Arch Biol Sci. 2022;74(2):191-199 https://doi.org/10.2298/ABS220405018J

Patterns of leaf morphological variation in *Quercus frainetto* Ten. growing on different soil types in Serbia

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Received: April 5, 2022; **Revised**: April 29, 2022; **Accepted**: May 3, 2022; **Published online**: June 10, 2022

Abstract: Leaf morphology is at a certain level defined by the ways in which plants adapt to different habitats, especially in large trees. In this study, morphological variations in leaf size and shape of the Hungarian oak (*Quercus frainetto* Ten.) growing on different soil types (lithic leptosol, vertisol, cambisol) were investigated in the central part of Serbia (Šumadija). The information on soil type was obtained using a digitalized soil map of the Republic of Serbia, while leaf traits were characterized by geometric morphometric methods. Landmark analysis and leaf measurements showed significant differences among the analyzed groups, with individuals growing on nutrient-poor, shallow soils having smaller leaves with greater lobation. The observed differences suggest that the levels of soil productivity influence variations in leaf patterns. More studies on a larger sample size and along a broader spatial scale are needed to fully understand the differences in the patterns of leaf morphological variation in *Q. frainetto*.

Keywords: Hungarian oak; *Quercus frainetto*; leaf size; leaf shape; soil type

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INTRODUCTION

Leaves possess important functional traits for transpiration and photosynthesis and are a fundamental component of a plant's ability to adapt to different habitat conditions. The leaf morphology variations result from plastic and adaptive responses to environmental differences, causing divergence between populations at both genetic and phenotypic levels [1]. Leaf morphological traits express different patterns within the same species and even within the same individual genotypes, particularly in large trees [2]. Foliar morphology is defined by the ways plants adapt to different habitats [3,4], especially in terms of climatic conditions and soil types [5,6]. The availability of nutrients in the soil is one of the main determinants of diversity within plant communities [7]. When exploring the link between soil and leaf nutrient concentration, the study of relations between soil fertility and canopy nutrient stock contributes to insight into plant adaptations to environmental factors [8]. Studies have shown that increasing altitude, reduced precipitation and the quantity of nutrients in the soil cause a reduction in leaf size [9,10], while the conditions of evaporative demand and soil water availability are reflected in different morphology-environment interactions [11]. Additionally, there is a clear relationship between plants and soil formation, and this trade-off is reflected in the plant feedback mechanism to soil nutrients – litter leaves of plants growing on nutrient-rich soils are also rich in nutrients and maintain high soil fertility after decay [12]. The quantification of the responses of leaf traits to different soil types is still limited and requires more investigation, especially in tree species that are affected by climate change, habitat degradation and poor management practices [13].

Species of the genus *Quercus* are suitable for the investigation of leaf morphological variation in different habitat types due to their wide area of distribution

How to cite this article: Jovanović M, Grbović F, Milovanović J, Nonić M, Šijačić-Nikolić m, Branković S. Patterns of leaf morphological variation in *Quercus frainetto* Ten. growing on different soil types in Serbia. Arch Biol Sci. 2022;74(2):191-9.

and a broad ecological niche [14,15]. Oaks are also more endangered, with nearly one-third of all oak species considered threatened with extinction [16]. The Hungarian oak (*Quercus frainetto* Ten.) is native to southeastern Europe and Asia Minor, with the widest distribution on the Balkan Peninsula as an element of sub-Mediterranean flora. It is believed that this species had a less intensive role in recolonization during the postglacial period due to its small distribution area [17]. This is a meso-xerophilous and highly light-demanding species, growing in habitats with hot summers and harsh winters. It prefers alluvial silica or lime soils and tolerates some waterlogging [18,19]. *Q. frainetto* is one of the edificatory species of the association *Quercetum frainetto-cerris* Rudski 1949, which is widely distributed in the Šumadija region in Serbia [20]. This association occurs on placoric sites with just enough water in the soil and represents a climatogenic forest in this area [21].

The evolution and genesis of the soil in Serbia were influenced by land relief and petrographic composition. Different soil types and their classes are present in this area, each possessing a characteristic set of morphological, chemical and water-physical properties that are responsible for different production characteristics [22, 23]. The pedological diversity of the Šumadija region is the result of the diverse parent material, the translational character of the climate, altitude diversity, characteristics of past and present vegetation, and the significant effect of anthropogenic factors. The region is characterized by mixed soil layers, with cases of adjacent areas having completely different soil profiles [24]. The greatest diversity of soil types is found in the central part of this region, in the vicinity of the city of Kragujevac. The characteristic diversity of soil types in this area has its basis primarily in a very diverse geological composition, both in terms of rock age and chemical properties. The area is divided into two parts: the lower part in which the parent rock plays a crucial role in the formation of soil types, and the mountainous part in which the geomorphological factor, regardless of the pedological substrate, affects the appearance of skeletal soil [25-27]. The basic type of land in Šumadija is vertisol, formed from Neogene sediment, which appears in the form of fragments on smaller areas. Vertisol is formed in climatic conditions in which the change of wet and dry period is well expressed, in a semi-humid to semiarid climate, mostly on flat and slightly wavy plain type of relief at 200-600 m above sea level, under natural vegetation of mixed deciduous (mostly oak) forests and grass communities [28]. Because they have undergone major changes, different types of cambisol are more common than vertisol. Cambisol (eutric) is a climatogenic type of soil of temperate-continental areas covered by the climatogenic plant community of *Quercetum frainetto-cerris* Rudski 1949 [27]. Cambisol develops on a hilly relief on terrains with plenty of lime and shady patches where the water drains quickly. The formation of this type of soil is significantly conditioned by the effect of the root system of the woody plants on the parent material [24]. Skeletal soils (lithic leptosol) appear at higher altitudes of the investigated area. These soils are mostly shallow, contain rock fragments, and are common on sites where the parent rocks are subjected to continuous erosion or weathering. Due to the different ways of soil formation, these soils vary in physical and chemical properties but are usually shallow and poor in terms of nutrient status. The appearance of skeletal soil is responsible for strong erosion phenomena in this area. The soil in parts of the Šumadija region is skeletal, and under these conditions, it can almost never be converted into genetic soil because its skeletal state is maintained by the constant removal of soil by water erosion [24,25,29]. Thus, this high soil diversity in Šumadija has conditioned the existence of different productivity levels, such as deep soils with thick topsoil and good subsoil properties, which are considered to be higher when compared to shallow skeletal soils [22]. Therefore, we investigated whether and to what degree leaf morphology is related to soil type in *Q. frainetto* populations from the central part of Serbia (Šumadija).

As leaf morphological traits can be related to habitat type and can change according to soil composition [9,11], we expected different patterns of leaf size and shape variation of *Q. frainetto* inhabiting areas with three major soil types that occur in the Šumadija region, lithic leptosol, vertisol and cambisol. The leaves of *Q. frainetto* are up to 20 cm long and up to 12 cm wide, often deeply lobed, with 7 to 9 lobes [19], displaying high morphological variability. According to the basic land quality characteristics of soil types in Serbia [22], lithic leptosol is identified with a land quality with serious limitations (unproductive soil), vertisol with moderate limitations (highly productive soil) and cambisol also with moderate limitations (medium to highly productive soil). Thus, we hypothesized that the lack of nutrients on shallow soils prone to erosion (skeletal soils or lithic leptosol), will produce differences in leaf traits of *Q. frainetto* when compared to more fertile and nutrient-rich soils such as cambisol and vertisol. The main objective of this study was to identify and evaluate the variability of *Q. frainetto* leaf size and shape, and to link the observed variability with soil types and their characteristics.

MATERIALS AND METHODS

Sampling and landmark configuration

In the autumn of 2021, we sampled 970 leaves (10 leaves from 97 randomly selected individuals) of *Q. frainetto* from areas of 3 different soil types in the central part of the Šumadija region (Supplementary Table S1, Supplementary Fig. S1A). For each locality, GPS coordinates were recorded and processed using Quantum GIS 3.24.0 [30] to obtain information on the soil type from imported digitalized maps [31, 32]. This revealed 3 types of soil present at the sampling sites as follows: (i) cambisol, (ii) lithic leptosol and (iii) vertisol (Table 1). These were also the dominant soil types in the study area (Supplementary Fig. S1B).

To minimize the risk of clone selection, the populations were at least 10 km apart and all sampled individuals were located at least 5 m from each other [33]. From each individual, 10 fully developed leaves were collected at a height of 8-10 m around the crown of each tree [34], mainly under shaded conditions. The leaves were pressed, herbarized and scanned by placing the abaxial surface facing upwards on an Epson Stylus DX4050 scanner at a resolution of 300 dpi.

Table 1. Physical and chemical characteristics of soil types used in this study (cambisol, lithic leptosol, vertisol) [24, 25]. Minimal and maximal values of each soil characteristic are represented.

Soil characteristic	Cambisol (eutric)	Lithic leptosol	Vertisol
pH (H_2O)	5.2-8.3	5.0-6.6	5.6-8.1
pH (KCl)	4.2-7.2	4.5-4.6	4.6-7.0
Humus content (%)	0.7-12.6	2.5-3.9	2.0-5.6
P_2O_5 (mg 100 g^{-1})	1.3-8.0	0.4-5.8	0.6-28.0
K_2O (mg 100 g^{-1})	3.9-14.2	1.5-12.6	19.0-59.6
Sand content (%)	25.8-75.1	40.0-68.0	11.9-31.2
Slit + clay content (%)	24.9-74.2	32.0-60.0	49.5-78.6

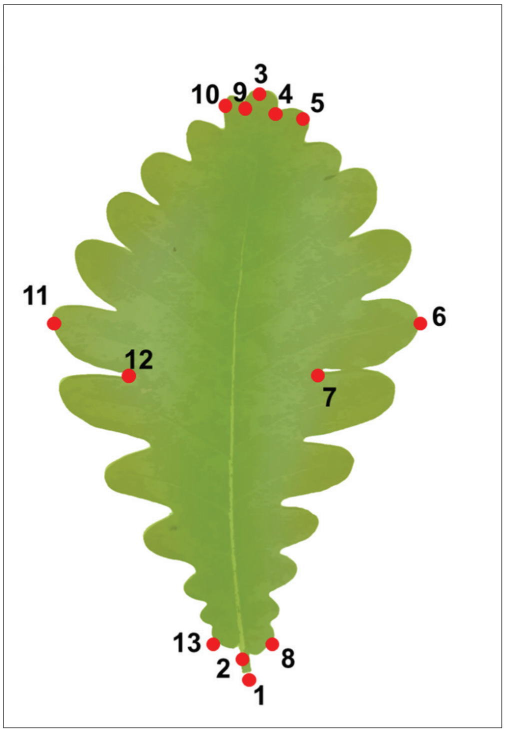

Fig. 1. Configuration of *Q. frainetto* leaf outline showing 13 landmarks: 1 – beginning of the petiole; 2 – junction of the blade and the petiole; 3 – apex of the leaf blade; 4 and 9 – base of the apical sinuses of the blade tip (right and left side); 5 and 10 – tip of the lobe immediately above the apex of the leaf blade (right and left side); 6 and 11 – tip of the lobe at the largest width of the blade (right and left side); 7 and 12 – base of the sinus immediately beneath the lobe of the landmarks 6 and 11; 8 and 13 – the first basal lobe of the blade (right and left side).

On the left and right sides of each leaf, 13 landmarks were recorded (Fig. 1) in accordance with the previously suggested methodology [34]. The first 3 landmarks (landmarks 1-3) were unpaired and distributed along the midrib of the leaves, while the other landmarks (landmarks 4-13) were paired and distributed symmetrically on both sides of the leaves. The coordinates of the landmarks for each leaf were recorded using tpsDig and tpsUtil software [35].

Statistical analysis

Generalized Procrustes analysis (GPA) was performed to minimize the sum of squared distances between the corresponding landmarks and to extract shape information by removing the data on size, location and orientation [36]. Procrustes ANOVA was performed to quantify leaf size and shape variation between the populations growing on different soil types [37], and canonical variate analysis (CVA) to further visualize differences between the groups. All analyses were performed in MorphoJ software [38].

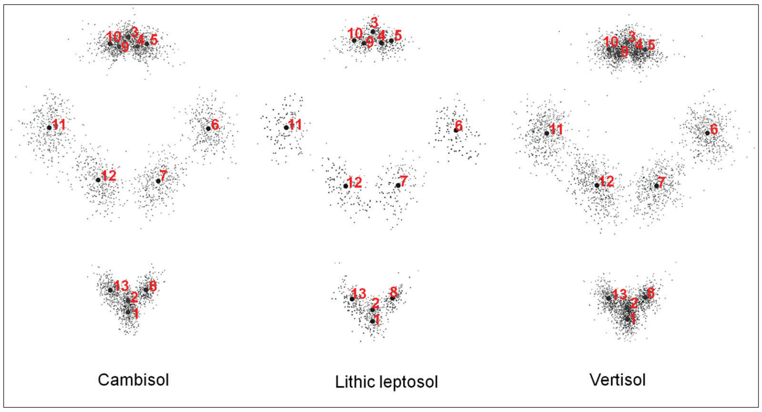

Fig. 2. Results of generalized Procrustes analysis of the leaf shape of Q. *frainetto* growing on different soil types.

Table 2. Results of Procrustes ANOVA of Q. *frainetto* leaves from different soil types.

	Effect	SS	MS	d.f.	F	P
Centroid size	Soil	131088906.65	1365509.44	96	7.01	<0.0001
Shape	Soil	3.40989577	0.001614534	2112	3.05	<0.0001

SS – the sum of squares, MS – mean squares, d.f. – degrees of freedom, F – F values, P – P values.

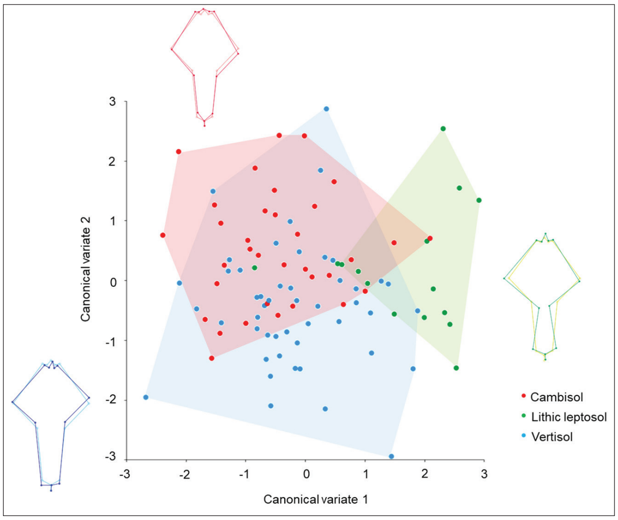

Fig. 3. Ordination of Q. *frainetto* individuals growing on different soil types along the first two canonical variates obtained by the canonical variate analyses. Shape changes along soil types are represented by wireframe graphs.

Morphological analysis of size and shape (MASS) [39] was used to calculate leaf length, width, area, length and width ratio (L/W ratio, aspect ratio), fluctuating asymmetry, roundness, circularity and solidity. The values of fluctuating asymmetry equal to zero suggested a perfectly symmetrical leaf, while values other than zero indicated skews to the left or the right side. Roundness was measured as the inverse of the aspect ratio, with completely round leaves having a value equal to one. Circularity was represented as the ratio of area to perimeter squared, with low values suggesting high lobation and high values suggesting entire leaves. Solidity was measured as the area divided by convex hull area, with values close to one suggesting little or no lobation and values less than one suggesting serrations or lobation. Differences in the obtained measurements were analyzed using analysis of variance (ANOVA), and a post-hoc Tukey HSD test was used for pairwise comparisons. Correlations between the obtained measurements were estimated using Pearson's correlation coefficient for the total sample. To prevent distortion of the results, the petiole was excluded from the MASS analysis [39].

RESULTS

The raw coordinate matrix obtained from image analysis of the 970 leaves was translated into a normalized matrix using Procrustes fitting. Subsequently, the configurations were rotated to the concentrated distribution around 13 landmarks (Fig. 1) based on the raw coordinate matrix (Fig. 2). Procrustes ANOVA of leaf size and shape showed that both shape and size exhibited statistically significant differences between Q. *frainetto* individuals growing on different soil types (Table 2). The CVA indicated a considerable overlap between individuals from different soil types, especially between those growing on cambisol and vertisol. Individuals growing on cambisol had narrow basal lobes of the leaf blade, while in those growing on vertisol this trait showed higher values. Individuals growing on lithic leptosol were separated from the others by having high values of the leaf blade's greatest width and pronounced lobes at the leaf's greatest width (Fig. 3).

Table 3. ANOVA results of *Q. frainetto* leaf measurements obtained by MASS.

Measurement	**F value**	**P value**
Length (cm)	103.25	<0.01
Width (cm)	62.46	<0.01
Area (cm^2)	87.09	<0.01
L/W Ratio	13.07	<0.01
Fluctuating asymmetry	7.47	<0.02
Roundness	11.95	<0.01
Circularity	43.06	<0.00
Solidity	49.74	<0.01

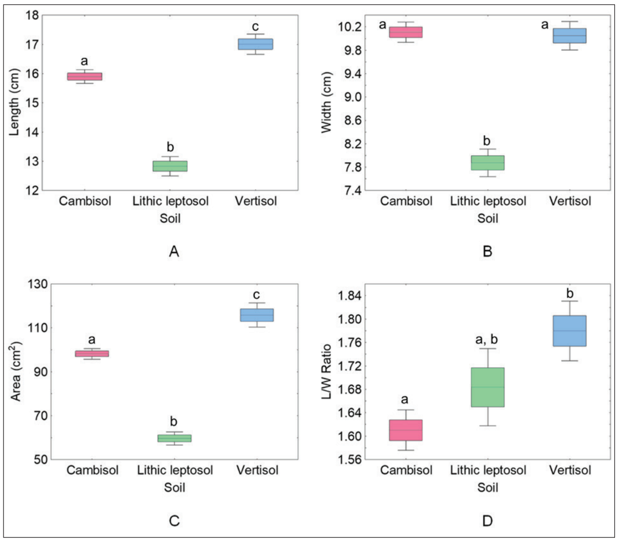

Fig. 4. Box plots of means, standard errors and 95% confidence intervals of leaf length (**A**), width (**B**), area (**C**) and length and width ratio – L/W ratio (**D**) for *Q. frainetto* leaves of individuals growing on different soil types, with the representation of homogeneous groups (a, b, c) based on the Tukey HSD post-hoc test.

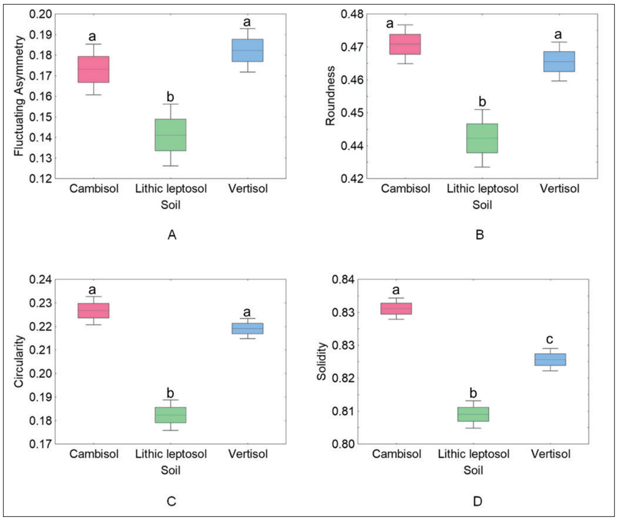

Fig. 5. Box plots of means, standard errors and 95% confidence intervals of fluctuating asymmetry (**A**), roundness (**B**), circularity (**C**) and solidity (**D**) for *Q. frainetto* leaves of individuals growing on different soil types, with the representation of homogeneous groups (a, b, c) based on Tukey HSD post-hoc test.

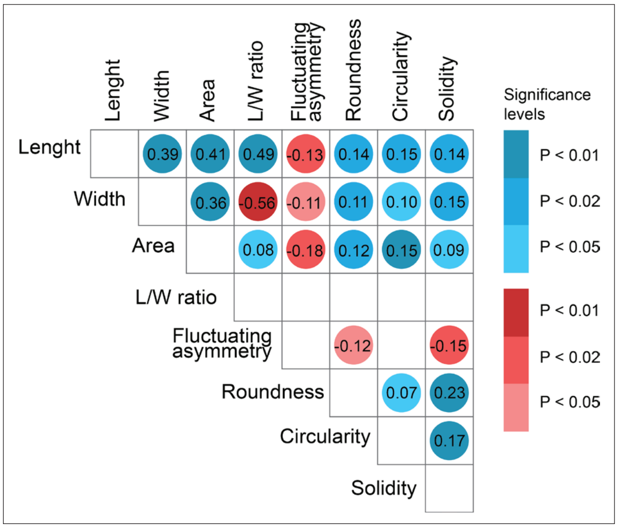

Fig. 6. Pearson's correlation coefficients of *Q. frainetto* leaf measurements. Positive correlations are displayed in blue and negative correlations in red. Only statistically significant correlations (P<0.05) are represented in different color intensities.

MASS showed a significant effect of soil type on leaf measurements (F=34.78, P<0.001). Statistically significant differences were obtained for all measurements (Table 3). The post-hoc Tukey HSD test showed that individuals growing on lithic leptosol had statistically significant (P<0.05) lower values of leaf length, width and area as compared to individuals growing on other soil types (Fig. 4A-C). Additionally, leaf length and area differed between individuals growing on cambisol and vertisol, with individuals growing on vertisol having higher values of these traits (Fig. 4A, C). Individuals growing on vertisol also had significantly higher values of length and width ratio (Fig. 4D). Individuals growing on lithic leptosol had significantly lower values of fluctuating asymmetry, circularity, roundness and solidity compared to individuals growing on cambisol and vertisol (Fig. 5A-D). The values of solidity differed significantly among all three groups, with individuals growing on cambisol having the highest values of this trait (Fig. 5D).

Pearson's correlation coefficient of the total sample suggested that the length, width and area displayed significant positive correlations with other measurements, except with fluctuating asymmetry where the correlation was significantly negative. Additionally, the length and width ratio (L/W ratio) showed a significant positive correlation with leaf length and area and a negative correlation with leaf width. Fluctuating asymmetry was significantly negatively correlated with leaf length, width, area, roundness and solidity. Roundness was positively correlated with leaf length, width, area, circularity and solidity. Circularity and solidity were also significantly positively correlated (Fig. 6).

DISCUSSION

This study demonstrated significant differences between leaf size and shape of *Q. frainetto* individuals growing on different soil types. Individuals growing on lithic leptosol, the most unproductive soil, were separated from individuals growing on soils of moderate and high productivity (cambisol and vertisol) by a smaller leaf size and higher lobation of the leaf blade.

Phenotypic plasticity is one of the ways in which plant species can adapt to new environmental conditions; leaf size is partially under genetic control, and its traits are considered the most plastic and are highly sensitive to local habitat conditions [40]. The leaf expansion process is controlled by both genetic characteristics (adaptation) and habitat conditions during leaf flushing [41]. Leaf size shows a tendency to decline with the increase in elevation, decrease in annual temperature and rainfall, and at sites with low soil fertility [9]. In this study, lower values of leaf length, width and area in individuals growing on lithic leptosol compared to cambisol and vertisol were recorded, suggesting that a smaller leaf size is favored on infertile soils, likely due to the plant's slower growth and a smaller investment in total leaf area. Nutrients in the soil represent important environmental factors that explain the variability of the functional traits of the leaves, which cause nutrient stress and the production of small leaves with a low leaf area and nutrient content [42]. At the study sites of the Šumadija region, skeletal soil (lithic leptosol) contains more than 35% (by volume) of rock fragments, pebbles, gravel and laterite concretions or ironstones with diameters greater than 2 mm. These soils are usually shallow (up to 30 cm), prone to erosion and low in nutrients; land aggregates are not formed, and if there are any, they are very unstable. Additionally, skeletal soil has a very unfavorable water regime. The amount of humus varies depending on the plant cover and ranges from 2.50-3.93% [24]. Land types that contain shallow and rocky soils have low productivity, most of the nutrients are in the organic matter on the surface and these environments are likely to favor a decline in leaf size. On the other hand, vertisol and cambisol in the study area are characterized by higher fertility, with the humus about 50-100 cm thick. The humus content in vertisol usually ranges from 3-5%, and under natural vegetation it can be 7-8%, and in cambisol is usually 4-7%. Vertisol and cambisol are moderately rich in nutrients (N, P, K), which depend on the parent substrate [24,25,28]. These nutrient-rich soils with more favorable water regimes provide suitable conditions for *Q. frainetto* individuals to develop larger leaves. In forest ecosystems, soil productivity is expected to be altered directly through reduced availability of water or through changes in nutrient availability [43]. Thus, in nutrient-poor soils, the small leaves ensure greater hydraulic conductance and represent a strategy for maximizing nutrient intake by driving nutrient flow from the roots [44]. Regarding the *Q. frainetto* populations analyzed in this study, the clear separation of individuals growing on more infertile soil suggests that soil productivity levels are indeed one of the important factors that influence leaf size, although other factors, such as water availability, must also be taken into consideration.

Landmark analysis of the leaf shape indicated *Q. frainetto* individuals growing on lithic leptosol had more pronounced lobes at the highest width of the leaf blade. These results were supported by the low values of roundness, circularity, and solidity, which indicated a high degree of leaf lobation [45]. Leaf geometry results from a balance between the need for energy uptake maximization and the minimization of the damage caused by environmental stresses [46]. Lobation in leaves is mainly explained by the light conditions, with lobed leaves having production costs that are adaptive in environments where competition for light is advantageous [47]. In the case of *Q. frainetto*, higher lobation of the leaf blade on unproductive soils suggests that these traits might be the result of the interaction with the evolution of smaller leaf size. Thus, when observing the relation of soil type and leaf lobation, the link between leaf morphology and soil productivity is not as straightforward as in leaf size. Moreover, fluctuating asymmetry values were the lowest in individuals growing on lithic leptosol and were correlated with higher leaf lobation. However, although individuals from lithic leptosol separated from the other two soil types, relatively low values of fluctuating asymmetry were recorded in each group, suggesting almost symmetrical leaves. Fluctuating asymmetry is a small and random deviation from bilateral symmetry that can represent an indicator of stress in natural populations [48]. It relies on the fact that the left and right sides of the bilateral structure have the same genome and develop similarly in a homogeneous habitat [49]. Thus, the obtained results suggest that smaller leaves with higher lobation displayed a higher ability to buffer their development against changes in the environment [50]. In the case of the *Q. frainetto* populations analyzed in this study, leaf size was likely a better indicator than fluctuating asymmetry of environmental impact on leaf morphological traits.

In the present study, both leaf size and shape of *Q. frainetto* showed differences in habitats with different soil types. However, even though these differences were statistically significant, the leaf trait-soil type relationship showed considerable overlap, suggesting that within-site variation could have been the largest source of variability [51]. In species characterized by a broad ecological niche, such as oaks, understanding the ways individuals adapt to local habitat conditions can be of critical importance when planning future conservation strategies, as past studies have shown that morphology can predict environmental conditions and population structure [13].

CONCLUSION

The results of this study indicate that levels of soil productivity influence the variation patterns of *Q. frainetto* leaves. A better understanding of the interactions of soil characteristics and leaf traits is an important topic that needs to be addressed in the future and should include larger samples of different oak species from diverse areas across a broader spatial scale. The results obtained herein lay the foundation for future research into the potential influence of soil type on leaf morphological variability in oaks.

Funding: The conducted research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No. 451-03-68/2022-14/200169, Agreement No. 0801-417/1 and Agreement No. 451-03-68/2022-14/ 200122.

Acknowledgments: We thank Sava Vasić (Faculty of Science, University of Kragujevac, Serbia) for the useful comments and suggestions regarding the manuscript.

Author contributions: Conceptualization, MJ and FG; methodology, MJ; software, MJ; validation, MJ, FG and JM; formal analysis, MJ; investigation, MJ and FG; resources, MJ; data curation, MJ and FG; original draft preparation, MJ, FG and JM; review and editing, MN, MŠN and SB; visualization, MJ; supervision, JM, MN, MŠN and SB; project administration, JM, MN, and MŠN; funding acquisition, FG, SB, JM, MN and MŠN. All authors have read and agreed to the published version of the manuscript.

Conflict of interest disclosure: The authors declare no conflict of interest.

Data availability: All data underlying the reported findings have been provided as part of the submitted article and are available at: https://www.serbiosoc.org.rs/NewUploads/Uploads/Jovanovic%20et%20al_7637_Data%20Report.pdf

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Supplementary Data

The Supplementary Material is available at: https://www.serbiosoc.org.rs/NewUploads/Uploads/Jovanovic%20et%20al_7637_Supplementary%20Material.pdf