Full Length Research Paper

Experimental measurement of the biomass of *Olea europaea* L.

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Olive (*Olea europaea* L.) is an evergreen tree extensively cultivated for its fruit in many warm parts of Southern Europe, North Africa, South Asia and Oceania. The objective of this paper was to obtain information on the total biomass productivity of the olive grove as well as on individual tree biomass productivity and the proportions of the tree components. The study was carried out in Central Italy and estimation of tree woody biomass and total biomass was made by direct measurement and through Intergovernmental Panel on Climate Change (IPCC) methodology. To determine the biomass of all the tree parts (including fruits and prunings) of the olive grove, the forestry estimation methodology named "model tree" was adopted and one representative tree was felled and uprooted; the biomass of main trunk, branches, leaves, twigs and roots was determined separately, using their volume, weight and density; also fruits and prunings of this tree were recorded. Wood density, biomass expansion factor, root/shoot ratio, carbon fraction of dry matter and carbon content of the single tree and tree component were calculated. The study showed that "model tree" methodology is suitable for olive grove; IPCC method can be successfully used with good reliability when direct measurement of biomass expansion factor and carbon fraction are obtained from a representative tree of the olive grove. The C stock evaluation methodology made in this research and the calculation of biomass expansion factor can be considered as the first scientific contribution in estimating productivity, CO_2 sequestration, carbon stocks and yield of olive groves.

Key words: Biomass, biomass expansion factor, Intergovernmental Panel on Climate Change (IPCC), tree volume, root/shoot ratio, *Olea europaea,* CO_2 sequestration.

INTRODUCTION

Olive (*Olea europaea* L.) is one of the most widespread fruit tree species in regions with Mediterranean climate, reaching 9.5 Mha worldwide in 2010 (FAO Statistics Division, 2012). The impact of this crop on the agricultural production of some regions is important, especially in Spain, Italy and Greece (the production of olives by these countries is 63% of world's production, FAO Statistics Division, 2012). In other traditional areas where olive is cultivated, such as North Africa and the Middle East, the interest for this species is increasing.

Recently, olive production has expanded into non-traditional areas (South Africa, New Zealand, Australia, Chile, etc.). In the face of such a wide diffusion of the crop, there is still very little knowledge regarding atmospheric CO_2 sequestration in the agro-ecosystem of olive groves and therefore its effective carbon sink offsetting role, mitigating the greenhouse effect. Climatic change and the management of agricultural lands and forests are interconnected processes of global interest; good agro-forestry practices can give an important contribution in combating climatic change (IPCC, 2007).

Climatic change has an important impact on the factors that influence agricultural production, which include temperature, carbon dioxide, precipitation and their interactions. Knowledge of the consequent effects can allow cropping choices that can be adjusted in order to optimize production and reduce the environmental impact

on the production systems (UN Report on Climate Change, 2007; IPCC Fourth Assessment Report, 2007). To follow this objective, the Intergovernmental Panel on Climate Change (IPCC) drew up guidelines called "Guidelines for National Greenhouse Gas Inventories", which called for the merging of the Agriculture and Land Use, Land-Use Change and Forestry sectors (LULUCF) into a single sector called Agriculture, Forestry and Land Uses (AFOLU) (IPCC, 2006).

In this context, also being able to accurately and precisely measure the carbon stock in agro-ecosystems is progressively acquiring great interest worldwide in order to quantify the estimations of the carbon reserves and the modifications of the biomass stock with respect to the framework convention of the United Nations on climatic change (IPCC, 2003; Tobin and Nieuwenhuis, 2007; Teobaldelli et al., 2009).

The stock of biomass carbon can be estimated using allometric equations (Teobaldelli et al., 2009), direct biomass measurement (Palese et al., 2004) or the Biomass Expansion Factor (BEF) (Lehtonen et al., 2004). With these methods, the total amount of aboveground biomass can be obtained, starting with woody biomass values (with respect to the trunk only, as already used in the forestry sector worldwide) previously estimated.

As reported in the literature, the BEF used in the forestry world varies according to the species, site of growth and the age of the tree, the density of the plantation and other various biotic and abiotic factors (Garcia et al., 2010; Fonseca et al., 2010; Gil et al., 2011.). It follows that if the BEF calculated for certain species is applied to other species and/or to different environmental and cultural conditions, there could be errors in the final results.

In the literature, there were no studies on the evaluation of *Olea europaea* CO_2 sequestration of olive groves, in terms of experimental methodology and carbon stock resulting data. Data in terms of C sink contribution were not available on annually removed fruits and prunings ("non-permanent" tree components), too; these data are relevant for quantifying the whole olive agro-ecosystem role in stocking CO_2.

The aim of this experimentation was to evaluate if the methodology used for forest trees can be used also for the olive tree, once cultivated in an agro-ecosystem. The adopted methodology would therefore be useful to evaluate its CO_2 sequestration. Using direct measurement, the biomass values and the carbon (C), that are necessary for using the IPCC method, were determined by defining: BEF, ratio of the belowground biomass to the aboveground biomass (R), carbon fraction in the dry biomass (CF) and base density (D).

As compared to forestry trees, for olive grove, the BEF and the other IPCC coefficients would result in lower variability of the data in relation to the various factors, due to the homogeneus conditions in the same olive grove (age, tree size and training system, cultural

practices, genetic variability, etc.). The variability is limited to the genetic diversity of the different *Olea europaea* cultivars cultivated.

In particular, in this study, the components co-involved in sequestering CO_2 in an olive grove representative of olive cultivation in Central Italy were investigated. Two different methodologies to estimate the biomass and the respective stock of CO_2 by the belowground and aboveground parts of the olive tree were used and compared: direct measurement (with uprooting of a "model tree") and the IPCC method. The obtained data can be useful for further studies tied to the carbon footprint of this tree cultivation.

MATERIALS AND METHODS

Study area

The study was carried out in a non-irrigated olive grove situated in Central Italy (Assisi - Perugia, Umbria region). The olive grove was planted in 2000. The trees of Leccino cultivar were trained in the vase system with a spacing of 5.5 x 5.5 m (about 330 trees per ha). The alkaline soil is loam/clay-loam with no stony components.

The area studied has a continental climate. The average temperature difference between the coldest month (January) and the hottest month (July) is 19 to 20°C (with an average diurnal thermal range of 10 to 11°C and an average annual air temperature of 13 to 14°C). Maximum temperatures can go up to 36°C and the minimum -7°C.

The annual average precipitation is 800 to -820 mm, mostly concentrated in the autumn, winter and spring. In summer, precipitation is very scarce; frequently prolonged drought can negatively affect the activity of the tree.

Selection of the "model tree"

Since in an olive grove the conditions are homogeneous with respect to tree age, cultural treatment and genotype, with consequent morphological uniformity of the trees (height and trunk diameter), we considered suitable, the "single model tree" biomass estimation approach (La Marca, 1999) that is used to estimate the biomass in forest stands, which, even though uneven, are divided into homogeneous diameter classes. The method is the most commonly used method in the Italian forestry sector, since it allows obtaining results that are very close to those obtained by direct measurement of the biomass components, with the least expenditure of time and money (Phillip, 1994).

To select the "model tree" the following parameters were measured on the 33 trees of the experimental plot: diameter at 80 cm above the ground level and the height of the trunk up to the scaffolding.

All data were submitted to analysis of variance using the SAS System for Windows program; significance was tested using Duncan's test. The tree with the closest measures to the average values was identified and uprooted on 27 May 2011 (after pruning) using an excavator. This "model tree" was subdivided into the various parts (large-diameter roots, root collar, trunk, branches, twigs and leaves) and the fresh weight was immediately determined in the field. Samples of each part were then dried in an oven at 105°C for 15 days to determine the moisture content and dry matter as suggested by Hultnas (2011) and Nystrom and Dahlquist (2004). In order to validate the data obtained in 2011, another tree with

similar measures was uprooted in 2012 (Brunetti, 2012). The data obtained in 2012 were the same with IPCC parameters of 2011, confirming that it is possible to use the "single model tree" biomass estimation approach for olive grove.

Determination of the biomass of the permanent structures (aboveground and belowground)

Two different methods were used to calculate the biomass of the tree and the respective CO_2 sequestration:

1) By directly determining the weight of the various components;
2) By estimating, with the IPCC (2003) "Stock change method", using the equation (technically defined "IPCC 3.2.2 equation" or "annual change in carbon stocks in living biomass in forest land remaining forest land"):

$$C = [V \times D \times BEF] \times (1 + R) \times CF$$

where: C = carbon of the calculated biomass (tons); V = commercial volume (m^3 ha^{-1}); D = wood basic density of the wood (t m^{-3}); BEF = biomass expansion factor to obtain the total aboveground biomass; R = ratio between belowground and aboveground biomass; CF = carbon fraction of the dry mass (conventionally equal to 0.5).

Total biomass by directly measuring the weight of the various components

After uprooting the "model tree" and taking care to retrieve most of the root system, as earlier described, the individual components, trunk, branches, twigs and root collar (above ground parts) and roots (below ground part) were weighed and samples of all parts were put in a ventilated oven at 105°C until they reached constant weight (about 15 days). To obtain the amount of organic C, the dry matter was multiplied by the values specifically elaborated (the measured CF) for each component, as shown in Table 2.

To determine the volume of the aboveground mass of the uprooted tree, the trunk and the individual branches were measured and the apparent density (m/V) was calculated. From the density values, the volume of the trunk and branches, necessary for calculating the BEF and D, were calculated.

Total biomass with the IPCC (2003) method: "Stock change method"

In order to apply the formula from the IPCC 2003 method, it is necessary to calculate the BEF, D and the root/shoot ratio (R) of the tree.

Calculation of BEF and D

The BEF was calculated with the aboveground/wood biomass ratio. The wood basic density D (t m^{-3}) was calculated based on oven dry weight of the biomass over the green volume of biomass as follows:

D = oven dry weight of plant sample/green volume of plant sample.

To calculate the belowground/aboveground ratio, the weight of the root system in kg of dry matter and the weight of the aboveground part in kg of dry matter were used.

Determination of the belowground biomass according to the "direct weighing" method

The CO_2 sequestered in the belowground part of the olive was calculated using the direct weight of the roots. Root samples were put in a ventilated oven at 105°C until constant weight to determine the dry weight and then the CO_2 and sequestered C were calculated (with the measured CF). The aboveground and belowground biomass ratio was calculated to obtain the R index.

Determination of the living biomass in the non-permanent structures according to the "direct weighing" method

The prunings (subdivided into leaves and wood) and the fruit production were considered in this category. The CO_2 sequestered in the prunings was estimated by determining the average dry weight (kg per tree) of the pruning material from the last three years. The CO_2 sequestered in the fruit production was estimated by determining the average dry weight of the fruit at harvest (kg per tree) from the last three years.

The CO_2 and the C from the fruit and prunings were calculated by multiplying the dry weight by the percentage of dry matter of each individual component, using the direct measurement of CF (Table 2) and the dry matter of the tree, assuming as default value, a fraction of carbon of the dry matter equal to 0.5 (IPCC, 2003).

Statistical analysis

Data were submitted to analysis of variance using the SAS System for Windows program; significance was tested using Duncan's test.

RESULTS

The tree with the closest trunk diameter and height shows the average values of the trees in the experimental plots with 16 cm diameter at 80 cm above the ground level and 122 cm for height of the trunk. The soil parameters are presented in Table 1. The Duncan test found no statistical differences for $p < 0.05$ among the trunk diameter and height of the 33 trees in the experimental plots.

The biomass calculated using the weight of the individual components of the "model tree" is shown in Table 2.

Total biomass determined directly

Apparent density (m/V), calculated from the volume and the aboveground mass was respectively, 930.14 kg m^{-3} for the wood of the branches and 940.52 kg m^{-3} for the wood of the trunk, a value very similar to the apparent density value of 950 kg m^{-3} reported by Giordano (1976). Using the direct measurements, the following two determinations were made:

1) using the data obtained from the CF - obtained by oven dry data (Table 2) and
2) using the data obtained from the CF - obtained from the IPCC formula, where CF = 0.5.

Using the measured CF, the C for the permanent structures was 0.0402 tons, while using the CF = 0.5, the C for the permanent structures was 0.0336 tons.

The amount of carbon calculated using the weight of the individual components of the "model tree" and the

Table 1. Soil analysis (0-20 cm) of the experimental olive grove.

Coarse fragments	Percentage
Fine soil	100
Coarse sand	14
Fine sand	20
Silt	38
Clay	28
pH	8.2
Total lime	31
Active lime	12.8
Organic matter	1.25
Total nitrogen	0.07
Organic carbon	0.73
	(ppm)
Assimilable phosphorus	20
Exchangeable potassium	114
Organic carbon (t ha^{-1} per year)	22.19
Density	0.73

Table 2. Weight of the "model tree" components and respective % as dry matter and CF (measurements done after pruning).

Tree component	Fresh weight (kg)	Dry weight (kg)	H$_2$O (%)	Dry matter (%)	CF
Root collar	20.32	12.02	40.8	59.2	0.592
Trunk	23.40	13.94	40.4	59.5	0.595
Branches	26.45	16.32	38.3	61.7	0.617
Twigs	16.78	10.00	40.4	59.6	0.596
Leaves	12.37	6.72	45.7	54.3	0.543
Total aboveground biomass	99.32	59.00			
Root (belowground biomass)	13.20	8.23	37.5	62.5	0.625
Total plant biomass	112.52	67.23			

amount of equivalent carbon dioxide (CO$_2$eq) are reported in Table 3. The CO$_2$eq, that is. the amount of CO$_2$ sequestered by the tree in the form of organic substance, is obtained by dividing the amount of C by a factor equal to 0.27 (ratio of the moles of carbon and the sum of the moles of carbon and oxygen).

The total organic C stored in the "model tree" was 33.61 calculated with the CF IPCC (0.5) and 40.23 with the measured CF (Table 2); therefore, the difference was 6.62 kg using the measured CF. The aboveground part was 35.09 and the belowground part was 5.14 kg using the measured CF.

Multiplying the amount of measured C in the "model tree" by the number of trees per hectare (330), 13.27 tons of C (11.57 t C ha^{-1} for the aboveground biomass and 1.69 t C ha^{-1} for the belowground biomass) is obtained. This is equivalent to 49.17 tons of CO$_2$ sequestered per hectare.

The amount of carbon and the average equivalent carbon dioxide per plant, calculated using the average weight of the fruit and prunings from the last three years (non-permanent structures) are shown in Table 4.

From these results, it can be deduced that the non-permanent structures sequester, an amount of C (13,00 kg) in comparison with the total abovegroung biomass after pruning (40.23 kg) is equal to 32.31% of the total biomass (and 37.14% of the aboveground biomass of 35.09 kg); the fruit production annually corresponds to 12.85% of the total biomass, while the prunings correspond to 19.46% of the total biomass.

For the total aboveground biomass, the dry weight with the addition of the values of the non-permanent structures (prunings and fruit) was 81.08 t dry matter. The respective amount of C, using the measured CF is 0.05347 tons (0.05347 tons × 330 trees per hectare = 17.645 t which is equivalent to 65.35 tons of CO$_2$

Table 3. Organic C and CO_2 of the "model tree" (measurements taken after pruning).

Tree component	C* (kg)	C** (kg)	CO$_2$eq* (kg)	CO$_2$eq** (kg)
Root collar	6.01	7.12	22.25	26.37
Trunk	6.97	8.29	25.81	30.70
Branches	8.16	10,07	30.22	37.30
Twigs	5.00	5.96	18.51	20.70
Leaves	3.36	3.65	12.44	13.52
Total aboveground biomass	29.50	35.09	109.23	128.59
Root (belowground biomass)	4.11	5.14	15.22	19.04
Total plant biomass	33.61	40.23	124.45	147.63

*With CF - IPCC 2003; **with measured CF.

Table 4. Organic C and CO_2 annually sequestered in the non-permanent structures.

Parameter	Fresh weight (kg)	Dry weight (kg)	H$_2$O (%)	Dry matter (%)	C* (kg)	CO$_2$ kg	C** (kg)	CO$_2$ (kg)
Fruit	16.71	9.28	44.43	55.57	4.64	17.18	5.17	19.15
Prunings	20.93	12.80 (9.53 branches + 3.27 leaves)	38.83	61.17	6.40	23.70	7.83	29.00
Total	37.64	22.08			11.04	40.88	13.00	48.15

*with CF - IPCC 2003; **with measured CF.

Table 5. Data for using the IPCC formula with respect to the "model tree".

BEF (aboveground/wood biomass ratio)	D (total biomass weight/fresh volume of biomass)	R (root /aboveground biomass)
1.13	0.562	0.139

sequestered per hectare). Using CF = 0.5, the C is 0.04464 tons.

For the total woody biomass, the dry weight of the aboveground biomass without the leaves but with the values for the pruning is 65.08 t dry matter. The respective amount of C, using the measured CF is 0.04803 tons (0.04803 tons × 330 trees per hectare = 15.85 t which is equivalent to 58.70 tons of CO_2 sequestered per hectare). Using the CF = 0.5, the C is 0.04 tons.

Total biomass estimated with the IPCC method

From the density values (930.14 kg m^{-3} for the wood of the branches and 940.52 kg m^{-3} for the wood of the trunk), the volume of the trunk and branches, which is necessary for calculating the BEF and the D, was calculated.

Calculation of BEF

With the aboveground biomass (dry matter) value equal to 59.00 kg and the woody biomass value (aboveground biomass minus the leaf biomass) equal to 52.28, BEF was calculated applying the formula for total aboveground biomass/woody biomass. The result of BEF is 1.13 (Table 5).

If prunings and fruit are considered, with the aboveground (dry matter) biomass equal to 81.08 kg and the woody biomass (total aboveground biomass minus the leaf biomass) equal to 61.81, then BEF is 1.31.

Calculation of D

Considering that the total biomass dry matter is 67.23 kg and the fresh volume of the total biomass is 119.64 dm^3, the D is 0.562.

Calculation of R

When the root weight was 8.23 kg dry matter and the aboveground biomass weight was 59.00 kg dry matter, the root/shoot ratio was 0.139.Using the IPCC 2003 method (applying Equation 3.2.3 of IPCC), the sequestered carbon from the aboveground biomass and the total biomass (below- and aboveground) is:

$$(0.0928 \times 0.562 \times 1.13) \times (1 + 0.139) \times 0.5 = 0.0337 \text{ tons.}$$

On the other hand, using the IPCC formula, but with the measured CF from the uprooted tree (equal to 0.595), the tons of C are equal to 0.0399.To obtain the amount of CO_2eq per hectare, the value obtained with the IPCC method is multiplied by the number of trees per hectare. With a CF of 0.5, the obtained value is 11.121 tons and with the calculated CF, the obtained value is 13.167 tons. These values, divided by 0.27 resulted into 41.188 tons CO_2eq per hectare (with IPCC CF) and 48.166 CO_2eq per hectare (with the measured CF) for the 11 years of the olive grove. The average annual CO_2 sequestration is 3.74 tons of CO_2 (with IPCC CF) and 4.433 tons of CO_2 (with the measured CF) without taking into account the prunings and harvested fruit.

If non-permanent structures (prunings and fruits) are taken into account, the obtained total value per hectare is 64.66 tons of CO_2, where 0.0399 tons of C with measured CF + 0.013 tons of C = 0.0529 tons of C × 330 = 17.457 tons of C / 0.27 = 64.66 tons of CO_2 for the 11 years of life of the olive grove. The average annual CO_2 sequestration is 5.878 tons, with a difference of 1.445 tons of CO_2 annually with respect to the amount without prunings and fruit (with measured CF).

DISCUSSION

Homogeneous cultural treatment and the same age and genotype originated in the olive grove similar trees, confirming the hypothesis that a "model tree" would be a suitable methodological approach in a uniform tree population as is generally the one that is found in an olive grove.

This was also confirmed by the data obtained with the other tree with the same measures uprooted in 2012, consequently, it is possible to affirm that one single "model tree" can offer consistency on the population results, at given homogenity with respect to age, cultural treatment and genotype.

The amount of C measured in the field (on the "model olive tree") was equal to 0.0402 tons of C; the amount of C estimated with the IPCC method (that use CF= 0.5) was equal to 0.0337 tons of C; the amount of C was estimated with the IPCC method, but adopting the measured "model olive tree" CF, was equal to 0.0399.

The total organic C stored in the model tree is underestimated adopting the value of 0.5 suggested by IPCC method (16% less), while it is close to the measured value with measured CF (1% less). It is therefore suggested to always calculate the CF when using the IPCC method for biomass estimation of Olive grove.

In general, a tree root system play an important role in stocking CO_2 since a considerable amount of carbohydrate substances is translocated and stored for relatively long periods in it, even if with annual cyclic variations. Our results, showed that the aboveground part was 35.09 kg and the below ground part was 5.14 kg (with a 14.65% ratio), using the measured CF shows that *O. europea* belowground biomass is reduced in comparison with the aboveground biomass.

Furthermore, considering that non-permanent structures annually sequester an amount of C equal to 32.31% of the total biomass, it is important to highlight that a correct evaluation of CO_2 sequestration of an olive grove should be done considering the annually removed amount of fruits and prunings, too. For a young olive grove (with age of about 11 years old), the fruit production annually harvested corresponds to 12.85% of the total biomass stocked in the tree, while the prunings corresponds to 19.46% of its total biomass. This is an important indicator of the effective carbon sink offsetting role of an olive grove, that can be evaluated starting from non-permanent structures annually removed from the tree.

Calculated olive BEF in this experimentation was 1.13. It is very interesting to know that this confirms the value obtained by Köhl et al. (2005) in their study carried out thus far on the BEF of olive. For the genus *Olea*, species *Olea ferruginea* Royle, also Abbas et al. (2011) determined the BEF; but unfortunately, the results are not comparable since they calculated the BEF for each component of the tree, but not for the entire aboveground part.

The amount of C estimated with the IPCC method was 16% less than that measured in the field (0.0402 tons of C); then, if the measured CF is considered, the difference is only 0.0003 tons of C, a difference of 1%. This is an important result since it shows the importance of a measured CF if IPCC method is adopted for a rapid estimation of olive grove biomass. The average annual CO_2 absorption of the "model tree" (if the prunings and fruit are taken into account) was 5.88 tons, that is, lower than the data found in the study on the estimation of the potential for absorption of C through the radiation-use efficiency (RUE) in other olive groves in the Mediterranean Basin: for example in southern Spain, it was 7 t ha^{-1} of CO_2 per year.

Further studies should be carried out in future on a more mature olive grove, in order to compare the two methods, since the different age of the plantations could be a reason for this difference.

Conclusions

Based on these results, the rapid evaluation of C sequestered by the tree parts using the IPCC 2003 method (0.0337 tons of C) and using the CF suggested by IPCC (CF = 0.5) gave a lower amount (-16%) than the direct measurement of the biomass from the model tree (0.0402 tons of C), for the aboveground as well as the belowground parts.

The rapid evaluation of C sequestered by the tree parts carried out using the IPCC 2003 method, and using the calculated CF from the uprooted tree, gave practically the same results (0.0399 tons of C) than the direct measurement of the biomass from the uprooted tree.These results are promising for use of the IPCC 2003 method for olive groves. In fact, It is cheap and saves time, but at the same time, gives very reliable results, provided that the CF used is obtained from a "model tree".

Furthermore, the amount of C present annually in the non-permanent structures (fruit and prunings) can be estimated reliably, as the proportions between the permanent and non-permanent structures were quantified annually (this is particularly useful for estimating the amount of C sequestered by the fruit). In fact, the non-permanent structures sequester an amount of C equal to 32.31% of the total biomass (and 37.04% of the aboveground biomass).

From the annual study of the experimental olive grove, the fruit annually harvested corresponds to 12.85% of the total biomass, while the prunings correspond to 19.46% of the total biomass. Since, calculation of the BEF of the model tree was key in estimating the rapid IPCC method, it it would be necessary to specify the BEF for different geographical areas, for different cultivars and for different aged trees.

In the present study, uprooting the "model tree" allowed the proportions of the belowground and aboveground parts to be defined (and the proportions among the aboveground parts), thus furnishing important data for determinations in other olive groves in central Italy.

Regarding the "model tree" methodology adopted in this contest, on the basis of the obtained results, it is possible to affirm that a single tree, if it is representative, and the olive grove is homogeneous, can provide a suitable estimate for the entire olive grove; this condition is true for almost all productive olive groves; above all, if they are monovarietal, and if more cultivars are present, it would be advisable to verify if they can be considered homogeneous and otherwise consider them separately.

This important conclusion is confirmed by the observation that estimation of total C ha^{-1} of the olive grove adopting the IPCC method (used in forest environment) with the calculated CF, was the same obtained using the data from direct measurement of the uprooted "model tree".

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