Forest Systems
25(2), e060, 12 pages (2016)
eISSN: 2171-9845
http://dx.doi.org/10.5424/fs/2016252-08062
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)

RESEARCH ARTICLE

OPEN ACCESS

Estimate of biomass and carbon pools in disturbed and undisturbed oak forests in Tunisia

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Abstract

Aim of the study. To estimate biomass and carbon accumulation in a young and disturbed forest (regenerated after a tornado) and an aged cork oak forest (undisturbed forest) as well as its distribution among the different pools (tree, litter and soil).

Area of study. The north west of Tunisia

Material and methods. Carbon stocks were evaluated in the above and belowground cork oak trees, the litter and the 150 cm of the soil. Tree biomass was estimated in both young and aged forests using allometric biomass equations developed for wood stem, cork stem, wood branch, cork branch, leaves, roots and total tree biomass based on combinations of diameter at breast height, total height and crown length as independent variables.

Main results. Total tree biomass in forests was 240.58 Mg ha^{-1} in the young forest and 411.30 Mg ha^{-1} in the aged forest with a low root/shoot ratio (0.41 for young forest and 0.31 for aged forest). Total stored carbon was 419.46 Mg C ha^{-1} in the young forest and 658.09 Mg C ha^{-1} in the aged forest. Carbon stock (Mg C ha^{-1}) was estimated to be113.61 (27.08%) and 194.08 (29.49%) in trees, 3.55 (0.85%) and 5.73 (0.87%) in litter and 302.30 (72.07%) and 458.27 (69.64%) in soil in the young and aged forests, respectively.

Research highlights. Aged undisturbed forest had the largest tree biomass but a lower potential for accumulation of carbon in the future; in contrast, young disturbed forest had both higher growth and carbon storage potential.

Keywords: Tree biomass; disturbance; allometry; cork oak forests; soil organic carbon stock.

Citation: Zribi, L., Chaar, H., Khaldi, A., Hanchi, B., Mouillot, F., Gharbi, F. (2016). Estimate of biomass and carbon pools in disturbed and undisturbed oak forests in Tunisia. Forest Systems, Volume 25, Issue 2, e060. http://dx.doi.org/10.5424/fs/2016252-08062.

Received: 21 May 2015. **Accepted**: 25 Apr 2016

Funding: This study was financially supported by INRGREF (Tunisia) and by the French Ministry of Foreign Affairs CORUS 2 project "Changements globaux et vulnérabilité des forêts méditerranéennes en Afrique du Nord".

Competing interests: The authors declare no conflict of interest.

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Introduction

Forest ecosystems play a crucial role in climate change mitigation by acting as sinks (e.g. Dixon *et al.,* 1994; Lal, 2004; Mohanraj *et al.,* 2011). Carbon dioxide from the atmosphere is accumulated in terms of the organic matter in soil and trees, and it continuously cycles between forests and the atmosphere through the decomposition of dead organic matter (Alexandrov, 2007). Forest ecosystems are estimated to store about 44% in biomass, 11% in necromass and 45% in soils (FAO, 2010) but this range varies across biomes.

Trees act as a sink for CO_2 by fixing carbon during photosynthesis and storing excess carbon as biomass (Nowak *et al.,* 2013). Forests also mobilize atmospheric carbon through plant respiration and organic material decomposition, although these losses are usually less than the gains (Fonseca *et al.,* 2011). The amount of carbon stored in a forest stand depends on its age and productivity (Alexandrov, 2007). Productivity estimates are fundamental in order to evaluate the potential of forest ecosystems and their capacity to sequester carbon. In undisturbed forests, total ecosystem carbon stocks generally increase with stand age as pools of living biomass, forest floor material (organic soil horizons), and mineral soil carbon accumulate through stand development before ultimately leveling off in older stands (Law *et al.,* 2004; Peltoniemi *et al.*, 2004; Powers *et al.,* 2012).

Root systems are an important fraction of plant biomass and play a significant role in forest net primary production (Martínez & Merino, 1987). In European Mediterranean ecosystems, root-shoot ratio of the *Quercus* species (R/S) ranged from 0.27 to 4.8 (Pausas, 1999; Cañellas & San Miguel, 2000; Ruiz-Peinado *et al.,* 2012). In Tunisia, Sebei *et al.* (2001, 2004) reported a cork oak root-shoot (R/S) ratio of 0.22, unfortunately without estimating the stump part. Researchers have traditionally paid only scant attention to root systems, due to the laborious and time consuming research methods involved (Levy *et al.,* 2004). Recently, however, substantial interest in tree root systems has been sparked by the need to accurately estimate the amount of carbon held in forests (Brunner & Godbold, 2007; Konopka *et al.,* 2011).

Although carbon storage in tree biomass can be quite high, assessments of carbon budgets should take into account the litter layer and soil, as these are also major storage compartments (Bauhus *et al.,* 2002, Cairns *et al.,* 1996, Balboa-Murias *et al.,* 2006). Currently, there is a high demand for estimates of current and potential future carbon sequestration in forests. Assessing carbon in the plant-soil system is required to be able to evaluate future strategies aimed at preserving and improving forest stands and therefore important associated environmental functions of these forests.

Cork oak (*Quercus suber* L.) is a sclerophyllous evergreen tree with special bark: cork. Cork oak forests are only found within the Mediterranean climate zones of Western Europe (Italy, France, Spain and Portugal) and North Africa (Morocco, Algeria and Tunisia); in all, they cover an area of 2,275,000 ha (37% in Africa and 63% in Europe) (Campos *et al.,* 2008). In Tunisia, *Quercus suber* forests cover approximately 10% of total forest land. They provide a wide range of environmental services, including biodiversity conservation, soil conservation, water table recharge and run-off control, fire prevention, desertification control and carbon sequestration. Our study was conducted in a cork oak forest with high cork productivity in Bellif located in north-western Tunisia (Nefza). The aims of the present study were (1) to develop equations for predicting total tree biomass as well as tree components for cork oak trees in Bellif, NW Tunisia (2) to estimate and compare above and belowground biomass of cork oak trees between a young (regenerated after a tornado) and an aged forest (undisturbed), and (3) to estimate and compare total carbon pools of these forests.

Materials and methods

Study site

The research was carried out in Bellif forest, said to be one of the most productive cork oak forests in Tunisia (Posner, 1988). In a monospecific and naturally regenerated cork-producing area of *Quercus suber*, two stands of different ages were selected for the study. Three plots of 25 m × 25 m were established in each stand. The first stand was a young site (YS) of even-aged cork oak trees. All trees were destroyed by the tornado of 1974 and were naturally regenerated in the same year. The second stand was an aged site (AS) with uneven aged trees ranging from 71 to 102 years. Major shrubs found in these stands were *Pistacia lentiscus* and *Erica arborea*. For each plot, tree density, height (H), life crown length (LCL) and diameter at breast height (DBH) were recorded. The leaf area index (LAI) was measured in May 2009 at each site using Licor-2000.

The study area is dominated by oligocene sandstone interspersed with clay layers, yielding brown soils with strong biological activity (Nouri, 2009). The soil type associated with this species is a Ferralsols (IUSS Working Group WRB, 2014). The main characteristics of the sites are listed in table 1.

The climate is typically Mediterranean, characterised by hot dry summers and warm winters. Precipitation mainly occurred from September to March and a drought period extended from May to August. The average annual precipitation was 1113 mm and the average annual temperature was 19.3°C. Maximum and minimum temperatures were 47.2 °C and 0.1 °C, respectively.

Cork oak tree biomass and carbon concentration

In order to evaluate the carbon stock in the studied forest, biomass of the different tree components (stem cork, stem wood, branch cork, branch wood, leaves and roots) will be estimated fitting regressions equations based on tree diameter at breast height under bark (DBH), total tree height (H) and life crown length (LCL). In order to obtain the most accurate estimation of biomass, all trees measured at each site were grouped by diametric class. Thus, sixteen *Quercus suber* trees (10 from the young and 6 from the aged site) distributed among these various diameter classes were each chosen from areas outside of but close to the stands in 2009. DBH, H and LCL of the selected trees were measured. The stem of each tree was fractioned into 0.5 m sections and weighed. Two cross sections from each tree (3–5 cm thickness) were taken at a height of 0.30 m and 1.30m from each tree for dendrochronological analysis. The wood discs were air dried in the laboratory and polished (40 to 500 grit). Tree-ring series of each dated sample were measured using a microcomputer with a 0.01 mm accuracy (Lintab™–

Rinntech™) in the Paleoenvironment and Paleoecology Laboratory (Mediterranean Institute of Ecology and Paleoecology, IMEP in France).

All leaves was clipped from branches and weighed in the field, and subsamples were brought to the laboratory for moisture determination. The cork was separated from the wood of each stem section and the branches. The stem and branches of each tree were weighed in the field both with and without cork to calculate cork weight. After cutting down the tree, an ellipse was identified on the ground taking into account crown projection and half the distance to neighboring trees. The whole ellipse area was excavated for belowground biomass estimation. Whole root systems were removed with an excavator, and the remaining part of the root system was then lifted out using a hand digger. Fresh belowground samples were then weighed in the field after cleaning. For each compartment, five samples were collected for dry weight and carbon analysis. In order to estimate dry matter content, samples were dried at 70°C until they reached a constant weight.

Samples of plant material (leaves, twigs, wood, cork and roots) were dried at 70°C for 48 h, crushed, then sieved to determine the carbon concentration using CHN O S in the National Laboratory of Chemical Metrology (National Research Institute and Physical-chemical Analysis, INRAP in Tunisia).

Soil and litter sampling and analyses

At the end of the measurement period, one profile was opened at each site (1.50 m). Soil samples were collected after the profile was defined and horizon boundaries were identified. To determine soil bulk density, five pseudo replicates of undisturbed soil samples were collected using 100 cm^3 stainless steel rings at each soil profile and each soil layer. The physicochemical analyses were carried out in the laboratory for Use and recovery of non-conventional water resources (National Research Institute of Water, Forests and Rural Engineering, INRGREF in Tunisia). The soil samples were dried, crushed and passed through a sieve (2 mm). Water holding capacity (Walker & Skogerboe, 1987) was determined using pressure plates; particle-size distribution was determined by the International Pipette Method (Burt, 2004). Organic carbon content was determined using a dichromate oxidation procedure described by Anne (1945). A correction factor of 1.32 was used to account for incomplete oxidation of organic C (Nelson & Sommers, 1996). Total nitrogen was measured by the Kjeldahl method (Bremner & Mulvaney, 1982).

SOC stocks for a given depth were calculated by summing SOC stocks by layer determined as the product of the bulk density (D_b), SOC concentration and layer thickness (Batjes, 1996). For an individual profile with *n* layers, we estimated the organic carbon stock with the following equation:

$$SOC_s = \sum D_{bi} C_i D_i \left(1 - CE\right) \quad (1)$$

where SOC_s is the soil organic carbon stock (kg C/m^2), D_{bi} is the bulk density (g/cm^3) of layer i, C_i is the soil organic carbon concentration (%) in layer i and D_i is the thickness of this layer (cm), and *CE* is the percentage of coarse elements (relative to the mass of the soil) (Brahim *et al.,* 2010; Martin *et al.,* 2011).

In each site, litter was collected from five squares (3 replications from each sampled plot and 2 replications from the outside area where we cut the chosen trees) of 0.25 m × 0.25 m to estimate litter stock (oak and understory vegetation litter). Subsequently, the samples were taken to the laboratory and dried at 70°C to a constant weight for moisture determination and carbon analysis. For organic carbon analysis of the litter, we applied the same method used for plant samples.

Statistical analysis

Equations predicting dry biomass content of each tree component (y_{SC}, stem cork biomass; y_{SW}, stem wood biomass; y_{BC}, branch cork biomass; y_{BW}, branch wood biomass; y_B, belowground biomass; and y_F, leaves biomass) were fitted using two steps.

In the first step, regression equations were fitted separately for each tree component using a nonlinear model with additive error, and DBH (Diameter at Breast Height), H (Total Height) and LCL (Life Crown Length = Total Height – Height of the first green branch) or CR (Crown Ratio = LCL/H) as independent predictors. The common and simple equation to estimate tree biomass component (y_i) was the power form:

$$y_i = e^{\alpha_0} DBH^{\alpha_1} + \varepsilon \quad (2)$$

where α_0 and α_1 are parameters to be fitted (Cienciala *et al.,* 2008) and ε is the independently and identically distributed (iid) error term. The inclusion into this equation of tree height H, often used to evaluate forest site fertility, as well as other dendrometric tree variables (CR or LCL), would improve the quality of the fit. The equation fitting tree biomass components (y_i) including these various dendrometric tree variables becomes (Parresol, 1999):

$$y_i = e^{\alpha_0} X_1^{\alpha_1} X_2^{\alpha_2} \ldots X_j^{\alpha_j} + \varepsilon \quad (3)$$

where α_j's are model parameters, X_j are tree dimension variables and ε is the random additive error term. Residuals were tested for homoscedasticity (constant variance) using: 1) studentized residuals *vs.* predicted values graphics, and 2) White (1980) and Breusch & Pagan (1979) tests. Heteroscedasticity was corrected using weighted nonlinear regression. Different weights, set as the reciprocal of tree dimension raised to various powers ($X^{-\omega}$), were tested iteratively. The optimal weight was the one that minimized Furnival's Index of Fit (Furnival 1961): $FI = [f'(y)]^{-1}\ RMSE$, where f' is the derivative with respect to biomass, the brackets signify the geometric mean and RMSE is the Root-Mean-Square Error. FI (Furnival, 1961) is a modified maximum likelihood estimator useful for comparing models with different dependent variables. The best model for each tree biomass component was chosen based on various statistical criteria: 1) Goodness-of-fit: R^2, Adjusted R^2, Mean Absolute Error (MAE) and Root-Mean-Square Error (RMSE), 2) normality of the error term (Shapiro & Wilk, 1965, test), and absence of collinearity. The goodness-of-fit statistics were calculated as follows:

$$R^2 = 1 - \sum\left(y_i - \widehat{y_i}\right)^2 / \sum\left(y_i - \overline{y_i}\right)^2 \quad (4)$$

$$Adjusted\ \ R^2 = 1 - \frac{n-1}{n-p}(1 - R^2) \quad (5)$$

$$RMSE = \sqrt{\sum\left(y_i - \widehat{y_i}\right)^2 / \left(n - p\right)} \quad (6)$$

$$MAE = \sum\left|y_i - \widehat{y_i}\right| / n \quad (7)$$

where y_i = actual, $\widehat{y_i}$ = predicted and $\overline{y_i}$ = mean biomass values, n = number of observations, and p = number of model parameters.

One measure of collinearity among independent variables in regression is the condition number, where a high value indicates collinearity. A condition number above 30 is considered to be indicative of collinearity (Belsley *et al.*, 2004).

In the second step, and in order to guarantee the property of additivity (*i.e.*, the sum of the estimations from the equations of the different biomass components should be equal to the estimation provided by the total biomass equation) (Parresol, 1999), the system of tree biomass component equations was simultaneously solved using the regression method developed by Zellner (1962) known as seemingly unrelated regression (SUR). In this method, different mathematical equations, with different independent variables and weights, were used for each tree biomass component (Canga *et al.,* 2013). The additivity was guaranteed by setting restrictions on the equation coefficients (Canga *et al.,* 2013). Since we were dealing with a system of nonlinear equations, the simultaneous fitting of the different biomass components involved the use of NSUR (Nonlinear Seemingly Unrelated Regressions) (Parresol, 2001).

In this study, both individual models fitted separately to each biomass component (1st step), using the nonlinear (weighted or non-weighted) least squares method (NLS), and simultaneously (2nd step) using the weighted NSUR method, were developed using PROC MODEL of SAS/ETS® (SAS Institute Inc., 2011). Assessment of biomass components at the stand level were performed using established models and dendrometric tree variables of the sampling plots.

The effects of the disturbance history (aged *vs.* young forest) on soil characteristics were tested using a one-way ANOVA. The Statistica 7.1 software (StatSoft, Inc., Tulsa, OK, USA) was used to perform statistical analyses. All the results further indicate mean values and their standard error (± SE).

Results

Stands characteristics

Given the proximity of the study sites, temperature and total annual rainfall were essentially the same in the Bellif forest. The sites did not differ significantly in micrometeorological conditions (Table 1), but they significantly differed in stand structure (stand density and DBH). Stand density was 603 and 475 stems ha^{-1} with mean tree diameter at breast height (DBH) of 27.8 cm and 40.6 cm in the young and the aged site, respectively. The age of forests ranged from 34 years in the YS to between 71 and 102 years in the AS.

The two soils were of similar texture but differed in other properties (Table 2). The total N and C contents in the top 30 cm of soil at the AS were significantly higher than in the YS. Soil carbon stocks are usually only reported for the top 30 cm or 1 m of soil. In our study, SOCs were 147.92 Mg C ha^{-1} and 109.56 Mg C ha^{-1} for 30 cm and 369.70 Mg C ha^{-1} and 246.25 Mg C ha^{-1} for 1 m in the aged and young sites, respectively (Table 2). In both sites, a significantly higher SOC stock was found in the aged forest than in the young one ($p<0.05$). For the whole profile (1.50 m), a value of 302.30 Mg C ha^{-1} was found in the YS and 458.27 Mg C ha^{-1} for the AS.

Allometric equations

Biomass equations for each component finally selected are shown in Table 3. According to the calculated condition numbers, it seems that the fitted regression had a moderate collinearity problem, which was

Table 1. Main characteristics of the young site (YS) and the aged site (AS) at Bellif forest (northwestern Tunisia). (Means ± SE).

	(YS)	**(AS)**
Site characteristics		
Latitude	37 04 49 39 33°	37 03 67 93 17°
Longitude	09 10 18 60 17°	09 06 29 92 50°
Altitude	125.95	120.3
Mean annual rainfall (mm)	1113	1113
Mean annual temperature (°C)	19.3	19.3
Cork oak rotation (years)	12	12
Bioclimate	Mediterranean subhumid	Mediterranean subhumid
Vegetation type	*Quercus suber* (even-aged)	*Quercus suber* (uneven-aged)
Shrub stratum	*Pistacia lentiscus, Erica arborea, Myrtus communis, Phillyrea latifolia*	*Pistacia lentiscus, Erica arborea, Phillyrea latifolia, Myrtus communis*
Stand characteristics		
LAI	1.88 ± 0.17	1.68 ± 0.30
Height (m)	12.9 ± 0.33 (8–15.9)	13.2 ± 0.20 (8.3–16.6)
DBH (cm)	27.8 ± 0.52 (22– 40)	40.6 ± 0.81 (25 –76)
Stem number (ha^{-1})	603	475
Annual ring width (mm) (1998–2008)	2.47 ± 0.26	2.13 ± 0.15
Age (years)	35	71 – 102
Cork removal	2000–1988	2005–1992–1980–1968–1956

Table 2. Soil chemical and physical properties of different soil layers in the two sites. C: Carbon, N: Nitrogen, SOCs: Soil Organic Carbon stock and WHC: Water Holding Capacity. (Means ± SE).

	Soil horizons (cm)	Soil bulk density ($g\ cm^{-3}$)	Gravel (%)	Clay (%)	Silt (%)	Sand (%)	C (%)	N (%)	SOCs ($Mg\ C\ ha^{-1}$)	WHC (mm)
Aged site (AS)	0–30	1.30 (0.004)[a]	8.76 (0.26)[a]	24.11 (0.67)[a]	33.22 (0.37)[a]	42.66 (0.84)[a]	4.15 (0.05)[a]	0.31 (0.01)[a]	147.92 (2.31)[a]	60.60 (1.31)[a]
	30–60	1.47 (0.006)[a]	7.86 (0.35)[a]	21.50 (0.73)[a]	48.12 (0.48)[a]	30.38 (0.75)[a]	3.70 (0.28)[a]	0.29 (0.00)[a]	150.87 (11.26)[a]	57.02 (2.08)[a]
	60–150	1.68 (0.010)[a]	2.36 (0.41)[a]	38.62 (0.71)[a]	30.47 (0.74)[a]	30.90 (0.79)[a]	1.08 (0.08)[a]	0.28 (0.00)[a]	159.47 (5.75)[a]	335.23 (3.81)[a]
Young site (YS)	0–30	1.23 (0.007)[b]	8.85 (0.58)[a]	25.36 (0.49)[a]	46.54 (0.27)[b]	28.10 (0.33)[b]	3.26 (0.02)[b]	0.28 (0.00)[b]	109.56 (1.64)[b]	57.18 (0.51)[b]
	30–50	1.28 (0.003)[b]	6.24 (0.52)[b]	21.74 (0.33)[a]	49.48 (0.37)[a]	28.78 (0.69)[a]	3.36 (0.03)[a]	0.29 (0.00)[b]	80.67 (0.59)[b]	43.30 (2.64)[b]
	50–150	1.27 (0.005)[b]	1.02 (0.30)[b]	37.28 (0.56)[a]	45.70 (0.24)[b]	17.02 (0.60)[b]	0.89 (0.03)[b]	0.10 (0.00)[b]	112.08 (4.14)[b]	238.80 (3.60)[b]

* Soil texture: clay loam.
For each parameter, letters (a and b) indicate statistically significant differences ($p < 0.05$) between the two stands (one-way ANOVA).

Table 3. Selected equations for each tree biomass component (, stem wood; , branch wood; , stem cork; , branch cork; , leaves; and, belowground biomass in Kg), using non-weighted or weighted regression, as well as *pr* of White's (*W test*) and Breusch-Pagan (*B-P test*) heteroscedasticity tests, Shapiro-Wilk W test (*W* test), condition number (CN) and goodness of fit statistics.

Component biomass	*weight*	*pr W test*	*pr B-P test*	*W test*	CN	RMSE	MAE	R^2	Adj. R^2
$y_{SW} = e^{-3.2433} DBH^{2.4134}$		0.4767	0.5696	0.2273	46.60	27.4581	17.8246	0.8633	0.8535
$y_{SW} = e^{-4.1886} DBH^{1.6962} H^{1.3323}$		0.1819	0.7526	0.8166	58.36	16.3393	11.9943	0.9550	0.9481
$y_{BW} = e^{-7.5788} DBH^{3.5021}$		0.1756	0.3494	0.0268	54.47	33.0006	23.2652	0.7726	0.7564
$y_{BW} = e^{-8.3637} \left(DBH^2 LCL\right)^{1.4059}$	$\left(DBH^2 LCL\right)^{-3.60}$	0.2621	0.6594	0.4046	40.33	29.3477	17.9669	0.8202	0.8074
$y_{SC} = e^{-7.5788} DBH^{3.5021}$	$DBH^{-2.28}$	0.1374	0.7527	0.1827	40.92	11.0113	8.5683	0.7689	0.7524
$y_{SC} = e^{-4.5075} DBH^{1.4698} H^{1.3296}$		0.2575	0.1088	0.9110	55.93	8.4275	5.9850	0.8743	0.8550
$y_{BC} = e^{-4.4855^*} DBH^{2.2495}$		0.6478	0.6755	0.1991	45.68	5.8765	4.6639	0.7736	0.7575
$y_{BC} = e^{-3.5835} \left(DBH^2 CR\right)^{1.0458}$		0.8380	0.2020	0.4146	41.50	5.1144	4.1211	0.8285	0.8163
$y_F = e^{-4.2891} DBH^{1.9694}$		0.0455	0.3873	0.0004	44.28	3.5465	2.8738	0.6208	0.5937
$y_F = e^{-3.8257} \left(DBH^2 CR\right)^{0.9651}$		0.1390	0.0392	0.0948	40.23	3.0165	2.2323	0.7257	0.7061
$y_B = e^{-1.1182} DBH^{1.7559}$		0.1578	0.0554	0.5546	43.35	18.0774	13.8689	0.8571	0.8469
$y_B = e^{-1.2074} DBH^{1.4976} LCL^{0.4420}$		0.3200	0.8704	0.9632	57.07	16.3462	12.4172	0.8915	0.8748

DBH, the Diameter at Breast Height (cm), *H*, the Total Tree Height (m), *LCL*, Life Crown Length (m) and *CR*, Crown Ratio (=LCL/H). All equation parameters were statistically significant at the 5% level, except for the constant α_1 of the y_{BC} model (*).

common since the independent variables were correlated. The presence of collinearity does not affect the efficacy of using the fitted model with new data as long as the independent variables follow the same pattern of collinearity in the new data as in the data on which the regression model is based (Kutner *et al.,* 2004).

The branch wood component biomass equation was the only one which had a slight problem with both heteroscedasticity (White's test *pr* Chi-square = 0.0383) and normality (Shapiro-Wilk W *pr* = 0.0968) of the model errors; this equation was fitted again using weighted regression. The selected weight factor was $(DBH^2LCL)^{-3.60}$ which had the lowest IF. For both stem wood and stem cork biomass, including height in the equations in addition to DBH improved the goodness-of-fit (Adjusted R^2 of the stem wood and stem cork biomass models, with DBH as the only independent variable, were 0.8535 and 0.7524, respectively. However, taking those same models, with DBH and H as independent variables, gave Adj. R^2 of 0.9481 and 0.8074, respectively). For the other tree biomass components, the inclusion of LCL or CR (= LCL/H ratio) instead of H in addition to DBH enhanced the quality of fit. The equations proposed to estimate both above- and belowground biomass of cork oak trees in Bellif were summarized in table 3.

Above and belowground biomass at stand level

The sampled stands differed in terms of disturbance history, and therefore stand age, and carbon pools of above- and belowground biomass. Total biomass ranged from (240.59 ± 19.27) Mg ha^{-1} for YS to (411.30 ± 26.69) Mg ha^{-1} for AS (Table 4) with significant difference ($p<0.05$) between stands. Moreover, the maximum carbon stock in the aboveground biomass was observed in the AS. Stand carbon stocks were significantly higher in stands with fewer trees (AS) than in the dense younger stands (YS) of the same forest. The great amount of biomass in the former stand seems to be attributable to the presence of trees larger than in the YS. For all the stands, there was no difference in biomass component distribution (Table 4).

Indeed, our estimates of aboveground biomass (169.68 ± 15.23 Mg DM ha^{-1} in YS and 312.49 ± 21.90 Mg DM ha^{-1} in AS) and root biomass (70.91 ± 4.04 Mg DM ha^{-1} in YS and 98.81 ± 4.98 Mg DM ha^{-1} in AS) lead to a low root/shoot ratio (0.41 for YS and 0.31 for AS).

Carbon fraction in the biomass and total carbon stocks

The carbon fraction for the different biomass components of cork oak trees (twigs, stem, branches, roots and leaves) varied between 44.07% (± 0.48) in roots and 56.2% (± 1.6) in cork. The average across biomass compartments was 47.72 % (± 2.2) (Table 5).

The total carbon stock was greater in the AS (658.09 Mg C ha^{-1}) than in the YS (419.46 Mg C ha^{-1}) (Table 4). The aged stand (AS) therefore contained 238.62 Mg C ha^{-1} more carbon than the young stand (YS), with an increase of 36.25%. Approximately 3/4 of the carbon in both

Table 4. Biomass (trees and litter) and carbon pools in a young and an aged forest of *Quercus suber* in Bellif, northwestern Tunisia. (Means ± SE).

Components	Young forest			Agedforest		
	Biomass (Mg ha^{-1})	Carbon (Mg C ha^{-1})	% contribution	Biomass (Mg ha^{-1})	Carbon (Mg C ha^{-1})	% contribution
Aboveground biomass	169.68 ± 15.23	82.52 ± 7.40	72.63	312.49 ± 21.90	151.96 ± 10.65	78.30
Stem wood	80.68 ± 7.07	37.18 ± 3.26	32.72	134.69 ± 5.66	62.07 ± 2.61	31.98
Stem cork	27.15 ± 2.31	15.26 ± 1.30	13.43	41.08 ± 1.44	23.09 ± 0.80	11.89
Branch wood	42.93 ± 4.88	19.78 ± 2.24	17.41	105.77 ± 12.45	48.74 ± 5.74	25.11
Branch cork	12.88 ± 0.77	7.24 ± 0.43	6.373	21.66 ± 1.83	12.17 ± 1.03	6.27
Leaf	6.03 ± 0.32	2.89 ± 0.15	2.543	9.29 ± 0.70	4.45 ± 0.33	2.29
Belowground biomass	70.91 ± 4.04	31.25 ± 1.78	27.50	98.81 ± 4.98	43.55 ± 2.19	22.44
Total tree biomass	240.59 ± 19.27	113.61 ± 9.11	100	411.30 ± 26.69	194.08 ± 12.54	100
Litter	7.46 ± 1.35	3.55 ± 0.64		12.01 ± 1.91	5.73 ± 0.91	
Total forest	248.05	117.16		423.31	199.81	
Total soil organic carbon 0-150 cm		302.30			458.28	
Total ecosystem		419.47			658.09	

Table 5. Carbon content (%) in the biomass of cork oak tree components and litter. (Means ± SE).

Carbon content	Means	SE	Minimum	Maximum
Leaves	47.97	1.37	44.26	52.39
Twigs	44.26	0.46	42.60	45.28
Cork	56.20	1.64	51.28	60.50
Wood	46.08	0.98	43.62	48.93
Root	44.07	0.48	43.24	45.82
Litter	47.72	1.82	44.08	56.20

young and aged forests was contained within belowground biomass and soil (Table 4). However, only 20% in YS and 23% in AS of the carbon was in aboveground biomass and 0.85% in YS and 0.87% in AS in the litter stock, with 79.52% in the YS and 76.26% in the AS accumulated in the soil. We can assume that there was a similarity in the proportional contribution of these two main pools between the two forests.

Discussion

Allometric regressions

Allometric relationship models were established in this study in order to predict biomass of the different components of the aboveground biomass (stem and branch woods, stem and branch corks, and leaves) and the belowground biomass of cork oak trees. The relationship models provide better fitting statistics and more accurate prediction of the cork oak tree biomass components, by using tree characteristics other than DBH. The additivity property, which was not satisfied in previously developed equations (Sebei *et al.,* 2001, 2004), was ensured in this study using the seemingly unrelated regression (SUR) method to simultaneously fit the system of equations of the various biomass components.

In this study, despite the fact that DBH was a very significant determinant in predicting tree biomass components, the additional inclusion of tree height, crown length or crown ratio as independent variables improved the allometric relationship models of all biomass components. Ruiz-Peinado *et al.* (2012) also found that the inclusion of tree height as a predictor variable significantly improved the goodness-of-fit of the biomass component models for cork oak trees, except for belowground biomass. The models developed in this study could therefore be applied in a wider range of stands since height and also crown length or ratio– provides information on growth conditions and site fertility (Wirth *et al.,* 2004).

The allometric models predict in a better way the stem wood biomass (Adj. $R^2 = 0.9481$) than those for the other biomass components. They had a lower predictive ability for leaves (Adj. $R^2 = 0.7061$), probably because of the high variability observed in this component resulting from differences in stand stocking and age.

The value of parameter α_1 in the $y = fct(DBH)$ simple equation for stem wood (2.41 ± 0.27; value ± SE) and stem cork biomass (3.5021 ± 0.32) of the sampled oak trees from Bellif forest in this study were higher than those for stem wood (1.96) and stem cork biomass (1.60) of oak trees from different sites in the Ain Draham region which have lower fertility (Sebei *et al.,* 2001). However, for belowground biomass, parameter α_1 was slightly lower for Bellif forest oak trees (1.76 ± 0.19) than that of oak trees from the Ain Draham region (1.96) (Sebei *et al.,* 2001). This finding suggests that the degree of biomass accumulation increases with site fertility for stem wood and stem cork biomass of cork oak trees, but slightly decreases for belowground biomass.

Stand biomass and root/shoot ratio

The aboveground biomass at stand level estimated in Bellif forest (169.68 Mg ha^{-1} in the young stands and 312.49 Mg ha^{-1} in the aged stands) was higher than in other studies of cork oak forests reported by Sebei *et al.* (2001, 2004) in Tunisia (48.9–113 Mg ha^{-1}) and Léonardi *et al.* (1992) in Italy (42.2 Mg ha^{-1}). In this study, estimations of aboveground biomass in the forest of Bellif are comparable with biomass estimations for the same kind of forest in Spain, with values ranging between 159 Mg ha^{-1}– 328 Mg ha^{-1} (Robert *et al.,* 1996) and 28.82 Mg ha^{-1} – 195.9 Mg ha^{-1}for only wood and cork biomass of cork oak trees (38 – 158 years) (Cañellas *et al.,* 2008) (Table 6).

Data on root systems of cork oak are scarce. Belowground carbon pool (70.90 Mg C ha^{-1} for the YS and 98.81 Mg C ha^{-1} for the AS) for the present study was higher than the values (11 – 25.8 Mg ha^{-1}) reported by Sebei *et al.,* (2001) for belowground biomass in cork oak forests in the Kroumerie. The difference between the values could be due to the method used for data collection and analysis. Sebei *et al.,* (2001) underestimated the belowground biomass of cork oak trees because they only estimated the coarse roots biomass at 40 cm in depth without estimating the stump biomass.

In this study, the root/shoot ratio was estimated to be 0.41 in the YS and 0.31 in the AS based on the estimates of stand-level biomass mentioned above. The estimated root/shoot ratio in our study was higher than

Table 6. Site characteristics in the Mediterranean region: forest type, age (years), DBH (cm), tree density (tree ha^{-1}) and aboveground biomass (Mg ha^{-1}).

Site	Forest type	Age	DBH	Tree density	Aboveground biomass	Ref
Bellif (YS)	cork oak forests	35	27.8	603	169.68	This issue
Bellif (AS)	cork oak forests	71-102	40.6	475	312.49	This issue
Ben Métir (Tunisia)	cork oak forests	81	14	723	80	Sebei *et al.* (2001,2004)
Ain Debba (Tunisia)	cork oak forests	92	16.2	322	48.90	Sebei *et al.* (2001, 2004)
Col des Ruines (Tunisia)	cork oak forests	113	20.2	528	113	Sebei *et al.* (2001, 2004)
Sicile (Italy)	cork oak forests	7-79	16.5	345	42.20	Léonardi *et al.* (1992)
Spain	cork oak forests	38	14.6	500	28.82	*Cañellas *et al.* (2008)
Spain	cork oak forests	118	51.3	70	195.50	*Cañellas *et al.* (2008)
Spain	cork oak forests	158	77.9	40	153.66	*Cañellas *et al.* (2008)
Quart (Spain)	cork oak forests	-	9.3-29	-	159	Robert *et al.* (1996)
St Hilari (Spain)	cork oak forests	-	24-57	-	328	Robert *et al.* (1996)

*Wood and cork biomass.

the value (0.20) reported by Ruiz-Peinado *et al.* (2012) for the same species in Spain. Ruiz-Peinado *et al.* (2012) reported that the estimation of root biomass was only undertaken on a few trees per species and diameter class due to the complexity and cost of the work involved, so one tree per diameter class was selected to estimate root biomass. Regardless of the differences in methodology of root excavation, the estimated root/shoot ratio in our study seems within the same range of available data on the root/shoot ratio for other *Quercus* species reported by Ruiz-Peinado *et al.,* (2012) for *Quercus pyrenaica* 0.35, *Quercus ilex* 0.32, *Quercus faginea* 0.35, *Quercus canariensis* 0.49, hardwood 0.466 and Pausas (1999) for *Quercus ilex* 0.45, 0.37 and 1.20 in Montseny (NE Spain) and 0.27 in Italy.

Carbon content in the biomass of cork oak forests

In this study, we estimated the amount of accumulated carbon in the biomass of the different components of the cork oak trees (Table 5). Many studies estimate carbon content in biomass by using a carbon proportion of 50% of dry weight (e.g. Matthews 1993; Jina *et al.,* 2008). Other authors believe that this value could introduce very large over- or under-estimates of carbon biomass into the calculation (Janssens *et al.,* 1999; Herrero *et al.*, 2011; Castaño & Bravo, 2012).

In our study, the average carbon content of cork was 56.2% (w/w), close to the one reported by Gil *et al.* (2005) which was 57%. However, the average carbon content of wood was 46.1% (w/w), slightly lower than the average carbon content of cork oak wood (47%) reported by Ibañez *et al.* (2002). This value was close to the range (45.7–60.7%) in subtropical/Mediterranean species reported by Thomas & Martin (2012).

Carbon stocks

In the present study, carbon stock of the total tree biomass at stand level averaged 27.08% (113.61 Mg ha^{-1}) in the YS and 29.49% (194.08 Mg ha^{-1}) in the AS of the carbon accumulated in the whole system (tree biomass, litter layer and total mineral soil). Carbon stock in the litter of the cork oak forest ranged from 3.55 Mg C ha^{-1} in the YS to 5.73 Mg C ha^{-1} in the AS, lower than those reported in the same forest ecosystem in Bellif, Tunisia (13 Mg C ha^{-1}) and Khroufa 10.12 Mg C ha^{-1} (Nouri, 2009). Estimated litter carbon pools for our study sites were consistent with the range reported in the same forest type by Nouri (2009) (7.06 Mg C ha^{-1}in Jouza and 3.37 Mg C ha^{-1} in Zouarâa, Tunisia), Andivia *et al.* (2010) (6.35 Mg C ha^{-1} in Huelva, southwestern Spain) and Caritat *et al.* (1996) (3.96 Mg C ha^{-1} and 4.62 Mg C ha^{-1} in northern Spain).

Average carbon storage in the mineral soil amounted to 109.53 Mg ha^{-1} and 147.89 Mg ha^{-1} at 0.3 m depth and 246.2 Mg ha^{-1} and 369.7 Mg ha^{-1} at 1m depth in the YS and the AS, respectively. In Tunisia, Brahim *et al.* (2010) estimated organic carbon stock by soils and delegations (from 1.2 to 199.8 Mg ha^{-1}) at 30 cm depth and (from 10.3 to 449.2 Mg ha^{-1}) at 1m depth. Batjes (2002) reported in Vertisols in Central and Eastern Europe 82 Mg C ha^{-1} at 0.3 m and 236 Mg C ha^{-1} at 1 m depth. In south-central Spain, the total carbon pool in the soil (Haplic Luvisol) was 120.45 Mg ha^{-1} of C (including forest floor and the top 30 cm of mineral soil) in Mediterranean maritime pinewoods (Ruiz-Peinado *et al.,* 2013).

Total carbon stock (including living aboveground biomass, belowground tree biomass, forest floor and the SOCs at 1.50 m depth) was 419.46 Mg ha^{-1} in the YS and 658.09 Mg ha^{-1} in the AS. These total carbon stocks were higher than those reported by Balboa-Murias *et al.* (2006) in four pedunculate oak stands in northwest Spain at 305.9 Mg ha^{-1}. In Belgium, Vande Walle *et al.* (2001) reported total carbon pools of 324.8 Mg ha^{-1} and 321.6 Mg ha^{-1} for an oak-beech stand and ash stand, respectively. Ruiz-Peinado *et al.* (2013) estimated 317 Mg ha^{-1} for maritime pinewoods. Their study only took into consideration the soil organic carbon down to a depth of 30 cm, whereas our study went down to 1.50 m. It is difficult to compare the carbon pool of our study forest because of the scarcity of studies carried out on this subject in the Mediterranean ecosystem. Furthermore, cork oaks, like other species, vary from one forest to another, depending on climate, soil, age, density and stand management (Lemée, 1978).

In the present study, a significant increase of approximately 36.25% in total carbon pool from young stands to aged stands was observed in the same soil type (0–150 cm depth). In fact, not unexpectedly, we found total aboveground biomass C stocks to be significantly higher in aged stands than in young stands. The great amount of biomass in the former stand seems to be attributable to the presence of trees larger than in the YS. A higher proportion of aboveground biomass in the higher diameter classes in the aged stands does indicate the important role played by large trees in carbon storage, but does not undermine the role of small trees (<30 cm DBH) which enhance future carbon stock because of their high carbon sequestration potential. These differences found in total carbon between young and aged stands highlight the importance of forest age in the potential of carbon sequestration. As a matter of fact, some studies have reported that the potential of forests to sequester carbon depends on forest type, forest age (Alexandrov, 2007) and tree class size (Ali *et al.,* 2014). Many other studies have found a similar increasing trend for both above- and belowground carbon stocks that addresses age dependence of forest biomass or carbon stocks (Peichl & Arain, 2006; Taylor *et al.,* 2007).

Cork oak is a slow-growing tree with a high longevity like other oaks, with a lifespan of 250-300 years (Acácio, 2009). The annual ring width of the last decade (1999-2008) ranged between 2.47 ± ± 0.26 mm for young trees (YS) and between 2.13 ± 0.15 mm for aged trees (AS). These findings corroborate that biomass in aged stands (71-102 years) continues to increase.

Many studies have reported that mature forests do not increase in biomass any further because the majority of the gross primary productivity is either used up in respiration or returned to the soil as litter; however, very few of these studies have used old forests as sources (Carey *et al.,* 2001; Pregitzer & Euskirchen, 2004; Acker *et al.,* 2002). Such mature natural forests thus do not significantly contribute towards carbon uptake, though they are important for regeneration and sustaining biodiversity (Baishya *et al.,* 2009). In contrast, Luyssaert *et al.* (2008) reported that young forests, rather than old-growth forests, are very often conspicuous sources of CO_2 because the creation of new forests frequently follows a disturbance to soil and previous vegetation, resulting in a decomposition rate of coarse woody debris, litter and soil organic matter that exceeds the NPP of the regrowth. These findings may explain the lower carbon stock found in the YS than in the AS, taking into account that the two sites are very near to each other and have the same soil type but differ only in disturbance history. The young forestwas naturally regenerated after a tornado (1974), so soil respiration probably exceeded regrowth and litterfall was reduced in young stands due to the small size of the trees in the first few years. According to Stevens & Van Wesemael (2008), carbon stock takes 50 years to be reconstructed after clearing tree stand growth. Seely *et al.* (2010) reported that after 50 years of stand growth, total organic carbon content may be restored to its initial state and then may increase with tree age. High carbon stock in the aged stand indicates that a natural forest without disturbance is the best carbon sink. Similarly, Luyssaert *et al.* (2008) reported that even old-growth forests are usually carbon sinks. Much of this carbon is lost into the atmosphere if disturbed, so this should be taken into account and old-growth forests left intact.

Conclusion

This study presented the first estimations of total carbon pools in a cork oak forest in Tunisia and showed that the total organic carbon stocks in the cork oak forests investigated range from 419.468 Mg C ha^{-1} in the YS to 658.09 Mg C ha^{-1} in the AS. The soil is the major reservoir of carbon in such ecosystem with 79.52% and 76.26% of the organic carbon was found in the YS and the AS, respectively within the 1.50 m soil depth. These stocks, which are relatively high, can be explained by a deep and fertile soil, a high soil water content, and a high mean annual precipitation associated with hot and dry summers, overall leading to extremely favorable conditions in the range of Mediterranean climates (Zribi *et al.,* 2015).

The high amount of carbon stocks found in the undisturbed site (AS) compared to the disturbed site (YS) demonstrate that studies on carbon stock should also pay attention to disturbance history which may affect carbon pools not only at tree level but also at soil level.

Acknowledgements

The authors would like to thank the staff at the Nefza station for their contribution to the field work and Madam Christie Chaar for correcting the English language of the manuscript.

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