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The effects of leaf litter chemistry and anatomical traits on the litter decomposition rate of *Quercus frainetto* Ten. and *Quercus cerris* L. *in situ*

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Abstract: This paper presents the results of a one-year decomposition experiment on *Quercus frainetto* Ten. and *Quercus cerris* L. leaf litter in natural conditions. The decomposition rate constant was 0.831 yr^{-1} (*Q. frainetto*) and 0.458 yr^{-1} (*Q. cerris*). For the initial chemical composition of the oaks' litter, differences were not found in concentrations of lignin and fats, waxes and oil fractions, but were found for water-soluble matter, hemicellulose and cellulose. Later decomposition stages indicated that lignin and fats, waxes and oil fractions influenced differences in both oaks' litter decay rates. Anatomical analysis revealed differences between the oaks in leaf mesophyll and epidermis but not in the entire leaf and lower epidermis. Results after 12 months of the experiment revealed that 48.04% of the entire leaf, 53.30% of mesophyll, 32.93% of lignified upper and 47.67% of lower epidermis in *Q. frainetto*, and 28.70% of the entire leaf, 31.60% of mesophyll, 25.17% of lignified upper and 20.93% of lower epidermis in *Q. cerris* were decomposed. Reduction in leaf thickness mainly was caused by the reduction of mesophyll parenchyma, composed of easily degradable plant materials. Leaf tissues with the most recalcitrant plant materials were lignified upper epidermis, covered by a thick cuticle composed of fats and waxes, and xylem within the leaf veins.

Keywords: oak forest; chemical composition; ecological interactions; leaf anatomy

INTRODUCTION

In terms of importance, decomposition can be compared with primary production as a fundamental ecosystem process. Theoretically, this process reduces organic matter to its inorganic components used by plants in the autotrophic production of organic matter. Factors that regulate the decomposition process in terrestrial ecosystems have been identified as (i) climate [1-3], (ii) soil components [4-6], (iii) the amount of litter input and the proportion and distribution of various plant parts [7], (iv) leaf litter chemistry [8,9], and (v) the decomposer community [1,10,11]. Temperature and water availability are the major controlling factors of litter decomposition rates on the global and regional scale [12], but the relative importance of litter quality compared to climate can change at lower spatial scales [13]. For instance, the decomposition rates vary across boreal forests more according to substrate quality rather than to climatic conditions [14]. Similarly, the prevailing effect of litter quality over climate has been reported for wet tropical forests [15,16]. In contrast, in Mediterranean ecosystems the dynamics of climatic conditions are critical for the decomposition of litter and organic matter in soil due to summer drought that limits microbial growth and activity [17].

The chemical composition of plant litter is commonly considered to indicate its quality as a resource for decomposer organisms. Therefore, it has been shown to be a major determinant of litter decomposition rates within and across terrestrial ecosystems [18,19]. Plant litter is an organic material composed of many different chemical compounds, mainly polysaccharides and lignin, as well as aliphatic biopolymers and tannins, with a wide range of turnover times [20,21]. Leaf litter contains 10-22% cellulose, 5-8% lignin, 10-19% hemicellulose, 2-15% raw protein

and 8-14% ash [22]. Early decomposition rates are strongly related to climate and the concentrations of water-soluble nutrients and carbohydrates in litter, while later decomposition rates are influenced more by lignin concentrations in litter [14,23]. Mass loss per unit time is often, though not always, greater in litters with higher nutrient availability, but reduced with increased lignin or polyphenol concentrations [24]. Leaf litter mainly consists of labile (e.g. cellulose and hemicellulose) and recalcitrant (e.g. lignin) compounds that decompose at progressively slower rates [20]. Cellulose is the main carbohydrate constituent of plant cell walls and is decomposed by a variety of microorganisms. Holocellulose, the sum of cellulose and hemicelluloses, comprises all insoluble polymer carbohydrates. Lignin, an aromatic compound, coats cell walls and combines chemically with cellulose to form lignocellulose. Lignocellulose is highly recalcitrant and only specialized organisms decompose it. Hence, its degradation is a limiting factor in decomposition processes [25]. Therefore, the above parameters can be useful indicators since the decomposition of these fractions may not occur independently of each other [24,26]. An inverse relationship was found between lignin concentration and the rate of decomposition [8]. Low lignin concentrations and high nutrient concentrations, such as N and P in litter, favor higher initial rates of litter decay [24,27]. Meentemeyer [28] found a clear interaction between the effect of lignin and climate. The C/N and lignin/N ratios in litter are widely used as litter quality variables [8,9,29]. The potential effects of global environmental change on the quality and turnover of litter and organic matter in soil have stimulated interest in models that simulate organic matter transformation in terrestrial ecosystems [16,30].

It has been established that the physicochemical features of leaf litter cause important interspecific variability in decomposition rates [1,31]. Comparative approaches to the links between decomposition and traits of living leaves proved the above observations [2,32,33]. These correlative studies were part of efforts aimed at obtaining a better understanding of the effects of individual plant species and functional types on ecosystem processes. For instance, Cornelissen et al. [32] found that traits of fresh leaves that provide structural or chemical protection remain operational in leaf litter and control interspecific variations in the decomposition rate. They also revealed the correlation between specific leaf area (leaf area/dry mass), litter nutrient concentrations and decomposition rates, with some exceptions owing to differences in leaf traits even within a single individual of the same species (e.g. sun and shade leaves).

Two different types of plant tissues are susceptible to decomposition: parenchymatic tissue (mesophyll, the bark of young twigs and fine roots) composed of cellulose walls, protoplast rich in protein and vacuole rich in dissolved sugars, organic acids, proteins and salts, as well as woody tissue (xylem as the woody part and sclerenchyma as the supporting tissue in stems, leaf epidermis, leaf ribs and barks) composed of cellulose, hemicellulose and lignin [20]. Cell walls consist of a variety of different compounds, mainly structural polysaccharides (cellulose and hemicelluloses with the ratio of cellulose:hemicelluloses commonly varying between 2:1 to 1:1), pectins, lignin, proteins and other minor components [34]. Hemicelluloses are thus the second most abundant polysaccharide group in plants and, depending on the tissue, generally account for 10-30% of a tissue's dry biomass [35]. The chemical composition and anatomical structure of plants are heavily influenced by ecological factors, as are all functions of an ecosystem, including decomposition [1].

The overall anatomical structure of the leaves of *Quercus* L. species is characterized by a lignified epidermis [36], mesophyll tissue composed of parenchymatic cells, and leaf veins composed of woody tissue (xylem) and supporting tissue (sclerenchyma). There are, however, differences in the anatomy of *Q. frainetto* and *Q. cerris* leaves [37,38].

The main objective of this paper was to analyze the influence of the chemical composition of leaf litter and anatomical leaf structure on the decomposition rate in natural conditions. We performed a one-year experiment and analyzed the leaf litter of *Quercus frainetto* Ten. and *Quercus cerris* L., which are predominant in oak forests of Serbia. The research focused on mass loss, the dynamics of the chemical composition of leaf litter, and changes in tissue thickness during the process of decomposition. Using light microscopy analysis during decomposition, we aimed to present the decomposition process visually and to locate the tissues in both oak species that took longest to decompose.

MATERIALS AND METHODS

Experimental site

The research was performed on Mt. Maljen in western Serbia (44° 10' N and 20° 05'E), 120 km southwest of Belgrade, in the central part of the Balkan Peninsula. The study area (a mixed forest of *Quercus frainetto* Ten. and *Q. cerris* L.) lies within the outer area of the Kosjerić locality at an altitude of 450 m, southwest facing, with a terrain-slope of 30°. The stand is 80 years old. The floor of high trees (14-18 m high) consists of *Q. frainetto* and *Q. cerris*. The floor of lower trees (3-6 m high) includes *Sorbus torminalis* (L.) Crantz, *Acer campestre* L., *Carpinus betulus* L. and *Fraxinus ornus* L. The floor of shrubs is formed by *Cornus mas* L. and *Crataegus monogyna* Jacq. The following species dominate in the herbaceous floor: *Poa nemoralis* L., *Brachypodium silvaticum* (Huds.) P.B., *Dactylis glomerata* L., *Lychnis coronaria* (L.) Desr., *Sedum maximum* Hoffm., *Hieracium sylvaticum* (L.) Grufb. and *Galium silvaticum* L. The soil is brown acid (Dystric cambisol, F.A.O., Typic dystrochrept, USA, [39], with a mull lake moder humus type [40]. The mean monthly temperatures ranged from -2.2°C to 22.3°C, and the mean annual air temperature was 11.12°C. Annual precipitation was 860.8 mm.

Decomposition study

Leaf litter decomposition rates for *Q. frainetto* and *Q. cerris* were determined using the litter bag technique. Litter bags measured 25×25 cm and were made of 2-mm plastic mesh. This mesh size allows for the movement of most soil fauna into the litter bag, facilitating decomposition and preventing loss of leaves. Ten grams of air-dried litter were placed in each bag. Exact weights were recorded and the bags were tagged with an identifying code and placed on the soil in autumn (October), with three replicates, covering 7 time periods (initial state and after 2, 4, 6, 8, 10 and 12 months of decomposition). A total of 36 leaf litter bags were placed. The leaf litter decomposition experiment was carried out over a 12-month period, until the following October. After completion of each experimental stage, the litter bags were promptly taken into the laboratory, carefully cleaned of soil and dried at 80°C for 24 h, and the leaf litter loss was measured. Once measured, the litter from each bag was used for chemical analyses.

Chemical analysis

For each time period the biochemical composition of the leaf litter was determined; fat, waxes and oils (gravimetrically after hydrolysis in ethyl ether for 24 h), water-soluble matter (gravimetrically after hydrolysis in H_2O for 2 h), hemicellulose (after hydrolysis in 2% HCl for 5 h, calculated by means of a standard glucose solution and the coefficient 0.9, which includes water loss following the polymerization of monosaccharides), cellulose (after hydrolysis in 72% H_2SO_4 for a 5-h period, calculated in the same way as hemicellulose), and lignin (as the lignin residue, gravimetrically, following the above hydrolyses) [41]. C and N (Kjeldahl method) were determined using the Ponomareva and Nikolaeva modification of the Anstat method [42]. The C/N and lignin/N ratios were calculated.

Anatomical analysis

Light microscopy analysis of fresh leaves and leaf litter included the thickness of the whole leaf, the mesophyll layer, the upper epidermal layer and the lower epidermis layer. For anatomical analysis, leaf samples were collected from litter bags, at three time periods (initially, after 6 months and after 12 months of the experiment), fixed in 70% ethanol and dehydrated with an ascending alcohol series and embedded in paraffin. Leaf cross sections (21 replicates for each time period, 7 replicates from each litter bag) from a central portion of the leaf, 10-20 μm thick, were cut on a slide microtome and stained in safranine (which stains lignified tissue elements, fats and waxes), and Alcian blue (which stains cellulosed tissue elements), [43]. Leaf cross-sections were measured and photographed with an optical microscope equipped with a calibrated micrometric grid.

Exponential decomposition model

Oak leaf litter decomposition was described using a single exponential Olson decay model [44], as follows:

$$\frac{Mt}{Mo} = e^{-kt}$$

where Mo and Mt are the resource weights at the start of the experiment and after time t respectively, and k is the decomposition rate constant. The values of r^2 express the variance explained by the exponential model.

Table 1. Initial chemical composition of oak leaf litter.

Leaf litter	fats, waxes and oils (mg g^{-1})	water-soluble matter (mg g^{-1})	hemi-cellulose (mg g^{-1})	Cellulose (mg g^{-1})	Lignin (mg g^{-1})	Lignin/N	C (mg g^{-1})	N (mg g^{-1})	C/N
Q. frainetto	102.60 (±6.88)	184.00 (±2.49)	120.20 (±3.50)	279.30 (±15.09)	176.43 (±8.30)	12.25 (±0.23)	480.00 (±0.35)	14.40 (±0.05)	33.33 (±1.02)
Q. cerris	107.20 (±1.04)	302.00 (±15.20)	89.50 (±7.75)	243.30 (±8.30)	191.20 (±4.88)	20.55 (±1.19)	390.20 (±0.51)	9.30 (±0.09)	41.95 (±3.95)
P	NS	***	**	*	NS	***	***	***	*

ANOVA, N=3, mean values are presented with standard deviations in parentheses. Levels of significance: *P<0.05, **P<0.01, ***P<0.001, NS = not significant.

Table 2. Decomposition rate of oak leaf litter during the experiment.

Leaf litter	After 2 months (%)	After 4 months (%)	After 6 months (%)	After 8 months (%)	After 10 months (%)	After 12 months (%)
Q. frainetto	14.98 (±1.27)	25.62 (±2.44)	35.64 (±2.84)	41.12 (±3.08)	45.16 (±2.57)	48.33 (±2.10)
Q. cerris	9.12 (±1.78)	15.36 (±1.87)	21.33 (±3.66)	25.16 (±1.96)	27.27 (±2.01)	28.52 (±1.53)
P	**	**	**	**	***	***

ANOVA, N=3, mean values are presented with standard deviations in parentheses. Levels of significance: **P<0.01, ***P<0.001

Statistical analysis

One-way analyses of variance (ANOVA) were performed to test the differences between the oaks during the experiment in terms of initial chemical composition, leaf tissue thickness, leaf litter loss, and changes in the chemical composition and leaf tissue thickness (subsequent tests of normality by the Shapiro-Wilk *W* test and Levene's test of homogeneity of variances showed non-significant values for all the reported ANOVA breakdowns).

RESULTS

Leaf litter quality

When comparing the initial chemical composition of oak litter, differences in water-soluble matter (P<0.001), hemicellulose (P<0.01), cellulose (P<0.05), the lignin/N ratio (P<0.001), C (P<0.001), N (P<0.001), and the C/N ratio (P<0.05) were observed, while no differences were found in the concentrations of fats, waxes and oils and lignin (Table 1). At the beginning of the experiment, decomposition indicators (C/N and lignin/N) had a more favorable ratio in the leaf litter of *Q. frainetto* than in that of *Q. cerris* (33.33:41.95; 12.25:20.55).

Leaf litter decomposition

After one year, the litter of *Q. frainetto* decomposed by 48.33%, and *Q. cerris* by 28.52% (Table 2). The decay process was well-approximated by the exponential decay model given in Eq. (1). As given in Table 3, the values of the decomposition rate constant (*k*) were 0.831 yr^{-1} for *Q. frainetto* and 0.458 yr^{-1} for *Q. cerris*. The *k*-value for *Q. cerris* was significantly lower than for *Q. frainetto* (P<0.001).

Table 3. Annual decomposition rate constant (*k*) and the 95% breakdown period of oaks.

Leaf litter	$k \pm$ S.E. (yr^{-1})	n	r^2	95% breakdown period (year)
Q. frainetto	0.831 ± 0.03***	19	0.93	3.61
Q. cerris	0.458 ± 0.02	19	0.82	6.55

ANOVA, ***P<0.001

Chemical compounds

The decomposition gradient of the chemical components of the examined types of leaf litter (*Q. frainetto*: *Q. cerris*) after 12 months of decomposition was: water-soluble matter (80.75%:74.55%) > hemicellulose (90.80%:53.11%) > cellulose (54.81%:30.92%) > fats, waxes and oils (46.19%:11.88%) > lignin (22.05%:8.05%) (Table 5). *Q. frainetto* hemicellulose (90.80%) deviated from this gradient as it decomposed faster than water-

Table 4. Changes in concentrations of chemical compounds[a] (mg g^{-1}) and changes in the weight (g) of leaf litter bags[b] during decomposition.

Time	Leaf litter	Fats, waxes and oils (mg g^{-1})[a]	Water-soluble matter (mg g^{-1})[a]	Hemicellulose (mg g^{-1})[a]	Cellulose (mg g^{-1})[a]	Lignin (mg g^{-1})[a]	Weight leaf litter (g)[b]
Initial	*Q. frainetto*	102.60 (±6.88)	184.00 (±2.49)	120.20 (±3.50)	279.30 (±15.09)	176.43 (±8.30)	10.00
	Q. cerris	107.20 (±1.04)	302.00 (±15.20)	89.50 (±7.75)	243.30 (±8.30)	191.20 (±4.88)	10.00
After 2 Months	*Q. frainetto*	116.20 (±3.21)	122.30 (±4.78)	68.20 (±6.55)	266.80 (±7.11)	204.00 (±3.10)	8.50 (±0.13)
	Q. cerris	116.76 (±1.19)	189.20 (±6.48)	92.10 (±5.36)	223.60 (±4.42)	209.60 (±5.45)	9.08 (±0.18)
After 4 Months	*Q. frainetto*	115.60 (±3.48)	113.00 (±6.61)	49.20 (±7.20)	261.00 (±4.95)	229.20 (±7.13)	7.43 (±0.25)
	Q. cerris	124.50 (±0.96)	156.50 (3.40)	94.20 (0.79)	221.80 (7.40)	224.00 (5.72)	8.46 (±0.18)
After 6 Months	*Q. frainetto*	113.90 (±3.03)	105.20 (±2.10)	28.40 (±2.55)	290.30 (±5.72)	261.50 (±6.33)	6.44 (±0.51)
	Q. cerris	130.00 (±0.88)	124.80 (±8.90)	98.80 (±2.27)	225.00 (±7.18)	239.10 (±8.00)	7.87 (±0.36)
After 8 Months	*Q. frainetto*	110.00 (±4.32)	81.10 (±3.50)	25.10 (±1.68)	271.60 (±7.52)	265.70 (±10.76)	5.89 (±0.44)
	Q. cerris	135.60 (±4.88)	112.60 (±3.10)	81.60 (±1.40)	232.70 (±7.30)	241.20 (±2.00)	7.48 (±0.58)
After 10 Months	*Q. frainetto*	108.20 (±5.04)	75.20 (±2.80)	23.60 (±3.12)	257.80 (±4.99)	268.40 (±4.47)	5.48 (±0.31)
	Q. cerris	134.50 (±10.96)	110.00 (±2.89)	64.20 (±5.03)	236.10 (±4.82)	243.70 (±7.47)	7.24 (±0.54)
After 12 Months	*Q frainetto*	106.70 (±6.79)	68.50 (±2.26)	21.40 (±1.83)	243.40 (±6.94)	270.00 (±2.76)	5.17 (±0.22)
	Q. cerris	132.46 (±3.80)	107.50 (±3.37)	58.50 (±2.12)	235.26 (±4.15)	245.60 (±3.77)	7.13 (±0.38)

N=3, mean values are presented with standard deviation in parentheses

soluble matter (80.75%), (Table 5, see data in Table 4). The fraction of fats, waxes and oils, the fraction of lignin in *Q. cerris*, and the fraction of lignin in *Q. frainetto* litter showed a high resistance to decomposition. All chemical components (except lignin in *Q. frainetto* and the fractions of fats, waxes and oils and lignin in *Q. cerris*) decomposed up to or above the level of the overall organic source (leaf litter) by the end of the experiment (Table 2 and 5).

Leaf tissues

Q. frainetto has thinner leaves, a thinner mesophyll, and a thinner cuticle (up to 4 μm) (Fig. 1A) compared to *Q. cerris*, which has thicker leaves, a thicker and more compact mesophyll, a thicker cuticle (up to 6 μm) and trichomes on the abaxial surface (Fig. 1B). At the beginning of the experiment, chlorophyll loss was noted in leaves since it had already decomposed during leaf senescence, as was lower tissue turgidity and the slow deformation of the mesophyll due to water loss. Differences between the samples of *Q. frainetto* and *Q. cerris* in terms of thickness of the entire leaf and lower epidermis were not noted. They were, however, noted in the thickness of the mesophyll ($P<0.01$) and upper epidermis ($P<0.05$) (Fig. 2).

After 6 months, differences between the oaks were not noted in the thickness of the upper and lower epidermis, while differences were noted in entire leaf thickness ($P<0.001$) and mesophyll thickness ($P<0.001$) (Fig. 2). If we assume that the thickness of the entire leaf, the mesophyll, and the upper and lower epidermis at the beginning of the experiment is 100% of the value, it transpires that 33.09% of the whole leaf, 38.31% of the mesophyll parenchyma, 22.75% of the lignified upper and 15.90% of the lower epidermis of *Q. frainetto* had decomposed. Cuticular waxes and lignified leaf vein

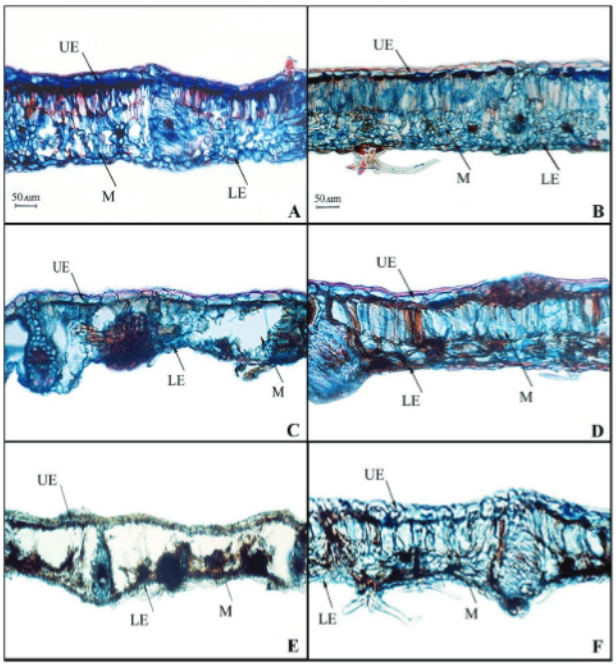

Fig. 1. Light microscopy of *Q. frainetto* leaf cross-sections (magnification: 400 ×), initial (**A**), after 6 months (**C**), after 12 months (**E**) of the experiment; *Q. cerris* leaf cross-sections (magnification: 400 ×) initial (**B**), after 6 months (**D**), after 12 months (**F**) of the experiment: UE – upper epidermis, LE – lower epidermis, M – mesophyll.

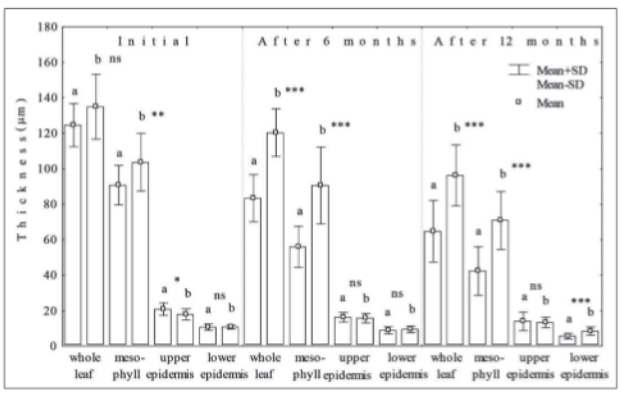

Fig. 2. Changes in leaf tissue thickness (μm) of *Q. frainetto* (a); *Q. cerris* (b) during the decomposition experiment, ANOVA, n=21, Levels of significance: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, NS – not significant.

tissues (xylem) were merely beginning to decompose (Fig. 1C). At the same time, only 10.83% of the whole leaf, 8.80% of the mesophyll parenchyma, 11.88% of the lignified upper and 12.23% of the lower epidermis of *Q. cerris* had decomposed. The continuous cuticle layer remained unchanged, as did the trichomes, xylem and the mesophyll to some extent (Fig. 1D).

At the end of the experimental period, differences between the samples of *Q. frainetto* and *Q. cerris* were not detected in terms of upper epidermis thickness, while there were differences in entire leaf thickness ($P<0.001$), mesophyll ($P<0.001$) and lower epidermis thickness ($P<0.001$) (Fig. 2). After 12 months, 48.04% of the whole leaf, 53.30% of the mesophyll, 32.93% of the lignified upper and 47.67% of the lower epidermis of *Q. frainetto* had decomposed. The upper surface cuticle of the leaf, the external lignified epidermal cell walls and leaf veins remained undecomposed. On the abaxial surface, only the outer lignified epidermal cell walls and trichomes remained undecomposed (Fig. 1E). At the end of the experimental period, 28.70% of the entire leaf, 31.60% of the mesophyll, 25.17% of the lignified upper and 20.93% of the lower epidermis of *Q. cerris* had decomposed, whereas the cuticle layer, trichomes and xylem remained almost unchanged (Fig. 1F).

At consecutive periods of 6 and 12 months, the leaves of *Q. frainetto* were more readily thinned than the leaves of *Q. cerris* (Fig. 2). Anatomical analysis also showed differences in the decomposition rates of the two oaks examined. Great similarity was found between the loss-percentages in the thickness of the entire leaves of *Q. frainetto* (48.04%) and *Q. cerris* (28.70%), and the loss percentages of leaf litter (*Q. frainetto* 48.33% and *Q. cerris* 28.52%) at the end of the experiment.

DISCUSSION

Theoretically, litter quality controls the potential rate of decomposition only as long as climatic and edaphic conditions remain constant [1]. In our study, leaf litter decomposition of *Q. frainetto* and *Q. cerris* was analyzed at a site where these species are predominant and where climatic and edaphic conditions for decomposition were identical for both species during the experiment. The results obtained point to a clear difference between the two species in terms of decomposition rates, which is induced by differences in chemical composition and anatomical leaf traits. Similarly, Pérez-Harguindeguy et al. [19] found large interspecific variations in leaf traits in the flora of central Argentina, which is strongly linked to variations in litter decomposition rates. The C:N ratio is an indicator that is usually taken into account when first observing and analyzing the decomposition process. It

Table 5. Percentage of loss of chemical compounds during the decomposition experiment.

Time	Species	Fats, waxes and oils (%)	Water-soluble matter (%)	Hemicellulose (%)	Cellulose (%)	Lignin (%)
After 2 Months	*Q. frainetto*	**3.72** (±0.57)	**43.51** (±1.44)	**51.83** (±3.28)	**18.72** (±2.30)	**3.18** (±0.66)
	Q. cerris	1.09 (±0.06)	43.07 (±1.39)	6.40 (±2.95)	16.52 (±1.36)	0.39 (±0.10)
	P	**	NS	***	NS	**
After 4 Months	*Q. frainetto*	16.25 (±3.32)	54.38 (±2.25)	69.65 (±3.61)	30.47 (±2.56)	4.92 (±2.04)
	Q. cerris	1.74 (±0.69)	56.11 (±1.25)	10.56 (±6.87)	22.87 (±0.62)	0.82 (±0.19)
	P	**	NS	***	**	*
After 6 Months	*Q. frainetto*	28.36 (±3.23)	63.17 (±0.55)	84.79 (±0.95)	32.97 (±2.43)	5.97 (±1.83)
	Q. cerris	4.54 (±0.29)	67.49 (±0.70)	18.10 (±5.10)	27.15 (±3.48)	1.27 (±0.43)
	P	***	*	***	NS	*
After 8 Months	*Q. frainetto*	36.77 (±1.98)	74.04 (±0.87)	87.70 (±0.48)	42.66 (±1.51)	12.63 (±3.05)
	Q. cerris	5.39 (±2.50)	72.09 (±0.63)	31.60 (±4.72)	28.46 (±0.20)	5.54 (±1.69)
	P	***	*	***	***	*
After 10 Months	*Q. frainetto*	42.15 (±.53)	77.60 (±0.52)	89.26 (±1.12)	49.35 (±1.88)	17.88 (±0.54)
	Q. cerris	9.19 (±6.79)	73.60 (±0.82)	48.02 (±1.40)	29.70 (±0.90)	7.65 (±1.27)
	P	**	**	***	***	***
After 12 Months	*Q frainetto*	46.19 (±2.88)	80.75 (±0.39)	90.80 (±0.52)	54.81 (±3.67)	22.05 (±0.90)
	Q. cerris	11.88 (±2.68)	74.55 (±2.01)	53.11 (±5.16)	30.92 (±1.56)	8.05 (±3.70)
	P	***	**	***	***	**

ANOVA, N=3 mean values are presented with standard deviations in parentheses. Levels of significance: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, NS=not significant

is commonly accepted that deposition will take place more slowly if this ratio is wider than 30 in the initial organic source [1,8,45]. In this study, the C:N ratio in the initial organic matter showed that deposition of the examined oaks' organic matter will occur at a slower pace, with the organic matter of *Quercus frainetto* decomposing more quickly than that of *Quercus cerris* (the C:N ratio of *Q. frainetto* to C:N for *Q. cerris* was 33.33:41.95). This was also confirmed by the lignin:N ratio, which in the initial organic matter of *Quercus frainetto* was 12.2 compared to 20.5 for *Quercus cerris.*

The functionally or morphologically defined compartmentalization of plant litter defines a metabolic (a labile or rapidly decomposing fraction) pool versus a structural (a resistant or slowly decomposing fraction) pool of plant litter. This concept recognizes that both cell structures decompose somewhat independently and that the physical structure of plant material at the microscale is an important attribute to its quality. Namely, the leaf litter quality that is inherited from living leaves has been repeatedly emphasized as one of the most important factors controlling the decomposition process [1,8,46,47]. The litter quality radically changes in the course of decomposition as a consequence of the different degradation rates of the various chemical constituents [14,48]. In both examined litters, a rapid decrease in water-soluble matter was to be expected. Storage materials, such as intracellular compounds in plant tissues, are a rapidly decomposing fraction and provide usable carbon and energy sources for microorganisms in the initial stages of decomposition [1,20]. However, the hemicellulose fraction in *Q. frainetto* decomposed to a greater extent than the water-soluble matter, which is the opposite case to *Q. cerris.* One possible explanation for this may be that

higher initial concentrations of the water-soluble fraction in *Q. cerris* leaf litter were readily available to microorganisms and that they decomposed before the relative decrease in hemicellulose occurred. At the same time, hemicellulose of *Q. frainetto* leaf litter had already begun to decompose due to the relatively small quantity of its water-soluble fraction. At the end of the experiment, hemicellulose decomposed to a greater extent in *Q. frainetto* than in *Q. cerris* ($P<0.001$).

Total hemicellulose concentrations differed markedly among functional types and tissues with the highest concentration in the sapwood of broadleaved trees (31% DW) and the lowest concentration between 10 and 15% DW in the leaves and bark of woody species, as well as in the roots of herbs. As for total hemicellulose concentrations, plant functional types and tissues exhibited characteristic ratios between the sum of cellulose plus lignin and hemicelluloses, with very high ratios (>4) in the bark of trees and low ratios (<2) in all the investigated leaves [35]. In a 3-year experiment with *Q. ilex* L., Fioretto et al. [17] did not find significant changes in lignin during the entire study period, whereas holocellulose, in contrast, began to decompose immediately. In our study, in both species hemicellulose decomposed more than cellulose, which confirms the findings of Swift et al. [1] and Delaney et al. [49]. At the end of the experiment, the cellulose in the leaf litter of *Q. frainetto* had decomposed to a greater extent than the cellulose in the leaf litter of *Q. cerris* ($P<0.001$). It seems that a higher initial lignin concentration directly influenced the slower decomposition of cellulose in *Q. cerris* leaf litter. Hemicellulose and lignin concentrations were reported to be negatively correlated with decomposition because these compounds are intimately associated within the cell walls of plants and although there is probably no chemical interaction between the two, the physical proximity of lignin may retard enzymatic attack on cellulose [1,5,23]. This is especially characteristic for leaf veins owing to the effect of lignin encrustation of cellulose that slows down microbial attack on this cellulose. This is why in both species the xylem of leaf veins resisted decomposition during the 12-month period of the experiment.

Lignin, together with fats, waxes and oils, takes longest to decompose. This was determined by earlier findings [31,32,50]. Lignin fills out the cell walls, which consist predominantly of cellulose and hemicellulose, thus providing structural rigidity, and it also protects the cell wall against microbial attack. The extremely slow decomposition of lignin can be the result of the polymerization of unstable components into far more complex humus matter, which may be analyzed as part of the lignin fraction [26]. In our study, lignin recalcitrance was noted in both types of analyzed litter. Lignin, however, was more readily decomposed in the leaf litter of *Q. frainetto* than in that of *Q. cerris*, which is in accordance with the earlier study in which the oak species exhibiting higher initial lignin contents displayed lower rates of leaf litter decomposition. For instance, the decomposition of *Quercus dealbata* litter is slower than that of *Q. fenestrata* [51]. This could be the result of a higher initial concentration of soluble nitrogen in *Q. frainetto* litter, which provides a higher pool of soluble compounds for the microbial community. Another explanation could be the different structures of the leaf lignin of *Q. frainetto* and *Q. cerris*, which indicates a different rate of lignin biodegradation. Berg and Lundmark [52] found structural differences of the lignin in different plant species, and also between species of the same genus. For instance, in leaves of *Q. dealbata, Q. fenestrata* and *Q. griffithii*, lignin concentrations vary between 3.8 and 6.0% DW [53]. The suppressing effect of lignin on litter decomposition rates can be described as a linear relationship at later stages of decomposition. A highly significant, positive correlation between lignin contents and litter decay rates was found, which can start at 20-30% for pine litter [54]. Our results showed 22% of decomposed lignin in *Q. frainetto* and 8% in *Q. cerris* after 12 months of the experiment. We also noted that lignin decomposition in both species depends on its position in the leaf tissue. During the experiment, lignin located in the outer epidermal cell wall, with a thick layer of cuticle above it (which protects the plant surface from desiccation and probably from the enzymatic attack of microorganisms), was preserved in both species even after a period of 12 months. It is the combination of lignin with cutin and waxes that could explain the slow decomposition of fats and waxes in both species. For instance, Quideau et al. [55] described the fresh leaves and leaf litter surfaces of *Quercus dumosa* Nutt., analyzed by scanning electron microscopy during 2 years of field exposure using litterbags. Likewise, lignin located in lignified tissues of leaf ribs (xylem) remains undecomposed for

a long time in both species. This could be due to the structure and chemical composition of xylem, i.e. the reason for this could be the manner of packing and the density of the fibrils of the cell walls of the xylem. Fibrils are composed partly of cellulose and partly of lignin, which impregnates and almost cements them [36]. The middle lamella as the binding substance between the cells of xylem consists of pectin and of lignin, which also contributes to slow decomposition. According to Fengel and Wegener [56], the middle lamella, together with the primary wall, has the highest lignin concentrations (40-60%). Lignin located on the lower surface of the leaf, protected with a thin layer of wax, is prone to microorganism attack, and in *Q. frainetto* litter it was about 48% decomposed after 12 months of the experiment, while the lignin in *Q. cerris*, protected by a thick layer of cutin and waxes (and with trichomes), only decomposed about 21%.

All the chemical components of *Q. frainetto* leaf litter, except the lignin, decomposed during the experiment at least up to the level of mass loss rates. In *Q. cerris* leaf litter, the fraction of fats, waxes and oils was also an exception, together with lignin. In our opinion, this is very indicative and implies that special attention should be paid to this fraction (fats, waxes and oils) in the future, at least as far as these two species are concerned. Looking at the initial chemical composition of the two litters examined, besides lignin, differences did not occur only in the concentration of fats, waxes and oils. Similar to lignin, it can be assumed that the decomposition rate of this fraction (fats, waxes and oils) is not only influenced by quantity, but also by its chemical structure and location within the leaf tissue.

The first protective mechanism on the outermost layer of leaves is the epidermis covered by the cuticle, which is composed of waxes, and within this there are layers impregnated with cutin. Cutin forms the framework of the cuticular layer, the main component of which is a long chain of fat acids. These layers (fats and waxes) act as chemical inhibitors of decomposition in two ways. Firstly, it is a result of the presence within them of components that have direct fungistatic effects, such as the ether-soluble acidic fraction of apple cuticle wax [57]. Secondly, the cuticle presents a mechanical barrier because it is composed of slowly degradable cutin [50]. Cutin and suberin, as analogous substances found in the walls of bark cells, are probably among the most recalcitrant of plant materials [1]. Therefore, in Mediterranean shrub species, where a thick waxy cuticle is a widely spread trait, cutin concentrations may be expected to be important for decomposition. Leaf toughness was further identified as a potentially good predictor of decomposition rate [50]. Suberins, resins and waxes that are slowly degraded during the later stages of decomposition have been reported as mainly regulated by the lignin content [58]. These are the reasons for the slow decomposition of the fraction of fats, waxes and oils in both the oaks. It is possible that the chemical structure of the fraction of fats, waxes and oils differs between the two oak species and that it is one of the crucial factors for the slower decomposition rate of *Q. cerris* leaf litter. Our results are in accordance with the findings of Swift et al. [1] and Gallardo and Merino [50] that the thickness and chemical composition of cuticles and suberized layers differ markedly between plant species and may thus provide a basis for differences in resource quality.

CONCLUSIONS

This study establishes a clear connection between decomposition, chemical composition and the anatomical traits of leaf litter. The litter of *Q. frainetto* was more readily decomposed than the litter of *Q. cerris* in natural conditions. Although there were no differences at the beginning of the experiment between the quantity of lignin and the fraction of fats, waxes and oils, these components had a crucial impact on the decomposition rates for both species. Research has proved that the fraction of fats, waxes and oils deserves more attention in the future. This fraction is part of the protective structure and resists biodegradation for a long time, meaning it can be of great importance to the process of decomposition. Research has also shown that, besides the chemical composition of leaf litter, the arrangement of chemical compounds in plant tissues is also of great importance. Tissues that withstand decomposition the longest were defined in the anatomical traits of both species. These are the lignified elements of leaf veins (xylem), the lignified upper epidermis and the cuticle. The results obtained in this study confirmed the fact that quantitative chemical traits can be used as a predictive tool for litter decomposability and ecosystem functioning and indicate that the combination of biological, physical

and chemical factors need to be examined to clarify the different decomposition rates and patterns of tree species. The potential global environmental changes will influence the organic matter quality of litter and soil and the decomposition process in the future. The degree to which tree species will tolerate or take advantage of these changing climatic conditions will depend on characteristics such as leaf traits, phenology and the allocation and storage of chemical compounds in their tissues, and thus the rate of litter decomposition.

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