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Mediterranean shrublands as carbon sinks for climate change mitigation: new root-to-shoot ratios

P. Carrión-Prieto[a], S. Hernández-Navarro[a], P. Martín-Ramos[b], L.F. Sánchez-Sastre[a], F. Garrido-Laurnaga[c], J.L. Marcos-Robles[a] and J. Martín-Gil[a]

[a]Agriculture and Forestry Engineering Department, ETSIIAA, Universidad de Valladolid. Avenida de Madrid, 44, 34004 Palencia, Spain; [b]Department of Agricultural and Environmental Sciences, EPSH, Universidad de Zaragoza, Carretera de Cuarte s/n, 22071 Huesca, Spain; [c]Department of Plant Production and Forest Resources. ETSIIAA, Universidad de Valladolid. Avenida de Madrid, 44, 34004 Palencia, Spain

ABSTRACT

Shrublands play an important role in the reduction of atmospheric CO_2 and contribute to the mitigation of the effects of climate change, due to their ability to act as carbon sinks and the large expanses of land involved. Two of the most representative shrub species in the Iberian Peninsula, *Cistus ladanifer* L. and *Erica arborea* L., were studied in terms of biomass distribution and carbon and nitrogen contents in the different fractions. With a view to fast and cost-effective estimation of radical biomass, a new procedure for easy root-to-shoot calculation based on vibrational data was proposed, resulting in an excellent agreement with the values obtained from conventional direct belowground and aerial biomass measurements: 0.23 for *C. ladanifer* and 0.54 for *E. arborea*. Carbon sequestration, estimated at 45 and 73 t CO_2 eq·ha^{-1} for *C. ladanifer* and *E. arborea*, respectively, was subsequently determined. Since these values are substantially higher than those of other shrubs, these two key species can be deemed particularly promising for ecological restoration and carbon offsetting.

KEYWORDS

Cistus ladanifer L.; climate change mitigation; CO_2 sinks; *Erica arborea* L.; shrubland; root-to-shoot ratio

Introduction

Under the right conditions, woodlands and shrublands play an important role in the reduction of atmospheric CO_2 due to their ability to act as carbon sinks [1]. However, the efficiency of the activities aimed at this reduction should maintain a positive balance between the absorbed and released carbon, and the ability to estimate these quantities and to gain insight into the carbon dynamics should then be regarded as an essential requisite [2]. It is in this context that the European Union is funding lines of research (such as project "CO_2 Operation," sponsored by the LIFE+ program) focused on demonstrating the viability of forestry and agroforestry carbon sequestration projects, extending the (carbon credits) green economy as an alternative for future development and significantly contributing to the fight against climate change.

At present, there are different approaches to estimate biomass and carbon stocks in forests, based on information from forest inventories and referred to factors or biomass equations. These formulas transform diameter, weight or volume data into carbon or biomass estimates [3].

According to Montero *et al.* [4], in Spain there is abundant information on some tree species [5–8], but that on bush and thicket formations is much more limited [9–15,57]. Ruiz-Peinado, Montero, and Del Rio [7] emphasized the importance of the role that shrubs play in water-limited agro-silvo-pastoral systems by providing shelter and forage for livestock, controlling erosion, maintaining biodiversity, diversifying the landscape and, above all, facilitating tree regeneration. Early successional shrublands have become dominant because of the abandonment of agricultural fields and the increase in wildfires frequency in recent decades [16]. Furthermore, the carbon sink capacity of shrubs could also help to mitigate the effects of climate change, since they account for a high proportion of the total plant biomass [7].

Some studies have evinced the existence of some variability in the carbon content not only between species but also between different biomass fractions [4,17–19,57], in spite of the fact that the overall average approaches 50% – the mean value proposed by Kollmann [20] and accepted by the IPCC-. Given the ecological role of these formations and their size in the Iberian Peninsula mountains, it is essential to quantify the biomass, carbon and nitrogen content differential ratios at both intra- and interspecies levels.

The study presented herein focuses on two of the most representative shrub species of the

CONTACT P. Martín-Ramos pmr@unizar.es

Figure 1. ***Cistus ladanifer* L., also known as gum rockrose or labdanum (left); *Erica arborea* L., also known as briar root or tree heath (right).**

Iberian Peninsula, namely *Cistus ladanifer* L. and *Erica arborea* L. (Figure 1), which occupy surfaces of over 2,100,000 ha and 2,400,000 ha, respectively [101]. They have a wide distribution, according to the Spanish Forest Map [102] and the Anthos Spanish Plants Information System [103], and also appear accompanying tree species, in such a way that they have their own codification in the Spanish National Forest Inventory [101]. Phytosociologically, these species are very important in pure Mediterranean shrublands and in siliceous soils [21], and *Cistus ladanifer* has a relevant role as an animal feed source [21]. These species are represented in various habitats of Council Directive 92/43/EEC.

Fractions of these two species, grown under the same conditions, have been assessed, placing particular emphasis on an accurate determination of their radical biomass, provided that the literature tends to underestimate root:shoot ratios (*R*), according to Mokany, Raison, and Prokushkin [22]. The aim of this work has been to obtain these ratios, since they are an expansion factor used for inferring belowground biomass from aboveground biomass measurements [23] and, provided that it is based on biomass and carbon, it can provide ecological values for the calculation of stock, production and ecosystem productivity that are close to reality.

Material and methods

Location

The study was carried out on a plot located in the municipality of Ayoó de Vidriales (42°07′10″N, 6°06′59″W), in the province of Zamora, Castilla y Leon, Spain (Figure 2). The chosen area (>1.2 ha) is a mixed shrubland in which the dominant shrub species are *Erica arborea* L. (*Ea*) and *Cistus ladanifer* L. (*Cl*).

With a continental climate – typical of the northern plateau of the Iberian Peninsula – temperatures are extreme, with monthly average temperature values ranging from −2 °C to 25 °C. Rainfall is scarce (about 440 mm per year), with a pronounced drought period from late May to mid-September. The soil belongs to Inceptisols (i.e. soils of relatively new origin, characterized by having only the weakest appearance of horizons, or layers, produced by soil-forming factors), suborder Ochrept (i.e. it is a young soil with thin, light colored horizons), with a xeric moisture regime (Xerochept).

Data sampling and fresh weight determination

Calculations for the estimation of biomass and carbon stocks may be obtained either by direct or by indirect

Figure 2. **Location of Castilla y León region in the Iberian Peninsula (left); location of Zamora province in Castilla y León region (center); location of the shrubland under study in Zamora province (right).**

methods [24]. Direct methods involve the destruction of heavy biomass, whereas in indirect methods regression models are used to estimate stored biomass and carbon from measurements of other variables – such as diameter at breast height (DBH), tree height (H) or age – making the process easier [25].

In the first part of this study, biomass was determined by a destructive method, which comprised the selection, felling and extraction of biomass for each of the species (conducted in December 2013). Selected samples corresponded to healthy individuals and featured similar characteristics to the rest of the population. The aerial part was separated from the roots using a saw and then, following an analogous procedure to that described by Ruiz-Peinado, Montero, and Del Rio [7], root systems were excavated using a tractor with a shovel and then spades were used to complete the job. For each plant, soil was excavated down in a circular area of twice the mean crown diameter. In addition to the main body of the roots, those remaining in the hole were also collected.

Twenty-five samples of each species were transported to the laboratory (ETSIIAA facilities, Universidad de Valladolid, Spain), where they were separated into different fractions and weighed (fresh weight). In the case of *Cistus ladanifer*, they were classified into leaves, fruits, thin branches (3–7 mm in diameter), thick branches (7–17 mm in diameter) and roots. On the other hand, for *Erica arborea* – given its morphology and the impracticality of leaf separation – they were divided into four fractions: leaves with flowers and fruits, fine material (<1 cm), thick material (<5 cm) and roots, in agreement with de Mello *et al*. [26].

Dry matter content

The dry matter (biomass) content was empirically determined by extracting subsamples from each fraction. The fractions of the aerial parts were dried in oven at 102 ± 2 °C until constant weight was attained (at which point the water content was assumed to be zero). The roots, because of their size, were weighed once their moisture was balanced with the environment (i.e. air-dried) and the results were crosschecked by comparison with those obtained for some fractions dried in the stove. The dry matter content for each component was calculated, in agreement with de Mello *et al*. [26], using the following expression:

$$\text{Dry matter } (\%) = \frac{W_{dry}}{W_{fresh}} \cdot 100 \qquad (1)$$

where W_{dry} is the dry weight (g) and W_{fresh} is the fresh weight (g).

Subsequently, each fraction was ground in a ball mill and homogenized to obtain 1-mm sieve powder (the fruits of the gum rockrose and some thick stem elements required a hydraulic press, given the resistance of the structure to grinding) for CHN analysis (discussed below).

Root-to-shoot ratio as indicator of the relationship between the belowground and aerial biomass

Root-to-shoot ratios can be applied to individual plants or to stands of vegetation at a local, landscape, regional or biome scale [22], and they are often considered constant or species/area-specific values in most studies [27]. The root-to-shoot ratio is defined by the IPCC [23] as the ratio of belowground (root) to aboveground (shoot) biomass – including leaves, thin branches and thick branches – as follows:

$$R = \frac{W_{root}}{W_{shoot}} \qquad (2)$$

where R is the root-to-shoot ratio (dimensionless), W_{root} is the root dry weight (g) and W_{shoot} is the aboveground dry weight (g).

The use of the root-to-shoot ratio as an indicator of the relationship between the belowground (root biomass) and aerial biomass (the sum of leaves, thin branches and thick branches biomasses) is particularly important, since it can serve as an estimator of belowground carbon based on a simple biometric survey of aboveground biomass with lower costs [28]. Consequently, realistic root-to-shoot ratios play a key role in the improvement of the accuracy of estimates of root biomass and, in turn, in the estimation of the effects of management and land-use changes in national inventories of greenhouse gas emissions [22].

The equations for obtaining the R value may vary from project to project. Individual standard values are frequently used, such as those proposed by Kauppi, Mielikainen and Kuusela [29], Kauppi, Tomppo and Ferm [30], Löwe, Seufert and Raes [31], UN-ECE/FAO [32], Federici *et al*. [33] and IPCC [23]. Nevertheless, it is known that these factors also vary depending on the species, the growth stage and the location: the R biomass ratio of adult plants in Mediterranean ecosystems tends to be higher than that in more temperate ecosystems, possibly as an adaptation to the summer dry season [34,35]. Consequently, calculations were performed under specific and identical conditions for the samples of the two species under study, in agreement with Sanquetta, Corte and da Silva [25].

Fourier transform infrared spectroscopy as new tool to determine root-to-shoot ratios

Fourier transform infrared (FTIR) spectroscopy is a useful analytical technique for the nondestructive characterization of biological specimens. It is regarded as a rapid and accurate method for the fast and

simultaneous qualitative and quantitative characterization of natural products and their constituents [36]. Molecular bonds with an electric dipole moment that can change by atomic displacement owing to natural vibrations are IR active. These vibrational modes are quantitatively measurable by FTIR spectroscopy [37,38].

The FTIR spectra of leaves, thin branches, thick branches and roots of *C. ladanifer* and *E. arborea* were collected in direct transmittance mode using a Thermo Nicolet iS50 spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) and the potassium bromide (KBr) pellet method. Pellets 13 mm in diameter were obtained by mixing 1.0 wt% sample into 200 mg of fine KBr powder and then finely pulverizing and putting into a pellet-forming die. A force of approximately 8 tons was applied under a vacuum of several mm Hg for several minutes to form transparent pellets. Spectra were recorded in the mid-infrared range (4000–400 cm^{-1}) at a spectral resolution of 4 cm^{-1}, taking 32 scans per sample. Background scanning and correction was carried out at 60 min intervals, using a pure KBr pellet for the background spectra to correct for infrared light scattering losses in the pellet and for moisture adsorbed on the KBr [39–41].

The vibrational data were analyzed with OMNIC v. 9.3.32 (Thermo Fisher Scientific) software, focusing on the fingerprint region (1900–800 cm^{-1}), in which most of the variations of infrared absorption occur. Within the fingerprint region, four wavenumbers were selected for the calculation of the shoot-to-root ratios: 1369 cm^{-1}, attributable to the C-H and C-O groups of the hexose ring in cellulose; 1458 cm^{-1}, consistent with the saccharide backbone; 1514 cm^{-1}, attributed to the C = C stretching vibration in the aromatic skeletal vibration in lignin; and 1730 cm^{-1}, assigned to ester linkage of the carboxylic group in hemicelluloses.

Root-to-shoot ratios for both species, *C. ladanifer* and *E. arborea,* based on vibrational data were calculated using the corrected peak areas at the four wavenumbers indicated above and Equation 3:

$$R_{\lambda} = \frac{A_{root}}{A_{shoot}} = \frac{roots}{(leafs + thin\ branches + thick\ branches)} \quad (3)$$

where R_{λ} is the root-to-shoot ratio (dimensionless) for each wavenumber; A_{root} is the area of the peak in the roots sample; and A_{shoot} is the aboveground peak area (i.e. the summation of the peak areas in the leaf, thin branch and thick branch samples). As noted above, these ratios are often regarded as constant or species/area-specific values [27].

It should be clarified that the peak height depends on the number of molecules present (concentration) and on the strength of the absorption (absorptivity).

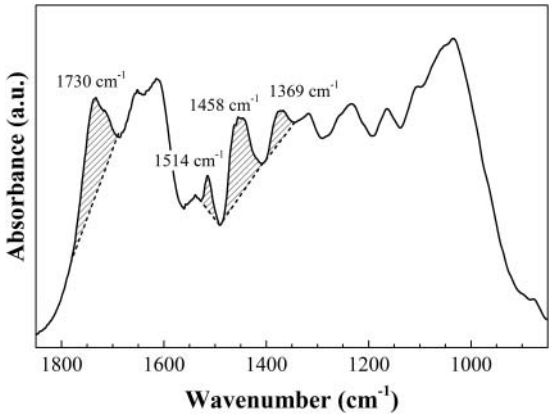

Figure 3. Fourier transform infrared spectrum of *Erica arborea* leaves with the corrected peak areas calculated at some selected wavenumbers (cm^{-1}).

Conversely, the area of the peak is regarded as a better indicator of concentration, because the final peak profile is the sum of all the individual elements. Whereas in some cases the peak height can be changed by a broadening problem, the area will remain unchanged, as the total number of molecules is constant.

The corrected areas (defined as the areas under the spectrum bordered with a baseline) in the spectral regions of interest were determined with OMNIC software, using the automatic baseline correction procedure prior to area calculation. Figure 3 shows how the areas at each selected wavenumber were calculated, taking the fingerprint region of the vibrational spectrum of *E. arborea* leaves as an example.

Carbon and nitrogen determination in the laboratory

The determination of the carbon and nitrogen concentrations was conducted using a LECO CHN-2000 analyzer (LECO Corp., Saint Joseph, MI, USA). Ethylenediaminetetraacetic acid (99%; CAS No. 60-00-4), purchased from Alfa Aesar (Thermo Fisher (Kandel) GmbH, Karlsruhe, Germany), was used for the analyzer calibration in four replicates from 0.09 to 0.12 g, while the weight of the samples of the two shrubs under study was always 0.10 g (measured with a precision scale). Samples were individually wrapped in tin foil and shaped into spheres and, subsequently, they were placed in an autosampler that loaded them into the apparatus. The automated system performed the combustion of samples at a temperature of 900 °C and the remaining products of combustion (CO_2, H_2O, O_2, N_2 and NO_x) were collected and mixed thoroughly. CO_2 and H_2O levels were monitored by two independent selective non-dispersive infrared detectors, and N_2 was determined by a thermal conductivity detector. The apparatus directly provided the weight-compensated results as a percentage of carbon and nitrogen content in each fraction.

There are many studies about the allocation of nitrogen in the plants, provided that it changes as a function of the species and time of the year. However, there is a strong linear relationship between plant nitrogen concentration and the fraction of mass allocated to leaves [34,42]:

$$P \cdot S = \frac{dN}{dt} = aN \tag{4}$$

where P is the net photosynthesis, S is the fraction of mass allocated to leaves, N is the nitrogen concentration, and a is a constant.

Indirect calculation of carbon stock

Aboveground biomass was estimated from the equations proposed by Montero, Pasalodos-Tato, López-Senespleda, *et al*. [43] for Mediterranean shrublands. These equations, deemed accurate for large expanses of territory, are based on data from the canopy cover and average height of different species of Mediterranean shrublands and provide tons of dry matter per hectare, differing according to taxonomic affinities. The species analyzed herein belong to the formations classified as 'gum rockroses and *Cistaceae* shrublands' [tr.] and 'briar roots and *Ericaceae* shrublands' [tr.], and their respective equations are as follows:

$$\ln(W_{a\ Cl}) = -2.596 + 0.957 \cdot \ln(H_{av\ Cl}) + 0.747 \cdot \ln(FCC_{av\ Cl}) \tag{5}$$

$$\ln(W_{a\ Ea}) = -2.921 + 0.984 \cdot \ln(H_{av\ Ea}) + 0.863 \cdot \ln(FCC_{av\ Ea}) \tag{6}$$

where W_a is the amount of aboveground biomass, in tons of dry matter per hectare (tons DW·ha^{-1}); H_{av} stands for the average height of the shrub expressed in decimeters (dm); and FCC_{av} represents the canopy cover of the shrub expressed in %.

The root biomass of each of shrub species was estimated by applying the value of the root-to-shoot ratios determined in previous sections to the aboveground biomass calculated for each hectare of shrubland. The average height was measured by a sample inventory with a range pole, and the canopy cover was determined through geographic information systems (GIS) tools, using low altitude remote sensing (LARS) data collected with a remotely piloted aircraft (RPA).

The total amount of carbon stored was predicted using the experimentally determined carbon content of each fraction. Thus, the tons of carbon per hectare of shrubland were estimated using Equation 7:

$$C_t = [W_{a\ Cl} \cdot (CC_{shoot\ Cl} + R_{Cl} \cdot CC_{root\ Cl}) + W_{a\ Ea} \cdot (CC_{shoot\ Ea} + R_{Ea} \cdot CC_{root\ Ea})] \cdot S \tag{7}$$

where C_t is the total assimilated carbon (tons), W_a is the aboveground biomass (tons·ha^{-1}), R is the root-to-shoot ratio, CC is the amount of carbon absorbed by each fraction of biomass (%) and S is the surface (ha).

Results and discussion

Biomass distribution and root-to-shoot ratios

Figure 4 shows the biomass distribution in each plant: *C. ladanifer* has 19% leaves, 1% capsules, 29% thin branches, 33% thick branches and 18% roots; versus *E. arborea* with 4% leaves, 20% thin branches, 41% thick branches and 35% roots. Thus, in *C. ladanifer* the aboveground biomass accounted for 3.98 g, roughly 81.8% of the total dry mass, whereas belowground (roots) accounted for 0.89 g, 18.2%. On the other hand, in *E. arborea* root biomass (8.06 g) represented 35.3% of the total dry mass, while the remaining 64.6% (14.72 g) corresponded to aboveground biomass.

Consequently, the root-to-shoot ratios, calculated as the quotients of 18.2 by 81.8 and 35.8 by 64.6, were $R = 0.22$ for *C. ladanifer* and $R = 0.55$ for *E. arborea*.

The distribution of biomass – and therefore of carbon uptake – differed in the two shrub species under study: whereas for *C. ladanifer* biomass and assimilated carbon were roughly similar in each of the components that the plant was divided into, for *E. arborea* significant differences were observed among the various fractions. This becomes evident in view of the values of R: while the aerial part of *C. ladanifer* accounted for over 80% of the dry weight of the plant, in *E. arborea* it was *ca*. 65%. This can be ascribed to the characteristics of the shrubs under study, such as the labdanum present in the leaves of the *Cistaceae* or the development of a root capable of holding the arboreal freightage of the *Ericaceae*. Moreover, it should also be taken into consideration that, under such environmental conditions, phenology reflects the strategy of plants to cope with the alternation of favorable and unfavorable seasons for assimilation and growth [44].

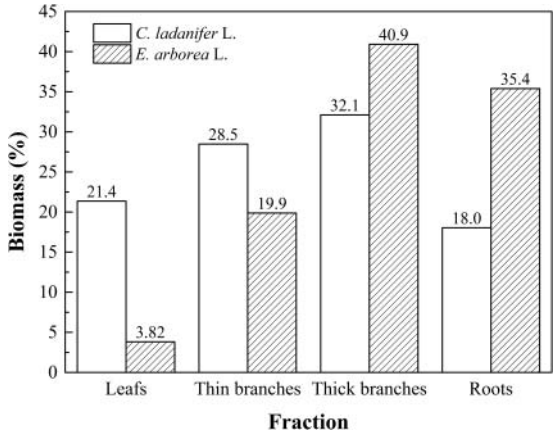

Figure 4. Biomass distribution for *Cistus ladanifer* L. and *Erica arborea* L.

Root-to-shoot ratios based on vibrational analysis

The infrared spectra of the different fractions of the two shrubs under study, *C. ladanifer* and *E. arborea*, are depicted in Figure 5. The main absorption bands and their assignments are listed in Table 1.

In the fingerprint region, four wavenumbers were selected: 1369 cm^{-1}, attributable to in-plane bending vibrations of the C-H and C-O groups of the hexose ring in the cellulose; 1458 cm^{-1}, associated with the alkane deformation of CH and CH_2 in the saccharide backbone; 1514 cm^{-1}, ascribed to the C = C stretching vibration in the aromatic skeletal vibration in lignin; and 1730 cm^{-1}, assigned to the ester linkage of the carboxylic group of ferulic and p-coumaric acids in hemicelluloses. The corrected area values for these selected peaks are summarized in Table 2. In general terms, peak areas for *E. arborea* were higher than those of *C. ladanifer*, in particular those associated to thick branches and roots (which are richer in lignin for *E. arborea*, Table 3).

Peak areas at 1369 and at 1514 cm^{-1} were lower than those at 1730 and 1458 cm^{-1}. For the peak at 1369 cm^{-1}, leaves and thin branches showed higher area values than those of roots and thick branches, due to their higher cellulose content. In *Erica arborea*, as regards the peak at 1514 cm^{-1}, thick branches and roots showed peak areas higher than those of leaves

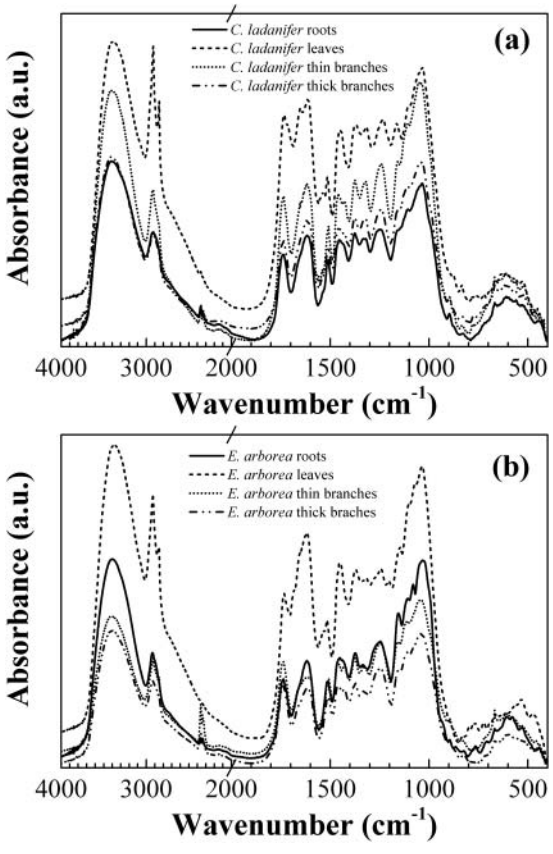

Figure 5. Fourier transform infrared spectra of the different fractions (leaves, thin branches, thick branches and roots) of (a) *Cistus ladanifer* and (b) *Erica arborea*.

Table 1. Main absorption bands in the Fourier transform infrared spectra of the different fractions of *Cistus ladanifer* and *Erica arborea* and their assignments according to the literature [45–48].

Wavenumber (cm^{-1})										Vegetal component	Bonds	Assignment
L *Cl*	t *Cl*	T *Cl*	R *Cl*	L *Ea*	t *Ea*	T *Ea*	R *Ea*					
1035	1050	1035	1035	1035	1050	1039	1031			Cellulose, hemicellulose, lignin	C–O, C=C and C–C–O	Aromatic C–H in plane deformation; plus C–O deformation in primary alcohols; plus C=O stretch (unconjugated)
							1081			Cellulose	C–H	C–O deformation in secondary alcohols and aliphatic ethers
1164							1155			Cellulose	C–H	C–O–C asymmetric valence vibration, C=O stretching in aliphatic groups
1233	1243	1243	1251	1243	1247	1247	1247			Lignin	C–H	C–C plus C–O plus C=O stretching (OH plane deformation, also COOH)
1317	1320	1320	1321		1331	1332				Cellulose, hemicellulose, lignin	C–O, CH_2	Condensation of guaiacyl unit and syringyl unit, syringyl unit and CH_2 bending stretching; CH_2 rocking vibration
1367	1375	1375	1375	1373	1375	1375	1374			Cellulose	C–H	In-plane bending vibration of the C-H and C-O groups of the hexose ring
1455	1458	1457	1456	1455	1464	1459	1452			Saccharide backbone	C–H	Alkane deformation relating to CH and CH_2, consistent with the saccharide backbone
1515	1508	1508	1509	1517	1508	1513	1513			Lignin	C=C	Stretching vibration in the aromatic skeletal vibration
1615	1618	1617	1617	1616	1617	1617	1618			Cellulose, lignin	O–H, C–O	Absorbed O–H and conjugated C–O lignin or cellulose
1733	1735	1735	1736	1733	1739	1740	1736			Hemicellulose, lignin	C=O	Ester linkage of the carboxylic group of ferulic and p-coumaric acids
2850				2852						Cellulose, hemicellulose, lignin	CH stretch	Symmetric CH_2 valence vibration
2919	2921	2920	2919	2923	2918	2919	2928			Cellulose, hemicellulose, lignin	CH stretch	
3389	3413	3421	3405	3374	3409	3409	3405			Lignin	O–H	Intramolecular O_3–H···O_5' in cellulose

Note: *Cl*: *Cistus ladanifer*; *Ea*: *Erica arborea*; L: Leaves; t: Thin branches; T: Thick branches; R: Roots.

Table 2. Corrected areas values for each fraction in *Cistus ladanifer* and *Erica arborea*.

Wavenumber (cm^{-1})	*Cistus ladanifer* L.				*Erica arborea* L.			
	Leaves	Thin branches	Thick branches	Roots	Leaves	Thin branches	Thick branches	Roots
1730	2.71	6.22	3.90	4.21	4.75	5.98	4.77	4.56
1514	0.84	1.30	0.86	0.85	0.79	1.20	1.75	1.97
1458	4.97	3.97	2.66	2.87	6.74	3.04	2.60	3.55
1369	1.30	1.26	0.93	0.88	1.44	1.03	0.94	1.15

Table 3. Percentages in terms of vegetal components for *Cistus ladanifer* and *Erica arborea* [49–51].

Vegetal component	*Cistus ladanifer*	*Erica arborea*
Cellulose (%)	54.9–55.7	37.3–41.1
Lignin (%)	24.5–34.2	39.3–40.1
Hemi-cellulose (%)	10.1–10.9	9.7–13.8
Extractive (%)	9.4–9.6	5.7–11.0

and thin branches, due the higher content of lignin of the former (mature wood has high condensed lignin structures with higher molecular weight than younger tissues) [52]. Conversely, for the peak at 1730 cm^{-1}, thin branches showed the highest area values because of their higher content in hemicellulose. In relation to this latter peak, which is a hemicellulose content indicator, the areas for both the thin and thick branches proved to be the sum of the areas under the three other peaks: $6.22 \approx \sum(1.30 + 3.97 + 1.26)$; $3.90 \approx \sum(0.86 + 2.66 + 0.93)$; $5.98 \approx \sum(1.20 + 3.04 + 1.03)$; $4.77 \approx \sum(1.75 + 2.60 + 0.94)$.

Regarding the 1458 cm^{-1} peak in leaves, the area values were the sum of the areas obtained for the other bands, for both *C. ladanifer* and *E. arborea* ($4.97 \approx \sum(2.71 + 0.84 + 1.30)$ and $6.74 \approx \sum(4.75 + 0.79 + 1.44)$, respectively). This can be explained by the fact that this band is an indicator of the overall polysaccharide content (hemicellulose, lignin and cellulose).

On the basis of previous correlations, it is possible to differentiate the roots from the other plant components, which can be grouped under the term 'shoot'. The root areas (A_{root}) ranged from 0.85 to 4.56, while shoot areas (A_{shoot}) were in the 3.41–15.50 range for both species (Table 4).

Root-to-shoot ratios based on vibrational data (R_{FTIR}) for *C. ladanifer* and *E. arborea*, for the four selected peaks, ranged from 0.25 to 0.33 and from 0.29 to 0.53, respectively (see Table 4). In comparison with the results based on belowground and aerial biomass ($R_{biomass} = 0.22$ for *C. ladanifer* and $R_{biomass} = 0.55$ for *E. arborea*), the closest values would be those associated with the peak at 1369 cm^{-1} (cellulose band) in the case of *C. ladanifer* ($R_{FTIR} = 0.25$) and with the peak at 1514 cm^{-1} (lignin band) for *E. arborea* ($R_{FTIR} = 0.53$). This is in agreement with the relative contents of vegetal components in the two shrubs under study: very high in cellulose for *C. ladanifer* and very high in lignin for *E. arborea* (Table 3).

Carbon and nitrogen concentrations

Carbon concentrations were analyzed for each the different components of the two shrubs (Table 5), omitting the one for *C. ladanifer* capsules due to its low representativeness and to allow comparison between the components of both species. The value of the carbon content (albeit slightly higher in the leaves) did not vary in a significant manner as regards the aerial and root parts, with values of $48.38 \pm 1.02\%$ for *C. ladanifer* and $50.56 \pm 1.38\%$ *E. arborea*. These values are in excellent agreement with those reported by Montero *et al.* [4] for the aerial part of the same species ($49.70 \pm 0.66\%$ and $51.43 \pm 1.17\%$, respectively), and the Spanish Forest Map 1:25,000 (MFE25) values ($49.64 \pm 1.04\%$ and $50.57 \pm 1.62\%$, respectively), and are very close to the 50% value proposed by IPCC [23].

In relation to the amount of nitrogen per unit mass, it was maximum in the leaves for both species (18.89 $mg{\cdot}g^{-1}$ and 10.46 $mg{\cdot}g^{-1}$ for *C. ladanifer* and *E. arborea*, respectively). The dissimilarity in the contents of N in the leaves between *Cl* and *Ea* (Table 5) can be explained according to Equation 4, provided that *S* is substantially higher in *C. ladanifer* than in *E. arborea* and that *P* is three times higher in April–May in species of genus *Cistus* (*Cistus incanus*) than in species of genus *Erica* (*Erica multiflora*) according to Catoni and Gratani [53].

Table 4. Comparison of root-to-shoot ratios for *Cistus ladanifer* and *Erica arborea* determined by two methodologies: using vibrational data (R_{FTIR}) and using the UN-ECE/FAO-IPCC procedure modified by Sanquetta, Corte, and da Silva [25] ($R_{biomass}$).

FTIR method	*C. ladanifer*				*E. arborea*			
Wavenumber (cm^{-1})	1730	1514	1458	1369	1730	1514	1458	1369
Root area	4.21	0.85	2.87	0.88	4.56	1.97	3.55	1.15
Shoot area	12.83	3.00	11.60	3.49	15.50	3.74	12–38	3–41
R_{FTIR}	0.33	0.28	0.25	<u>0.25</u>	0.29	<u>0.53</u>	0.29	0.34
UN-ECE/FAO-IPCC modified by Sanquetta								
$R_{biomass}$	0.22				0.55			

Note: The underlined R_{FTIR} values are the closest to those obtained by the UN-ECE/FAO-IPCC method, modified by Sanquetta.

Table 5. C content, N content and C:N ratios for *Cistus ladanifer* and *Erica arborea*.

	Cistus ladanifer L.				*Erica arborea* L.			
	Leaves	Thin branches	Thick branches	Roots	Leaves	Thin branches	Thick branches	Roots
C content ($mg \cdot g^{-1}$)	500.72	481.20	475.60	477.76	528.20	493.38	502.62	498.24
	(0.37)	(0.27)	(0.59)	(0.47)	(0.22)	(0.13)	(0.31)	(1.23)
N content ($mg \cdot g^{-1}$)	18.89	8.42	2.66	3.62	10.46	3.41	3.78	3.40
	(0.02)	(0.00)	(0.18)	(0.02)	(0.03)	(0.18)	(0.04)	(0.16)
C:N ratio	26.50				50.52			
	(0.05)				(0.14)			

Note: All values are given in average ± standard deviations (in brackets).

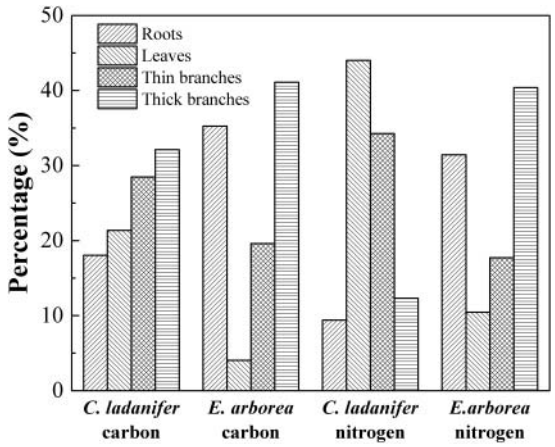

Figure 6. Contribution of the different components to the total carbon and nitrogen stocks, for *Cistus ladanifer* and *Erica arborea*, taking into consideration the biomass distribution.

The distribution of nitrogen content, which resembles an inverse pyramid, reaching its maximum in the leaves and gradually decreasing as we move toward the root, where it presents its minimum, is consistent with that reported by García Rosa [57] for fractions of *C. ladanifer* of different ages. Nonetheless, the value obtained in this study (1.89%) is higher than that found by García Rosa [57], around 0.91%.

In the gum rockrose, the second highest nitrogen content – albeit substantially lower – corresponded to the thin branches (8.42 $mg \cdot g^{-1}$), followed by the roots (3.62 $mg \cdot g^{-1}$) and by the thick branches (2.66 $mg \cdot g^{-1}$). Conversely, in the briar root the leaves were followed by the thick branches (3.78 $mg \cdot g^{-1}$) and by thin branches and roots (with almost identical values: 3.41 and 3.40 $mg \cdot g^{-1}$, respectively). If the nitrogen content is analyzed considering the biomass of each fraction (Figure 6), it may be observed that the behavior is very different in the two species: the values for the analyzed fractions of *C. ladanifer* followed the order leaves >thin branches >thick branches >roots, which was almost the reverse of that in *E. arborea*: thick branches >roots >thin branches >leaves.

Carbon stocks

Upon calculation of the aboveground biomass for each of the species, according to Montero *et al.* [43] (Equations 5 and 6), the total amount of carbon stored (Table 6) was estimated with Equation 7, using the previously determined root-to-shoot ratios (Table 4) and the carbon concentrations in each of the fractions (Table 5). The carbon dioxide equivalent was obtained by direct conversion of the carbon stock using the ratio of their atomic weights (44/12).

In relation to the biomass (dry matter) values, the obtained value for *C. ladanifer* (25.45 t $DW \cdot ha^{-1}$) was significantly higher than those reported by Alías Gallego *et al.* [17] (13.82 t $DW \cdot ha^{-1}$) and García Rosa [57] (17 $t \cdot ha^{-1}$), but was similar to those reported by Basanta [54] (27.26 t $DW \cdot ha^{-1}$) and Terradas [55] (25 t $DW \cdot ha^{-1}$).

It should be mentioned that although the aboveground biomass value for *E. arborea* (25.61 t $DW \cdot ha^{-1}$) was only 19% higher than that reported by Navarro [14] (21.39 t $DW \cdot ha^{-1}$), the total biomass value (39.70 t $DW \cdot ha^{-1}$) would be 85% higher than Navarro's estimation. Therefore, regardless of whether biomass multiplied by carbon concentration or carbon equations are directly used for carbon quantification, omitting the belowground biomass would be misleading, as stated by Koehler, Watzlawick, and Kirchner [56], and it would seriously affect the accuracy of carbon stock estimates.

The carbon stock values for *C. ladanifer* (10.07 t $C \cdot ha^{-1}$ and 2.19 t $C \cdot ha^{-1}$ for the shoots and roots, respectively) were – as expected – higher than those reported by Alías *et al.* [9] and García Rosa [57]: 8.05 t $C \cdot ha^{-1}$ and 1.41 t $C \cdot ha^{-1}$, respectively. Nonetheless, these differences may be ascribed to the fact the latter are average values for specimens of very different ages (ranging from 0–2-year-old to 25–55-year-old specimens), whereas the specimens studied in this work were much more homogeneous (25–35-year-old specimens) and the associated biomass would be at its maximum (as noted by García Rosa [57]).

Table 6. Estimated biomass (dry matter), carbon stock and carbon dioxide equivalent.

	Biomass (t $DW \cdot ha^{-1}$)			**Carbon stock** (t $C \cdot ha^{-1}$)			**CO_2 eq** (t CO_2 eq$\cdot ha^{-1}$)		
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
Cistus ladafiner	20.86	4.59	25.45	10.07	2.19	12.27	36.94	8.04	44.97
Erica arborea	25.61	14.09	39.70	12.84	7.02	19.86	47.07	25.73	72.80

It is also worth noting that the biomass values for both species were significantly higher than those of other shrubs: *ca*. 16, 14, 8.36 and 3.17 t·ha^{-1} for *Asparagus albus*, *Genista* sp., *Rosmarinus officinalis* and *Retama sphaerocarpa*, respectively [57]. In turn, the carbon sequestration associated to *C. ladanifer* and *E.arborea* shrublands would also be significantly higher.

Conclusions

In this work, different fractions of two shrub species present in significant volumes in Mediterranean areas, namely *Cistus ladanifer* and *Erica arborea*, have been studied using several techniques. A faster, cheaper and less time-consuming method for root-to-shoot ratio calculation based on vibrational data has been proposed: by using the areas under selected peaks in the infrared spectra, an excellent agreement with the results from UN-ECE/FAO-IPCC/Sanquetta *et al*. methodology ($R_{biomass}$) was obtained, attaining the best correspondences for the peak at 1369 cm^{-1} (cellulose band) in the case of *C. ladanifer* ($R_{FTIR} = 0.25$; $R_{biomass} = 0.22$) and for the peak at 1514 cm^{-1} (lignin band) for *E. arborea* ($R_{FTIR} = 0.53$; $R_{biomass} = 0.55$). The elemental analysis confirmed that the percentage of carbon in the aerial and radical fractions did not differ in a significant manner, so the use of a 0.5 global value for the entire plant can be deemed appropriate for both species. The percentage distribution of the biomass showed significant differences between the two species. As regards carbon storage, since carbon content did not depend on the analyzed fraction but was directly related to biomass, it could then be directly quantified from the aerial biomass (which is relatively easy to determine) using the root-to-shoot ratios. The carbon stock values (12.27 and 19.86 t C·ha^{-1} for *C. ladanifer* and *E. arborea*, respectively) were substantially higher than those of other shrubs, evincing the importance of these two shrubs species for the mitigation of climate change and their suitability for ecological restoration purposes, in particular for poor soils.

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Conflict of interest

The authors declare no competing financial interests.

ORCID

S. Hernández-Navarro http://orcid.org/0000-0002-6556-8567
P. Martín-Ramos http://orcid.org/0000-0003-2713-2786
J. Martín-Gil http://orcid.org/0000-0001-9921-2465

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