclusion, all of the selected trees have been introgressed by oak taxa that are distributed in the Pannonian wood–steppe zone. This introgression could be the major driver for better adaptation to local site conditions. The hairiness of the trees is likely an advantageous adaptive trait in the face of the extreme ecological conditions of grasslands, such as poor and dry soils, summer heat, and drought. Oak genotypes lacking hairiness may be less well adapted to extreme ecological conditions. This could explain the absence of non-hybrid sessile oaks (*Quercus petraea sensu stricto*) from grasslands in the Tolna region. The introgression of the genome of the Dalechamp oak may also contribute to the fitness of hybrids, as *Quercus dalechampii* is known for its high frost resistance. Winter frost may be more intense on grasslands, particularly on hilltops and ridges (Figure S5), where the selected trees grow. Frost-resistant hybrids might be more advantageous in extreme cold winter temperatures.

Most of the trees have been regenerated through vegetative reproduction, specifically by root or stump suckers. This method of reproduction demonstrates a high adaptive potential to xeric site conditions. However, coppiced trees were reproduced naturally, thereby ensuring the preservation of the native genetic resources of the natural populations. Due to vegetative reproduction, the genotypes of coppiced trees are likely to be considerably older than the trees themselves, depending on the cycles of coppicing (Figure S5).

The species composition per tree was diverse (Tables 3–5). However, the most common species were *Acer campestre* (59%), *Crataegus monogyna* (72%), and *Ligustrum vulgare* (69%), representing at least 64-% of the trees. Many forest-specific species were also recorded exclusively in four site plots, including *Campanula persicifolia*, *Corydalis solida*, *Galium schultesii*, *Geum urbanum*, and *Euonymus europaeus* (Site plot No. 3), *Iris variegata*, *Scilla vindobonensis*, and *Chamaecytisus hirsutus* (Site plot No. 5), *Limodorum abortivum*, *Ruscus aculeatus*, *Astragalus glycyphyllos*, *Galium sylvaticum*, *Glechoma hederacea*, and *Helleborus dumetorum* × *odorus* (Site plot No. 8), and *Cephalantera longifolia*, *Colutea arborescens*, *Fraxinus ornus*, and *Rosa gallica* (Site plot No. 9). Various studies reported that solitary trees or wood-pastures in Europe, basically composed of temperate *Quercus* spp. [39,41] or Mediterranean oaks [29,30], might have a relevant role in the preservation of biodiversity, [28,29,45–47] and emphasized their importance to serve specific microhabitats for various plant, fungal and animal species [21,31–39,45,47]. Some wood-pastures preserve relic genotypes of rare forest fruit species such as *Pyrus pyraster* or *Malus sylvestris*, respectively, as well as endangered species (e.g., *Fraxinus* spp.), due to recent ash declines [47]. Solitary trees and wooded pastures have an important dispersal role in natural succession and are effective sources of seedlings [30,39–41,46].

The impact of land-use type on the abundance of woody, dicot herbaceous, and monocot herbaceous plants did not demonstrate a statistically significant effect. However, we found a significantly strong positive correlation ($R = 0.46$, $p < 0.01$) between the abundance of woody plants and the frequency with which the maps depicted forest or forest boundary land-use types. This finding suggests a noteworthy historical association between the presence of woody plants and the occurrence of forest or forest boundary land-use types. A total of 44 vascular plant species were recorded on the 39 site plots, including character elements of various forest associations and 13 plant species specific to the Pannonian wood–steppe vegetation zone. In addition to the oak wood character species, a few protected plants were also found among the relic oak trees. Table 5 shows that a total of four geophyton orchid species (*Orchis purpurea*, *Limodorum abortivum*, *Cephalantera longifolia*, and *Cephalantera damasonium*) were recorded across four site plots (No. 5, 8, 9 and 17), with at least three plants per plot. These orchid species are associated with forest ecosystems, but they are intolerant to dark shadows and require moderate solar radiation. In general, geophyton orchid species are indicators of habitats without radical disturbances. All four site plots were optimal habitats for orchids in terms of their ecological conditions (soil and light) and species compositions. As a result, the diversity of orchid species was more than reasonable.

The recorded macrofungi diversity of microhabitats was specifically associated with oak trees and their wood material. However, most of the fungal species were polyphagous, but one species (*Aleurodiscus disciformis*) was taxon specific to *Quercus* spp. and two species (*Peniophora quercina*, *Vuillemenia comedens*) were dominantly restricted to *Quercus* spp. All of these species are xylophagous saprotrophs and live on dead wood material. In many cases, this material is essential for various saproxylic fungal or animal organisms (insects, birds, etc.), and the microhabitats of wood-pastures or solitary trees might mitigate biodiversity loss [45]. However, the saprotrophic and parasitic fungal species (Table 5) could not be recorded in the absence of woody microhabitats due to lack of living or dead wood material. The relatively low number of fungal taxa, which were found and recorded, could be explained by the extremely dry period of the years 2020–2022. Actually, mycorrhizal fungi were not recorded due to the long drought period, but indirectly, the presence of species of the Orchideaceae family, especially *Limodorum abortivum*, indicated possible mycorrhizal relationships.

Overall, our results supported the hypothesis that relic trees harbour native plants and fungal diversity. While the micro-sites of individual solitary trees may only preserve a limited number of species, the complex of relic trees may operate as a network and preserve a relatively high diversity of Pannonian woodland or wood–steppe character species.

Most of the diversity of shrubs in grassland areas can be attributed to natural succession. Previous studies have reported similar results to ours, indicating that the colonisation of woody species on grasslands is a common occurrence when regular grazing or mowing has been interrupted or ceased [36,41,48] Shrubs can effectively alter microclimatic conditions in shrubby patches, thereby creating a suitable environment for oak trees to thrive. Figure S3 illustrates an example of how the oak trees can colonise grasslands. Over the past 30 years, the area of wooded grasslands in the Tolna region has expanded by hundreds of hectares as a result of natural succession. The saplings or root suckers found near the relic oak trees (No. 2, 5 and, respectively, No. 8) could support the hypothesis that relic oak trees are essential for the restoration of xeric or steppe–wood ecosystems. However, forest genetic resources are also expected to play a pivotal role in the future ecosystem restorations [49].

In the context of climate change, the region has already been identified as a 'hotspot' due to the cumulative effects of both the continental and (sub-) Mediterranean climatic conditions. The native plants in the Tolna region are adapted to a range of climatic extremes, including prolonged summer heat events, long drought periods in all four seasons, frequent late and/or early frosts, and infrequent but intense winter frosts (−15 to −20 °C). This is due to the high diversity of the continental and sub-Mediterranean flora elements reported in the literature [50–52].

Geographically, the Tolna region is located at the crossing point of the Pannonian steppe, Pannonian oak wood–steppe, and pre-Illyrian (xeric) oak wood vegetation zones. Model studies [24,26] suggest that climatic conditions in the Pannonian Basin (and particularly in this region) are undergoing a significant change. The direction and impact of these changes are difficult to predict, but it is highly probable that the steppe climatic zone will cover a larger surface area in the near future. Additionally, the extent of forest cover may be reduced, or the species composition of forests may be altered. Forests on the xeric limits [24,25] would probably be eliminated or partially disappear. In such cases, woody patches may play a more prominent role within the landscape.

Our results support the hypothesis that relic oak trees harboured a high number of forest character elements. This diversity is not correlated with the distance from forests but significantly linked with the period of forest cover in the past (land-use type). Solitary trees, groups of trees or small forest patches could harbour most of the recent species' diversity of forested lands, such as the relic oak trees have done for long periods of time. However, mosaic-like patterns of steppe and steppe–wood vegetation elements often occur in Hungary [48]. The higher elevation zones are generally covered by secondary woodlands consisting of shrub and tree species, while the steppe elements dominate in lower elevation zones [48].

5. Conclusions

Recent genetic studies [42,43] have shown that hybridisation and introgression within the *Quercus* section were the main drivers of adaptation to local ecological conditions during the postglacial period. The adaptive potential of oaks has been considered as a dominant element of their secondary evolution [44]. The relic hybrid oak phenotypes may be optimally adapted to the extreme local geographic, climatic and soil conditions. Most of the relic trees were coppiced in the past, and their genotypes were selected by human factors such as grazing and wood harvest as well.

In areas where the grasslands have been less intensively used over the past three decades, oak saplings were observed to be growing near the relic oak trees. These saplings were described as hybrids, similar to the parent trees. The natural migration of hybrid oak saplings can be explained by the optimal fitness of their hybrid genotypes on grasslands.

The adaptive potential of these hybrids may be useful in the context of climate change. Forest populations on xeric limits are likely to develop into steppe vegetation [24,26]. In the Pannonian region, oak genotypes with xeromorphic characteristics, such as hairiness and rugged bark, will be more advantageous. The downy oaks sensu lato (*Quercus pubescens* and *Quercus virgiliana* and their hybrids) have a relevant role to play not only in the forestry sector but also in grassland management. Vergilius oaks and its hybrids are well adapted to loess-based soils and can grow as a solitary tree or even in groups on a wood-pasture. The oak trees have various environmental effects at the local level, such as tempering summer heat, increasing air humidity, moderating soil erosion, and providing shelter for diverse species. Additionally, their acorns can be used as valuable feed for cattle. Considering these aspects, the Vergilius oak may be a valuable species for natural grassland management, in the context of climate change as well.

These relic trees have served as refuges for small patches of the natural vegetation and preserved a relatively high species diversity. The relic tree mosaics can also serve as small flagstone habitats, providing opportunities for natural succession and migration. Our results indicated a positive correlation between the frequency of forest land-use or the presence of forested land boundaries and the richness of forest woody plants (Pearson's R = 0.46, n = 37, $p < 0.01$).

The richness of forest dicot herbs is significantly lower in areas where the land was previously used for arable land and/or vineyards without being followed by forest (Student's t: t(35) = 2.90, $p < 0.01$). This might be explained by the land-use type, since dicot herbs were considered as weeds in both cases. Consequently, dicot herb plants were continuously regulated/reduced by weeding, preventing the accumulation of their seeds in the soil. In contrast to this, the number of monocot herbs was positively correlated with arable lands.

Relic trees are valuable genetic resources for the forestry sector. The in situ conservation of these trees not only preserves their genetic resources but also their microhabitats, which are important for dynamic conservation purposes. The adaptive potential of oaks is determined by hybridisation and introgression through pollen flow. Relic trees, with their large crowns, can produce a significant amount of pollen. The recorded trees were predominantly found on hilltops and ridges, where they were consistently exposed to wind, facilitating effective pollen flow over both short and long distances. Relic trees may play a crucial role in the mating system of larger oak forest populations in the region due to their pollen flow, which is essential for dynamic genetic conservation purposes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d16070401/s1, Figure S1: Detailed maps of the site plots with recorded trees. The blue dots and ID numbers are referenced with coordinates listed in Table S2; Figure S2: A blossoming plant stock of *Limodorum abortivum* on site plot No. 8 in summer 2020. The leaves and stipules have just started to form into green due to increasing chloroplast activity. (Photo: Horváth, Cs.); Figure S3: Natural succession on grasslands: in front, a young oak sapling and shrubs nearby the site plot No. 2. and a former wood-pasture (site plot No. 1) transformed into a forest with closed canopy shift (secondary wood) in the background. (Photo: Bordács, S.); Figure S4: Landscape mosaic of grasslands in Mucsi. Regularly mowed meadow (front left), a reconstructed wood-pasture (front right) and various phases of succession are in the background, transforming grassland into secondary wood. (Photo: Bordács, S.); Figure S5: A coppiced oak tree growing on grassland in Mucsi (Site plot No. 9) used as marker tree in the past made the boundary line visible. The flat hilltop is regularly mowed (front right) and deeply eroded slopes are transformed into wooded land (left background). (Photo: Bordács, S.); Table S1: Descriptor list of morphological and phenotypic traits used for taxonomic characterisation. The descriptors were summarized by a synthesis of publications referenced in the text; Table S2: Georeferences of trees recorded and analysed.

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