

The Effects of Soil Type, Exposure and Elevation on Leaf Size and Shape in *Quercus cerris* L.

Marija Jovanović^{1,*}, Jelena Milovanović¹, Marina Nonić², Mirjana Šijačić-Nikolić², Ivona Kerkez Janković², Filip Grbović³

(1) Singidunum University, Environment and Sustainable Development, Danijelova 32, RS-11000 Belgrade, Serbia; (2) University of Belgrade, Faculty of Forestry, Kneza Višeslava 1, RS-11000 Belgrade, Serbia; (3) University of Kragujevac, Faculty of Science, Radoja Domanovića 12, RS-34000 Kragujevac, Serbia

* Correspondence: e-mail: marija.jovanovic.202@singimail.rs

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ABSTRACT

One of the main environmental factors that influence plant species and community diversity are soil types, exposure and elevation. This study aimed to evaluate differences in leaf size and shape of *Quercus cerris* L. along environmental gradients in the Šumadija region in Serbia by using geometric morphometrics methods. The results showed significant differences between *Q. cerris* individuals inhabiting sites with different soil types, exposures and elevations. Individuals growing on nutrient deficient soils had smaller leaf size, elongated petiole, wide leaf blade, and higher values of fluctuating asymmetry compared to individuals growing on nutrient-rich soils whose leaf size was larger, more variable in shape and had lower values of fluctuating asymmetry. Additionally, individuals inhabiting higher elevations had elongated and narrow leaves and short petioles. Leaf size was also greater in individuals from lower elevations and north-exposed sites. The results of this study suggest that leaf morphological traits are affected by habitat differences and exhibit considerable plasticity in response to environmental demands.

Keywords: Turkey oak; habitat differences; leaf morphometrics; intraspecific variability

INTRODUCTION

The development of plant form and structure is regulated by genes and affected by the environment (Barkoulas et al. 2007, Fritz et al. 2018). Plants have the ability to display phenotypic plasticity to optimize resource utilization under different habitat conditions (Liu et al. 2020), and leaf morphology can represent one of the main determinants that reflect the status of the whole plant, thus being an excellent tool for ecological studies. Leaf traits vary across habitats with different climatic conditions (Olsen et al. 2013) and can provide insights into the evolutionary changes that enable plant adaptation to local environments.

In this study we investigated whether and to what degree leaf morphological traits of Turkey oak (*Quercus cerris* L.) are influenced by soil types, land exposure, and elevation. Oaks are suitable models for this kind of study, since they are represented by the large area of distribution and a broad ecological niche (Nixon 2006, Di Pietro et al. 2016, Jovanović et al. 2022a). Oaks are common, often dominant vegetation elements that include many ecologically diverse species which are widely distributed (Simeone et al. 2018).

The natural range of *Q. cerris* is southern Europe and Asia Minor. This species is characterized by good adaptability to different habitat conditions, it is tolerant to drought, air pollution, and it grows on a wide range of soil types. It can be found up to 1900 m above sea level, in hot climates on semi-shaded exposure, and in colder climates on sun-exposed sites (de Rigo et al. 2016). Although *Quercus* is one of the most commonly investigated genera regarding leaf morphology, studies of leaf morphological variability in *Q. cerris* are quite rare (e.g., Sisó et al. 2001, Čermák et al. 2008, Karavin 2014, Jovanović et al. 2022b).

One of the main environmental factors that influence the diversity of plant communities is soil type, as well as exposure and elevation gradients. Soil nutrients, for example, influence variation in plant morphology, including leaf functional traits (Su et al. 2021, Jovanović et al. 2022a). Litter leaves of plants growing on nutrient-rich soils are also rich in nutrients and maintain high soil fertility after decay (Gong et al. 2020). Previous studies have shown that increasing elevation, reducing precipitation and the amount of nutrients in the soil affect the reduction in leaf size (McDonald et al. 2003, Milla and Reich 2011), while the evaporative demand and

availability of water in the soil reflect different interactions of the morphology and the environment (Nicotra et al. 2011, Wang et al. 2019, Salehi et al. 2020, Kahveci 2023). Exposure also influences vegetation patterns (Yang et al. 2020) – at different exposures, differences occur in air and soil temperature, evaporation and transpiration, wind speed, and solar radiation (Bennie et al. 2008). In general, the polar-facing slopes are more humid and colder, with higher content of organic matter and deeper soil, while the equator-facing slopes are hotter and dryer, with lower levels of soil nutrients and more pronounced erosion. These conditions significantly influence vegetation through the modification of the local environment, causing differences in the morphological traits of the plants inhabiting such areas (Moeslund et al. 2013). Lastly, elevation also significantly influences vegetation distribution and attributes. Elevation gradients affect plant development and growth as they cause variations in environmental factors such as air temperature, solar irradiance, and rainfall (Liu et al. 2020). Additionally, plants are impacted by changing air pressure in different elevations – the reduction in air pressure at higher elevations lowers the partial pressure of the oxygen, decreasing the water vapour pressure and increasing the atmospheric transmissivity to solar radiation (Xu et al. 2021), further influencing photosynthetic parameters by affecting the trade-offs between these competing requirements. In habitats situated at greater elevations and on soils low in nutrients, plants show the tendency to decrease leaf area and increase leaf thickness (Liu et al. 2020). Although leaf traits can influence the fitness of trees on physiological, biochemical, morphological, and developmental levels (Donvan et al. 2011), intraspecific studies on how soil types, exposure and elevation affect leaf morphology in oaks (*Quercus* spp.) are scarce and mainly include physiological responses related to leaf nutrient composition changes in different environments (Li et al. 2006, Singh and Todaria 2012, Du et al. 2017, Azizi et al. 2020).

This study aimed to evaluate differences in leaf size and shape of *Q. cerris* along different soil types, elevations and exposures in the Šumadija region in Serbia. The leaves of *Q. cerris* vary in size and shape, are mostly oblong or oblong-elliptical, widest in the middle of the leaf blade, normally 9 to 12 cm long and 3 to 5 cm wide, with 7 to 9 pairs of triangular lobes (de Rigo et al. 2016). In the Šumadija region, *Q. cerris* is one of the edificatory species of the Hungarian oak and Turkey oak forests (*Quercetum frainetto-cerris* Rudski 1949), which are climatogenic forests typical for Serbia (Vukin and Rakonjac 2013, Jovanović et al. 2022b). The Šumadija region is characterized by high habitat diversity (Pavlović et al. 2017) – different soil types and elevation differences, along with significant areas under natural forest vegetation, make this site suitable for the investigation of the potential influence of habitat conditions on plant morphology. In the Šumadija region, small waterways dissected the relief, developing a hilly layout, with areas ranging from 100 to 1130 m above sea level. This region is characterized by high pedological diversity, with vertisol, cambisol and lithic leptosol being the most common soil types. Vertisol is formed in areas in which the change of wet and dry periods is well expressed, mostly on flat and slightly wavy relief, at 200–600 m of elevation, under natural vegetation of mixed deciduous

forests and grass communities (Čirić 1991). Cambisol (eutric) is the climatogenic type of soil of temperate-continental areas under the climatogenic plant community *Quercetum frainetto-cerris* Rudski 1949 (Brković 2015). It develops on a hilly relief on terrains with a lot of lime and shady sides where the water drains quickly (Pavlović et al. 2017). Skeletal soils (lithic leptosol) appear at the higher elevations of the studied area (300 m above sea level), where the relief is well developed. These soils are shallow, with rock fragments, common in areas where the parent rocks are subjected to continuous erosion. Thus, as leaf traits vary with the physical environment in predictable ways (Xu et al. 2021), the main objectives of this study were to evaluate leaf size and shape variation patterns between *Q. cerris* populations, and to link the observed patterns with habitat differences recorded for each population.

MATERIALS AND METHODS

Sampling

In the autumn of 2021, 138 randomly selected adult individuals of *Q. cerris* were sampled from nine natural populations in the central part of the Šumadija region in Serbia (Table 1, Figure 1). Šumadija, the central region of Serbia, occupies about 5800 km², covering the area between the Sava and the Danube in the north, Great Morava in the east, West Morava in the south, and Kolubara in the west (Šikanja 2019). *Q. cerris* leaves were sampled within Hungarian oak and Turkey oak forests (*Quercetum frainetto-cerris* Rudski 1949). To minimize clone selection risk, all sampled individuals from the selected populations were located at least 5 m from each other (Li et al. 2021, Jovanović et al. 2022c). From each individual, 10 fully developed leaves were collected (1380 in total), at the height of 8 to 10 m around the crown of each tree (Viscosi 2015), mainly under shaded conditions. Leaves were herbarized and scanned by placing an abaxial surface facing upwards on an Epson Stylus DX4050 scanner, with a resolution of 300 dpi.

Spatial Data

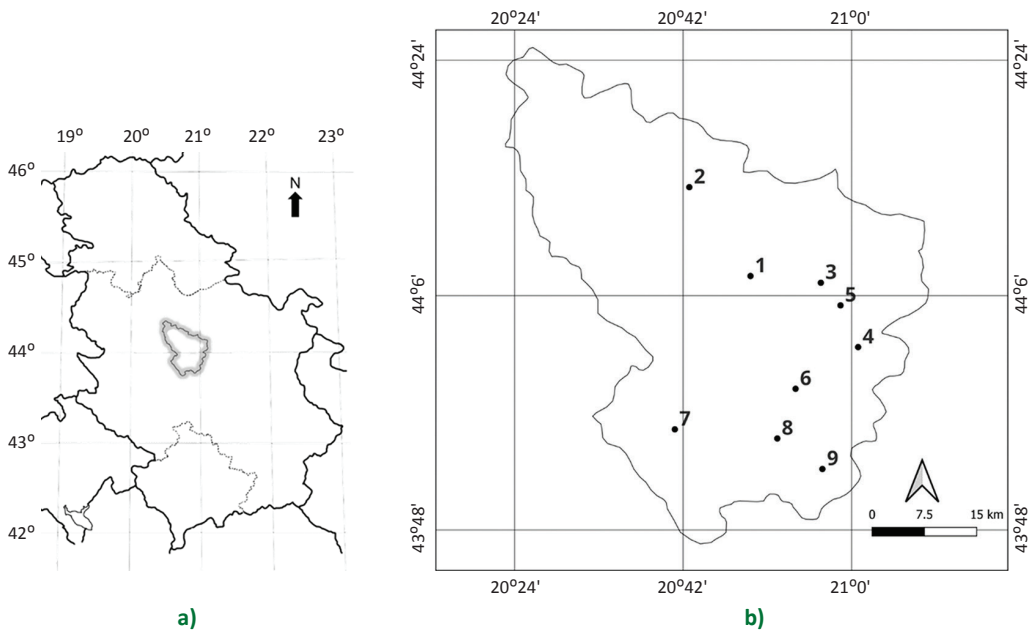
At each of the sampling sites, which were at least 10 km apart, latitude and longitude were recorded and used for obtaining information on soil types, exposure and elevation using QGIS 3.24.0 (QGIS Development Team 2022). The information on soil type was obtained from the imported digitalized maps using the data from Mrvić et al. (2013) and the Republic Geodetic Authority of Serbia (www.geosrbia.rs) which revealed three soil types at sampling sites – cambisol (eutric), vertisol, and lithic leptosol (Figure 2a). The information on the exposure and elevation (Figure 2b, c) was obtained by the SRTM Downloader Plugin in QGIS – Shuttle Radar Topography Mission (NASA Earth Data – <https://urs.earthdata.nasa.gov/>). This revealed six exposure groups – north, northeast, northwest, east, south and west, and nine elevation groups ranging from 288 to 444 m above sea level.

Landmark Configuration

On each leaf, 13 landmarks were recorded (Figure 3), following the methodology suggested by Viscosi (2015) using tpsDig and tpsUtil software (Rohlf 2015). The first three

Table 1. Sample size (N), soil type, exposure, elevation, latitude and longitude of the sampled populations of *Q. cerris*.

Population (Figure 1)	N	Soil type	Exposure	Elevation (m)	Latitude	Longitude
1	15	Vertisol	Northeast	288	44°7'30"	20°49'12"
2	13	Vertisol	West	222	44°14'18"	20°42'40"
3	18	Cambisol	Northeast	202	44°6'59"	20°56'43"
4	13	Cambisol	South	347	44°2'3"	21°0'42"
5	21	Vertisol	North	150	44°5'15"	20°58'49"
6	15	Vertisol	West	232	43°58'51"	20°54'1"
7	18	Cambisol	Northwest	272	43°55'44"	20°41'7"
8	18	Lithic leptosol	Northwest	373	43°55'2"	20°52'4"
9	7	Lithic leptosol	East	444	43°52'41"	20°56'53"

**Figure 1.** (a) the Šumadija region in Serbia, and (b) the enlarged sampling area showing sampling localities. Characteristics of each sampling locality (population) are presented in Table 1.

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.

Statistical Analyses

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 information on size, location, and orientation (Savriama 2018). Procrustes ANOVA was performed to quantify leaf size and shape variation (Klingenberg 2003). In this analysis, centroid size (square root of the sum of the squared distances of all landmarks from their centroid)

was used as a measure of size (Rohlf and Slice 1990). The Canonical Variate Analysis (CVA) was performed to further visualize the differences between groups.

Fluctuating asymmetry (FA) was calculated by digitizing the leaf's left and right side separately and combining the two datasets into one. Procrustes ANOVA was performed on the combined dataset using individual and side as classifiers. When fluctuating asymmetry is present, the interaction of individual \times side is significant in the Procrustes ANOVA. The intensity of fluctuating asymmetry was measured by extracting the MS values from the interaction of individual \times side from the Procrustes ANOVA for each group (Benitez et al. 2020). All statistical analyses were performed in MorphoJ software (Klingenberg 2011).

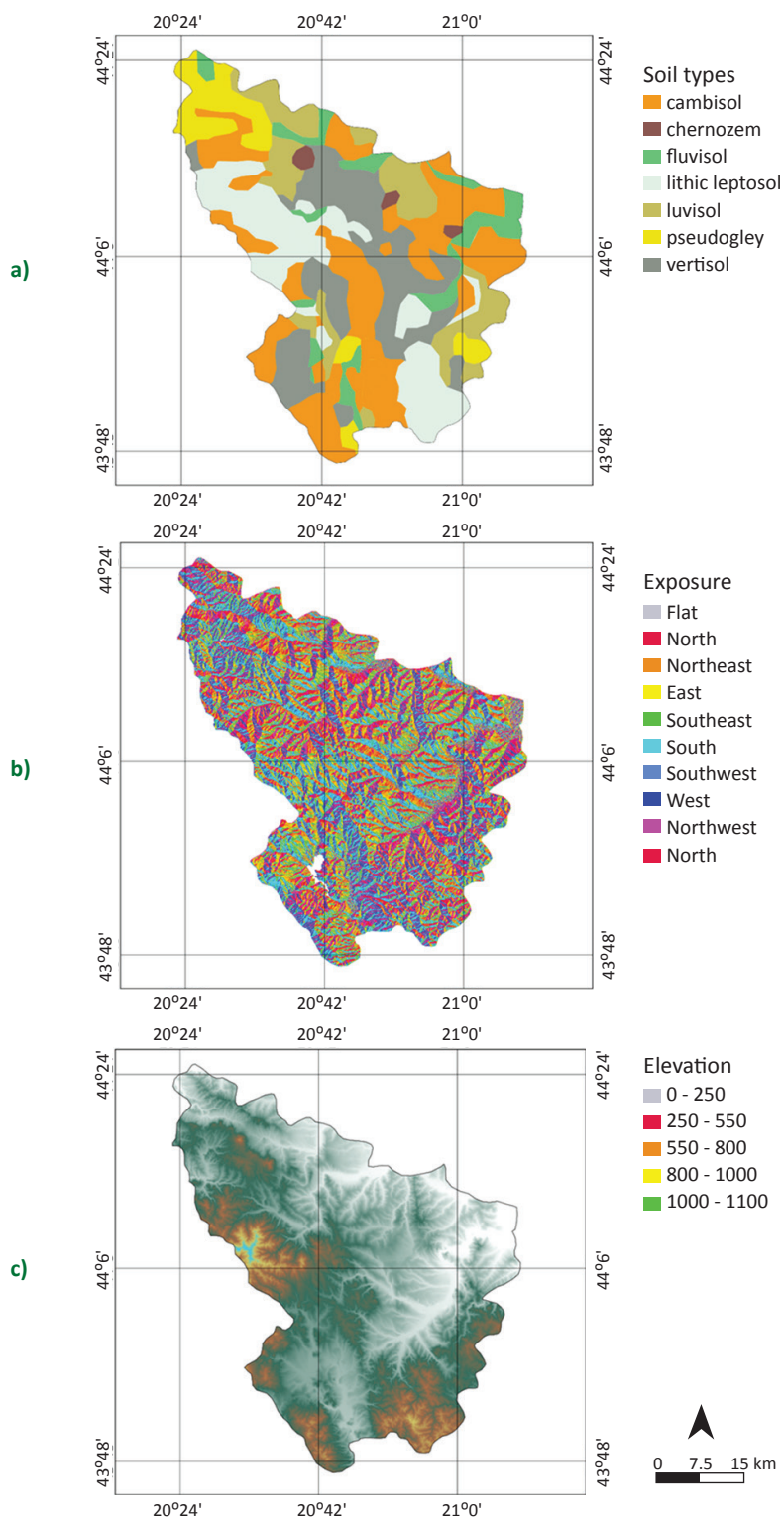


Figure 2. Spatial data of the study region: (a) soil map, (b) exposure map, and (c) elevation map.

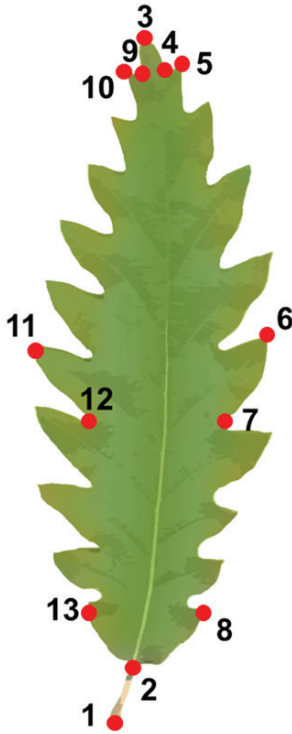


Figure 3. Configuration of *Q. cerris* leaves showing 13 landmarks: 1) beginning of the petiole, 2) junction of the blade and the petiole, 3) apex of the leaf blade, 4) and 9) base of the apical sinuses of the blade tip (right and left side), 5) and 10) tip of the lobe immediately beneath the apex of 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).

RESULTS

Procrustes ANOVA of leaf size and shape indicated that both size and shape show significant differences between individuals growing on different soil types, expositions and elevations (Table 2). Additionally, ANOVA of the centroid size indicated a statistically significant effect of soil type

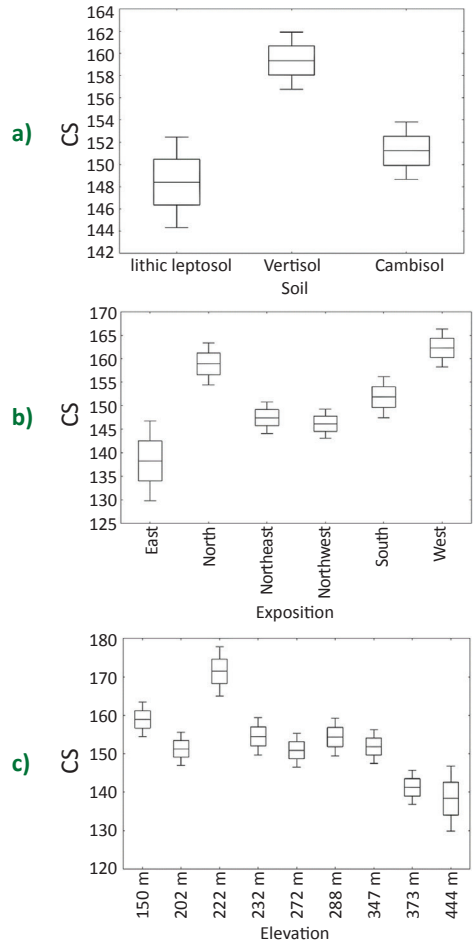


Figure 4. Means, standard errors and 95% confidence intervals of leaf centroid size (CS) of *Q. cerris* individuals growing on: (a) different soil types, (b) expositions, and (c) elevations.

($F=23.11$; $P<0.01$), exposition ($F=12.74$; $P<0.01$) and elevation ($F=14.24$; $P<0.01$) on leaf size (Figure 4). Individuals growing on lithic leptosol, on east exposition, and at higher elevations, had lower values of centroid size compared to individuals growing on cambisol and vertisol, north and west expositions and at lower altitudes, which had higher values of centroid size.

Table 2. Results of the Procrustes ANOVA of *Q. cerris* leaves of individuals growing on different soil types, expositions and elevations.

		SS	MS	df	F	P
Soil type	Shape	0.622	0.014	44	14.40	<0.01
	Size	67803021.26	3390160.63	2	24.99	<0.01
Exposition	Shape	1.42	0.01	110	13.51	<0.01
	Size	9226782.09	1845356.42	5	13.76	<0.01
Elevation	Shape	2.19	0.01	176	13.37	<0.01
	Size	15550775.03	1943846.88	8	14.98	<0.01

Canonical variate analysis indicated considerable overlapping between individuals growing on different soil types (Figure 5). However, some grouping patterns were observed – individuals growing on vertisol had narrower lower part of the leaf blade, higher lobation and shorter petiole compared to individuals growing on cambisol and lithic leptosol.

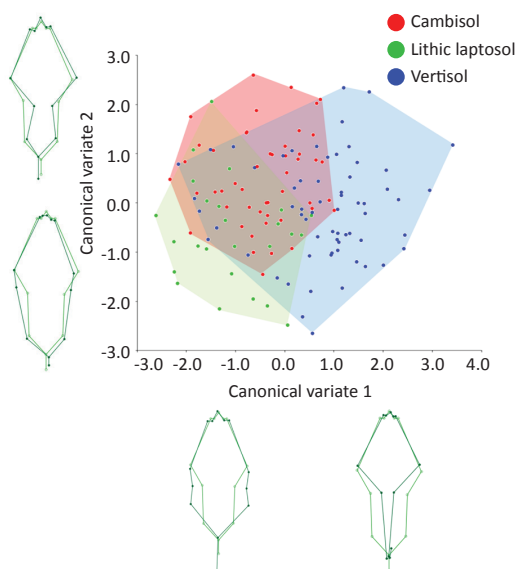


Figure 5. Ordination of *Q. cerris* individuals growing on different soil types within the first two canonical variates obtained by the canonical variate analyses. Shape changes along different soil types are represented by wireframe charts.

Individuals growing on lithic leptosol had rounder leaf blades, with lesser lobation, elongated petiole and a wide leaf blade. Individuals growing on cambisol had a wide upper part of the leaf blade and an elongated petiole. Canonical variate analysis also showed some grouping patterns in regard to exposition (Figure 6). Individuals growing on the east-exposed sites differed from others by having narrow leaf blades and elongated petioles. Individuals growing on west-exposed sites had shorter petioles and a narrow lower part of the leaf blade. Individuals growing on north, northeast and northwest-exposed sites grouped based on a long petiole and a wide leaf blade. Individuals growing on south-exposed sites had a wide upper part of the leaf blade and a short petiole. For different elevations, despite the considerable overlap, some grouping patterns were also observed (Figure 7). In general, individuals from higher elevations had elongated and narrow leaves and short petioles, compared to individuals growing on lower altitudes which had shorter and wider leaf blades and elongated petioles.

Procrustes ANOVA for leaf side of each individual (interaction individual × side) showed significant differences ($F=3.25$; $P<0.01$), indicating the presence of fluctuating asymmetry. The highest levels of fluctuating asymmetry were recorded in individuals growing on lithic leptosol, north-exposed sites and at lowest altitudes (Figure 8).

DISCUSSION

The results of this study showed significant differences in leaf size and shape between *Q. cerris* individuals inhabiting sites with different soil types, exposures, and elevations, suggesting that leaf morphological traits are

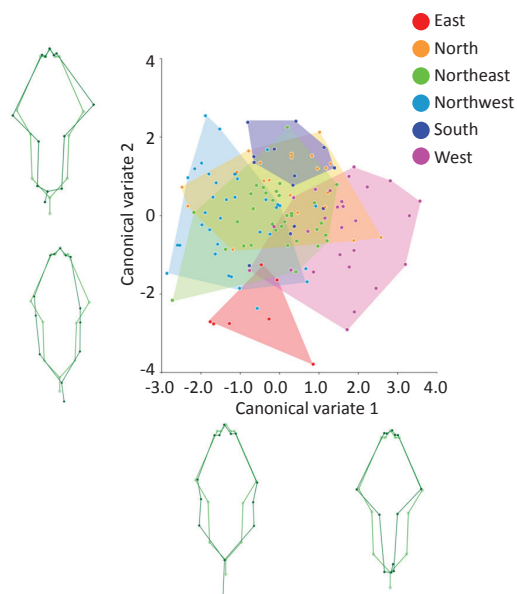


Figure 6. Ordination of *Q. cerris* individuals growing on different expositions within the first two canonical variates obtained by the canonical variate analyses. Shape changes along different exposures are represented by wireframe charts.

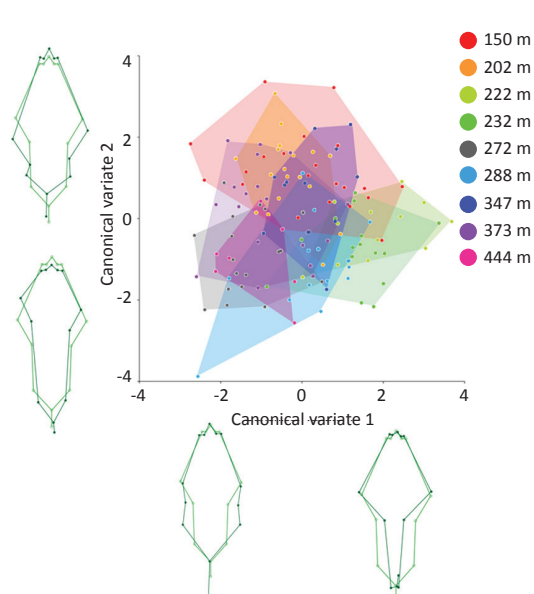


Figure 7. Ordination of *Q. cerris* individuals growing on different elevations within the first two canonical variates obtained by the canonical variate analyses. Shape changes along different elevations are represented by wireframe charts.

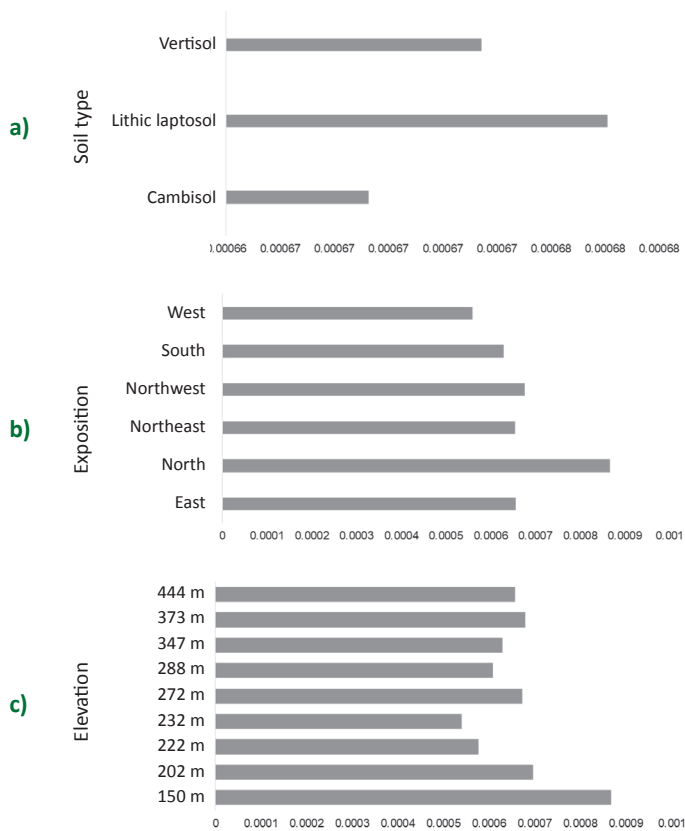


Figure 8. Values of fluctuating asymmetry of *Q. cerris* individuals growing on: (a) different soil types, (b) expositions, and (c) elevations.

affected by habitat differences and exhibit considerable plasticity in response to environmental demands. Leaves of individuals growing on nutrient-rich soils (cambisol and vertisol) were larger compared to the leaves of individuals growing on skeletal soils (lithic leptosol). Individuals from lithic leptosol also differed from others by more round leaf blade, less pronounced lobation, elongated petiole and a wide leaf blade. Additionally, the leaves of individuals from higher elevations were smaller compared to the ones growing at lower elevations, with elongated and narrow leaves and a short petiole. Individuals growing on northern exposures had larger leaf sizes, elongated petioles, and wide leaf blades, contrary to the individuals growing on southern exposures, which had smaller leaves and shorter petioles.

In the Šumadija region lithic leptosols are relatively variable in physical and chemical properties and are usually poor in nutrients, and have poor water regime (Veljović 1967, Pavlović et al. 2017, Jakšić et al. 2021), while cambisol and vertisol are moderately rich in nutrients (N, P, K) and have more favourable water regimes. This high soil diversity in the Šumadija region in Serbia conditioned the existence of different productivity levels – productivity of deep soils is considered to be higher compared to shallow skeletal soils (Ličina et al. 2011, Jovanović et al. 2022b). Thus, cambisol

and vertisol (nutrient-rich soils with more favourable water regimes) present at lower elevations provide suitable conditions for *Q. cerris* to develop larger leaves. Moreover, leaves of shaded plants tend to be slightly larger than those of full-sun plants (Stanton et al. 2010), as larger leaf areas receive light energy for photosynthesis at sites where light levels are low, explaining larger leaves at the lower elevations of the study area. When observing the connection between leaf traits and expositions, north-exposed sites have a higher content of soil nutrients and are moist, which influences leaf size increase (Moeslund et al. 2013), as suggested by the larger leaves, with a broad leaf blade and an elongated petiole at the northern expositions of the study region.

Environmental factors, including air temperature, radiation, and soil nutrients vary with elevation – temperature decreases, and precipitation and radiation increase with higher altitudes (Guo et al. 2018). Soil nutrients also change at different elevations – soil organic carbon (C) concentration may increase, while the availability of soil nutrients, such as N and P, may decrease with increasing elevation (He et al. 2016). Smaller leaf sizes are favoured at higher altitudes, characterized by higher insolation and higher light-capturing surface built by the plant per unit investment of dry mass, optimized to maintain

a positive carbon balance and influence the fitness of the whole plant (Pan et al., 2013). Additionally, larger leaves in northern exposures shed heat more slowly and are heated above air temperature more compared to smaller leaves. Transpiration is also effective in shedding heat – when there is less total foliage per unit ground area, more leaves are exposed to direct radiation (McDonald et al. 2003). In general, smaller leaves are advantageous at high intensities of solar radiation, while larger leaves which have less efficient energy exchange capacity are advantageous in habitats with lower irradiance (Wang et al. 2019). The differences between leaf traits along different habitats in this study indicate that in *Q. cerris* morphology can reflect environmental demands, although it must be noted that other factors, such as genes and development, also play important roles in the observed leaf variability.

Fluctuating asymmetry values were the highest in individuals growing on nutrient-poor soils, in both high and low altitudes, and in north-exposed sites. The differences in fluctuating asymmetry between traits are explained by varying levels of developmental stability, which can be related to trait functionality, selection mode, and stress associated with the development processes (Aparicio and Bonal 2002). Fluctuating asymmetry is a reliable measure of plant stress at local scales and it can be used as a biological tool for monitoring environmental quality (Cornelissen and Stiling 2010). This small and random deviation from bilateral symmetry (Cornelissen et al. 2003), used as an indicator of developmental instability, is more pronounced if the plant's adaptive mechanism fails to buffer stress (Graham et al. 2010). In this study, higher values of fluctuating asymmetry recorded in nutrient-deficient, shallow soils (lithic leptosol), suggest that the ability of *Q. cerris* to buffer environmental stress is reduced, causing deviations between the left and the right side of the leaf. In the Šumadija region, lithic leptosol is present at higher altitudes where stress levels are also higher compared to the lower elevations. High values of fluctuating asymmetry were also recorded in north-exposed sites, which are characterized by decreased insolation, lower temperature, and higher precipitation. Thus, the connection between high values of fluctuating asymmetry and more severe environmental conditions is not as straightforward, suggesting that other phenomena, such as phenotypic plasticity, can be more sensitive to stress than fluctuating asymmetry (Graham et al. 2010). A plastic response regarding leaf morphology to varying environments has been found to be higher in many *Quercus* species, enabling relatively quick adaptation to different environmental conditions (Blue and Jensen 1988, Ashton and Berlyn 1994, Kusi 2013). Plastic responses enable *Quercus* species to cope with adverse environmental conditions by modifying leaf morphological traits, such as water transport, heat reduction, prevention of photochemical damage, and the preservation of minimum photosynthetic rate (Dickson and Tomlinson 1996).

Although the results of this study showed differences in leaf size and shape between *Q. cerris* individuals growing at different environmental gradients, leaf trait relationship with environmental conditions showed considerable over-

lapping, suggesting that within-site variation may also be the source of the observed variability (Gong and Gao 2019). Plant and climate relationships at the intraspecific level are influenced by ecotypic variation of plant traits and their plasticity (Royer et al. 2008), and these relationships can be used as indicators of the levels of environmental stress present in a certain area. Thus, understanding how plants adapt to different habitat conditions is important in conservation strategies, especially when dealing with species that have large areas of distribution and a broad ecological niche (Šijačić-Nikolić et al. 2021). In such cases, leaf morphology can be used as a reliable indicator of habitat quality (Xu et al. 2021).

CONCLUSIONS

This study showed that leaf morphology is related to environmental conditions of *Q. cerris* from the Šumadija region in Serbia, as geometric morphometrics analysis revealed differentiation of *Q. cerris* individuals growing on different soil types, expositions and elevations. The observed differentiation was determined by both leaf size and shape. However, the results indicated the environmental impact on the variation patterns of the leaf in *Q. cerris* on a relatively small spatial scale. Future studies should include a larger sample of different oak species from diverse areas across a broader area of distribution to better understand how habitat influences leaf morphology. Along with the environmental influences, the variability of leaf morphology can also be attributed to genetic factors, making molecular analyses another priority in future research.

Author Contributions

MJ and JM conceived and designed the research, MJ carried out the field measurements, MJ and FG processed the data and performed the statistical analysis, MŠN, MN and IKJ supervised the research and helped to draft the manuscript, MJ and FG wrote the manuscript, and all authors provided comments.

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Conflicts of Interest

The authors declare no conflict of interest.

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