

Which is the contribution to the carbon sequestration of the forest ecosystems in the Castelporziano Reserve? Evidences from an integrated study on humus and vegetation

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Abstract Soil is a major carbon sink or source on terrestrial ecosystems. Despite their great importance, humus forms, which constitute the small portion above the soil, have been often neglected in local studies and in international research projects. In the present work we evaluated the organic carbon stocked in the different humus forms in a Mediterranean lowland forest, to highlight the carbon concentration in different vegetation types, particularly between evergreen and deciduous woodlands. Results showed that the carbon stock stored in the organic and organo-mineral horizons of humus and soil, expressed in Tons/Ha, had a wide range for each vegetation type, reflecting the high diversity of the forest vegetation and the variability within each type. The vegetation with the highest value of carbon stock despite its small extension was represented by humid woodlands dominated by *Fraxinus oxycarpa*, a relic forest type occurring in the dune slacks within the study area, which gives, therefore, an

important contribution to the climate warming reduction. We demonstrated as the humus forms play a role in the carbon sequestration in a forest ecosystem; therefore, it may be important to add the evaluation of carbon stock when carbon concentration is evaluated for the soil and above and plants below ground biomass.

Keywords Carbon stock · Humus forms · Mediterranean ecosystem · Forest vegetation · Global warming

1 Introduction

Understanding carbon cycle feedbacks in an ecosystem is central to the debate on climate change (Xuguang et al. 2013). The carbon stock sequestration from forest and soil has a relevant importance to mitigate the effect of global warming. These two components have clearly the majority of carbon stock in a terrestrial ecosystem (Batjes 1996; Penne et al. 2010). All interactions taking place in the soil between plants, microbes and animals are under the control of a particular environment, where these organisms live and evolve together. These interactions contribute in turn to the humus form build-up and maintenance, stemming in an integrated view of the topsoil as a key component of terrestrial ecosystems. The quantity and quality of organic matter falling on the ground, or resulting from the death of subterranean parts of plants, depend on the availability of carbon dioxide in the atmosphere, soil nutrients and through fall sun, heat and water, herbivores and various injuries (Ponge 2013).

The humus forms are the fraction of the topsoil that is strongly influenced by organic matter, corresponding to the sequence of organic and underlying organo-mineral horizons (Green et al. 1993; Brêthes et al. 1992). The thickness

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of forest floor and the structure of organo-mineral horizons, which are under the paramount influence of ecosystem engineers such as earthworms (Wironen and Moore 2006), can vary according to the age of the trees (Bernier and Ponge 1994; Godefroid et al. 2005; Chauvat et al. 2007), plant successional processes (Scheu and Schulz 1996) and undergo cycles at the scale of centuries in naturally regenerating late-successional forests (Salmon et al. 2008).

The humus forms may be indicative for environmental changes since they evolve together with the whole ecosystem (Zanella et al. 2001; Ponge 2003; Gobat et al. 2003).

Global stock of carbon in humus forms may play an important role in the global change mitigation level. At the global level in tropical forest the litter decomposition process is particularly rapid (Powers et al. 2009; Wieder et al. 2009), with the difference of tropical mountain forest where cold climatic conditions reduce the litter decomposition increasing the layer thickness. Temperate forest by contrary have a lower decomposition process with humus layer thicker compared with tropical forests. In temperate forest the sequestration of carbon above ground is several orders of magnitude compared with desert, grassland, or shrub vegetation (Curtis et al. 2002). The temperate forest has recently received particularly attention as source or sink in the global carbon cycle (Fan et al. 1998; Canadell et al. 2000).

The global stock of carbon has a plausible range between 1,462 and 1,548 billion tonnes in the topmost metre, 2,376 and 2,456 in the top 2 m (Batjes 1996) and 2,344 billion tonnes in the upper 3 m (Jobbagy and Jackson 2000). At global level the concentration of carbon in the humus forms is difficult to evaluate, due to the variation of humus layer, therefore, most of the studies have been conducted at local or regional level. The humus carbon sequestration in temperate forest has been often neglected; in the Mediterranean Region there are few studies (Andreotta et al. 2011, De Nicola et al. 2014) on the carbon sequestration despite its importance in the general contribution to mitigate the effect of global warming. Mediterranean ecosystem is a unique biome that occurred in different continents. In the Mediterranean basin it borders Europe, Africa and Asia. It is recognized by a marked seasonality and a pronounced dry season of more than five months (Di Castri and Mooney 1977). Vegetation types change from north Africa to the south edge of Europe and the variation is a consequence of geological and climatic history, as well as the evolution of each plant family. This explains the different species composition and species richness according to the area taken in consideration. The Italian peninsula encompasses the complexity of the Mediterranean ecosystem with different vegetation types. The complexity of the vegetation is mirrored by a

complexity of humus forms (Zanella et al. 2001; De Nicola et al. 2013). Recently, terrestrial humus forms in forest ecosystems have been well investigated because of their involvement in carbon cycle and possible consequences on climate warming. Sartori et al. (2007), Garlato et al. (2009a, b) studied soils of Central-East alpine forests; Bonifacio et al. (2011) considered West alpine regions; Ascher et al. (2012) worked in Central Alps; Andreotta et al. (2011) and De Nicola et al. (2007, 2013) realised a synthesis in a large Mediterranean area. All these authors measured interesting differences in humus forms, in terms of functioning and as natural sites of organic carbon stock. Mull, Moder and Amphi forms were found everywhere, whilst Mor are absent in Mediterranean and rare in Alpine forest ecosystems.

Italy has recently developed projects on humus forms classification in different forest ecosystems (Zanella et al. 2001; De Nicola et al. 2007, 2013) but measurements of C sequestration and the related implications in the ecosystems only recently became one of the most important aims in the applied researches on the environmental theme (Andreotta et al. 2011; De Nicola et al. 2014).

In this paper we present one of the results of the long-term research project carried out in the last years in the Castelporziano Reserve (Rome). We aim to evaluate the carbon stock in humus forms in different vegetation types, particularly between evergreen and deciduous woodlands.

2 Methods

2.1 Study area

The study area is located in the evergreen and deciduous Mediterranean lowland oak forest in Castelporziano Reserve (CPR) (Latium). It represents a residual lowland vegetation of Mediterranean forest along the Italian coast. Its plant species composition and community diversity make of CPR a “hotspot” of biodiversity in the Mediterranean basin. CPR has 970 species of vascular plants and 45 plant communities in an extension of 5,800 ha (Pignatti et al. 2001). From a geological standpoint the area is occupied for the most part by highly pedogenized Wurmian eolian sands; the rest of CPR is covered by recent sands, alluvial deposits and, marginally, by pyroclastic materials. Furthermore, in relation to geomorphological and lithological diversity, a large variety of landscapes are present (Dowgiallo and Biondi 2001). The different geomorphological units are represented by Recent Dune, Ancient Dune and Tuffs, hosting different ecosystems, representative of three biomes coexisting at short distance. The oldest biomes, dating back some 100,000 years, are represented by (1) deciduous forest with *Quercus cerris* and *Quercus*

frainetto; (2) *Laurus nobilis* and *Carpinus betulus* forest, a relic of *laurisilva* (Pignatti et al. 2001); while the more recent biome, referable to a time scale of 1,000 years, is the evergreen oak forest with (3) *Quercus ilex* and/or (4) *Quercus suber* (De Nicola et al. 2013); lastly (5) an azonal humid forest spreads in the Recent Dune slacks dominated by *Fraxinus oxycarpa* and *Populus alba*. The species of these different ecosystems in some cases create ecotonal zones of significant extension (Guidotti et al. 2010). The deciduous forest (1) is the most heterogeneous vegetation type due to the coexistence of more humid sectors temporarily flooded and more xeric ones with the presence of mediterranean evergreen species such as *Erica arborea* and *Phillyrea latifolia*. These characteristics are typical of the old lowland forest spreading along the Tyrrhenian sub-coastal belt (Testi et al. 2004).

2.2 Vegetation survey

The classification of vegetation is based on the CPR vegetation map (Pignatti et al. 2001), utilized to select the five different forest vegetation types in which we conducted an integrated study on humus and vegetation:

(1) *Quercus cerris* and *Quercus frainetto* deciduous forest (EQF); (2) *Laurus nobilis* and *Carpinus betulus* mixed woodland (LC); (3) *Quercus ilex* evergreen forest (QI); (4) *Quercus suber* evergreen forest (QIS) and (5) *Fraxinus oxycarpa* humid woodland (FRO).

The area occupied by each vegetation type differs considerably (Table 2).

2.3 Humus and soil analysis

A total of 60 soil/humus profiles were opened and analysed. Among them, a subset of 32 samples, the most representative of the five vegetation types, were selected and better investigated taking samples of each horizon, then submitted to laboratory observations and measures (Table 1). Following the methods of the Italian Soil Science Society (1985) and the USDA Soil Survey Staff (1975, 1993, 1998), for each sample of the 60 soil/humus profiles, we conducted: microscope observation, pH measurement, organic carbon (OC) (Walkley Black method), total nitrogen (N_{tot}) (Kjedajl method) in organo-mineral horizons. In the subset of 32 profiles we additionally measured: OC (Walkley Black method) and N_{tot} (Kjedajl method) in organic horizons; sand and clay contents in organo-mineral horizons (ISO 13317-1: 2001).

The stock of OC is obtained by the product between the percentage of OC and bulk density (bD) of each analysed horizon and subtracting the percentage of volume occupied by coarse fragments (100-frag/100). A coefficient of 10 (cm²/g × Kg/m²) [=10,000 (cm²/m²)/1,000 (g/Kg) =

Table 1 List of the 32 soil and humus profiles: humus form classified, vegetation units (acronyms) and description, thickness for each single horizon and total amount of OC (tons/hectare) in OF + OH + A horizons are reported

Vegetation types	Carbon stock in OF + OH + A (tons/hectare) (differences)	Mean values	Dominant humus form	Extension of vegetation (hectare)
EQF	19–101 (82)	45 ± 21	Moder–Amphi	2,000
QI	30–70 (40)	57 ± 15	Amphi	500
QIS	20–50 (30)	39 ± 14	Amphi–Moder	230
FRO	56–97 (41)	74 ± 17	Mull	142
LC	35–43 (8)	39 ± 4	Mull	50

The OL organic horizon was not considered because of the seasonal variability of its thickness

10 cm²/g × Kg/m²] was introduced to provide an appropriate measurement unit (Kg/m²):

$$\text{OC}_{\text{ineachhorizon}} (\text{Kg/m}^2) = \text{OC} (\%) \times \text{bD} (\text{g/cm}^3) \times \text{depth} (\text{cm}) \times (100 - \text{frag}/100) \times 10 (\text{cm}^2/\text{g} \times \text{Kg/m}^2).$$

Bulk density (bD) was estimated by the use of “pedo-functions” coined by Hollis et al. (1996). This formula provide bD in function of the percentage of organic matter (OM = 2 × OC) in organic horizons (OF, OH), and the percentages of sand (S) and clay (C) in organo-mineral horizons. Compared to the values calculated with a measured bD, the average OC result of these organic horizons was higher when using the pedo-function. However, because the volume of these organic horizons was strongly related to the local conditions (vegetation cover, seasonal variations, micro-topography, macrofauna disturbance) it was always difficult to measure; the obtained values of “real” bulk density were often less trustworthy than the ones estimated by the reported pedo-function (Garlato et al. 2009a).

The results presented in this study consider the 32 complete samples. At each of the 32 sites we associated a phytosociological survey to categorize the vegetation type.

The humus forms were classified in the field according to European Humus Forms Reference Base 2011 (Zanella et al. 2011a), recently modified following Jabiol et al. (2013). The classification consists to observe the sequence and morphological characters of the following humus organic (OL, OF, AH) and organo-mineral layers (A):

- OL—on-decomposed litter;
- OF—accumulation of partly decomposed litter;
- OH—accumulation of zoogenically transformed/well-decomposed material/litter;

- A—the topmost mineral horizon, often referred to the ‘topsoil’. This layer generally contains enough partially decomposed (humified) organic matter that gives the soil a darker colour than the underlying horizons.

The humus forms were classified using two hierarchical levels of classification. The first level units, utilized in this study, are known as equilibrium points (Jabiol et al. 2004), kept in their instable position by ecological attractors in a continuum pattern (Ponge 2005; Ponge and Chevalier 2006; Trap et al. 2011): (1) neutral and biologically active Mull, developing in good climate conditions on neutral parent materials; (2) less active Moder, dominating in colder, dryer or acid less favourable conditions; (3) seasonally active Amphi, which take place in periodically hard climate (Zanella et al. 2011a, b; Ponge et al. 2011).

3 Results

Based on the European Humus Forms References Base, the 60 terrestrial humus forms surveyed in CPR were assigned to 26 Mull, 15 Moder and 19 Amphi. Among them, in the more homogenous sites, the humus/soil profiles were very similar in horizon thicknesses and morphological characters, while in the heterogeneous sites, mainly corresponding to EQF, complex mosaics of humus forms were observed in dynamic patches connected each other's. For this reason, the terrestrial humus forms diversity of the CPR could be well described only with the subset of 32 humus forms: 12 Mull, 10 Moder and 10 Amphi.

These forms gave a broad overview of the humus forms variability in the CPR, related with the different vegetation types:

- Moder–Amphi are dominant in the *Q. cerris*/*Q. frainetto* woodlands (EQF);
- Amphi–Moder are dominant in the *Q. suber* woodlands (QIS);
- Amphi is dominant in the *Q. ilex* woodlands (QI);
- Mull is dominant in the *Fraxinus oxycarpa* woodlands (FRO);
- Mull is dominant in the *Laurus nobilis* and *Carpinus betulus* forest (LC).

Results showed that the carbon stock stored in the organic and organo-mineral horizons of humus and soil, expressed in tons per hectares, had a wide range for each vegetation type, reflecting the variation of the different vegetation types and the variability within each (Table 1).

The vegetation with the highest mean value of carbon stock was represented by the humid woodlands (FRO) with 74 tons per hectare. The second vegetation type was represented by the evergreen forest (QI) with 57 tons per

hectare. The following vegetation was deciduous forest (EQF) with 45 tons per hectare. The remaining last two forest types were the evergreen forest (QIS) and the mixed woodlands (LC) with 39 tons per hectare, respectively (Table 2). Furthermore the deciduous forest EQF had the highest range of values among the samples collected with the lowest of 19 and the highest of 101 tons per hectare. At the same time EQF represents the largest and heterogeneous forest type in the CPR, with 2,000 hectare of extension (Table 2).

The carbon stock concentration in organic (OF + OH horizons) and organo-mineral (A) horizons showed a clear difference between the woodlands QI and FRO *versus* EQF, QIS and LC (Fig. 1). We did not take into account the OL organic horizon because of the seasonal variability of its thickness. The comparison among the group QI and FRO as well as the group of EQF, QIS and LC did not show any statistical difference. Whereas a comparison between the two groups QI + FRO and EQF + QIS + LC showed a clear statistical difference (t test = 2.5, $p < 0.01$).

4 Discussion and conclusion

The aim of the present study was to assess the carbon stock concentration in the different vegetation types and humus forms in the CPR.

Within the carbon stock sequestration, litter production and quality are the main drivers of potential for soil to sequester carbon. In fact the more recalcitrant litter compounds as ligno-cellulose complex, nitrogen content involved in the humification processes received particular attention by scientists starting from Berg (1997), Melillo (2002) until Chertov (1997). The process of soil carbon sequestration starts with litter deposition and the decomposition by macrofauna and thereafter by heterotrophic microorganisms follow. Soil microbial community drives thus the path of carbon mineralization on both short and long temporal scales, thus on humus formation until the final step is achieved where organic matter is ultimately chemically and physically stabilized within the mineral matrix.

The mosaics of CPR vegetation are mirrored by a variability of humus forms and the amount of carbon stocked varied according to this high diversity. Even though the area has the status of Reserve, it has been under some degree of disturbance as drainage projects, and overpopulation of wild animals as boar and deer (Focardi et al. 2001). The disturbance clearly influenced the seedling reproduction of herbaceous plants and juveniles tree species, at the same time it alters the litter decomposition and the humus formation. In CPR the categories of humus

Table 2 The 32 soil and humus profiles grouped according to the five different vegetation types

Locality	Veg.Unit	Vegetation description	Humus form	OL (cm)	OF (cm)	OH (cm)	A (cm)	Tons/hectare (OF + OH + A)
1 Capocotta- Rimessone sud	EQF	<i>Quercus cerris</i> moist woodland	Moder	1	0.75	0	7	19.66
2 Farnete part. 131 area 3	EQF	<i>Quercus cerris</i> warm woodland	Amphi	4	2.75	1.5	5	24.12
3 Figurone	EQF	<i>Quercus cerris</i> moist woodland	Mull	5	3	0	6.5	26.90
4 Farnete part. 134 area 3	EQF	<i>Quercus cerris</i> with <i>Erica arborea</i> woodland	Moder	0.75	1.95	1.75	6.5	27.85
5 Tellinaro 2003	EQF	<i>Quercus cerris</i> woodland	Moder	2.25	0.45	0.5	7	27.88
6 Acquedotto	EQF	<i>Quercus cerris</i> with <i>Erica arborea</i> woodland	Moder	7	4	1.25	4.5	29.36
7 Capocotta 1	EQF	<i>Quercus cerris</i> moist woodland	Mull	3	1.5	0	6	30.19
8 Banditella	EQF	<i>Quercus cerris</i> moist woodland	Moder	1.25	0.75	0	12	32.52
9 Tellinaro	EQF	<i>Quercus cerris</i> moist woodland	Moder	7	2	0.45	6	34.47
10 Farnete part. 134 area 1	EQF	<i>Quercus cerris</i> with <i>Erica arborea</i> woodland	Moder	0.55	0.4	0	7	39.15
11 Strada valle Renaro	EQF	<i>Quercus cerris</i> moist woodland	Mull	2.5	1.25	0	10.5	42.45
12 Farnete part. 134 area 2	EQF	<i>Quercus cerris</i> woodland	Moder	0.75	1.5	3.5	1	45.47
13 Capocotta- Quarto dei Frati	EQF	<i>Quercus cerris</i> warm woodland	Amphi	0.45	0.35	0.75	16	48.71
14 Campo di Rota sud	EQF	<i>Quercus cerris</i> warm woodland	Amphi	0.75	0.6	0.75	13	53.35
15 Figurella	EQF	<i>Quercus cerris</i> moist woodland	Mull	0.75	0.65	0	8	53.95
16 Scopone	EQF	<i>Quercus cerris</i> with <i>Erica arborea</i> woodland	Amphi	0.4	0.4	0.75	28	54.43
17 Capocotta 2	EQF	<i>Quercus cerris</i> warm woodland	Amphi	1	0.45	0.45	10	70.89
18 Capocotta- Rimessone	EQF	<i>Quercus cerris</i> moist woodland	Mull	0.25	0.35	0	15	86.34
19 Tellinaro- Giordano	EQF	<i>Quercus cerris</i> with <i>Erica arborea</i> warm woodland	Amphi	2	1.5	3.5	12.5	101.46
20 Alneto	FRO	<i>Fraxinus oxycarpa</i> woodland	Mull	1	0.35	0	7	56.33
21 Frassineto 1,2,3	FRO	<i>Fraxinus oxycarpa</i> woodland	Mull	3.25	0	0	11	68.18
22 Frassineto 4	FRO	<i>Fraxinus oxycarpa</i> woodland	Mull	4.4	0.35	0	14	97.88
23 Valle Renaro 3	LC	<i>Laurus nobilis</i> and <i>Carpinus betulus</i> woodland	Mull	1	0.1	0	14	35.87
24 Valle Renaro 1	LC	<i>Laurus nobilis</i> and <i>Carpinus betulus</i> woodland	Mull	2.5	0.75	0	9	43.11
25 Scopone- Icceta	QI	<i>Quercus ilex</i> woodland	Mull	1.25	0.35	0	16.5	31.42
26 Ponte Guidone 2	QI	<i>Quercus ilex</i> woodland	Amphi	1.25	0.75	0.65	15	58.60
27 Ponte Guidone 1	QI	<i>Quercus ilex</i> woodland	Amphi	2.25	1.5	0	17.5	67.90
28 Icceta 1	QI	<i>Quercus ilex</i> woodland	Amphi	0.75	0.35	2.5	37	70.78
29 Sughereta Santola	QIS	<i>Quercus suber</i> woodland	Moder	0.75	1.5	1.25	3.5	20.97
30 Spagnoletta 1	QIS	<i>Quercus suber</i> woodland	Moder	2.5	1.75	0	9.5	31.06
31 Spagnoletta 2	QIS	<i>Quercus suber</i> woodland	Mull	3.5	1.85	0	10	50.21
32 Sughereta Grascete	QIS	<i>Quercus suber</i> woodland	Amphi	2.5	1.5	4	19	55.29

In each type the extreme values of carbon stock—in bracket the interval difference between maximum and minimum—and the mean value with the relative standard deviation are shown. Dominant humus forms and the extension for each vegetation type are also reported

EQF *Quercus cerris*/*Quercus frainetto* deciduous forest, QI *Quercus ilex* evergreen forest, QIS *Quercus suber* evergreen forest, FRO *Fraxinus oxycarpa* humid forest, LC *Laurus nobilis* and *Carpinus betulus* mixed forest

observed, Mull, Moder and Amphi, are approximately equally represented with 12, 10 and 10, respectively. They change considerably in relationship with the homogeneous and/or heterogeneous sites of the forest types.

Carbon concentration is a central topic for international programmes of global warming mitigation as REDD + (<http://www.un-redd.org/>). The stock of carbon

in forest and in soil has been studied for a long period in temperate areas (Curtis et al. 2002; Liski et al. 2002) as well as in tropics (Powers et al. 2009; Gibbs et al. 2007). By contrary the evaluation of carbon stock in humus forms has been often neglected and recently has been considered its potential role for the total carbon stock in a forest ecosystem (Janzen 2006). To our knowledge in central

Italy there are no yet available data to compare with. Bonifacio et al. (2011) estimated in North West Italian woodlands with a range of carbon stock for conifer (f.i. *Picea abies*, *Larix decidua*) and broadleaf forest (*Castanea sativa*, *Alnus glutinosa*, *Fagus sylvatica*) of 2.7–9.5 kg m⁻². Despite the difference on forest types and on methodological approach on the vegetation survey between the two studies, which cannot be properly compared, the range of carbon stock in CPR is quite the same; from 1.9–10.1 kg m⁻². This range is represented by the ample differences observed in the deciduous forest of EQF. This vegetation type is the more heterogeneous with the widest cover area and the humus form varies between Moder and Amphi. The other four forest types have a carbon stock, which is within the EQF range (Table 2). Forests with Mull humus form have a range from 5.6–9.7 kg m⁻² and 3.5–4.3 kg m⁻², FRO and LC, respectively. Although the geographic, methodological and vegetation differences of the two studies, we can underline that there is a similar trend of variability within the humus forms and about the carbon concentration. The work from Bonifacio et al. (2011) is based on an altitudinal gradient between 365 and 2,027 m, whereas CPR is a single locality on an altitude gradient between 0 and 85 m. a.s.l. and the similar mesoclimatic conditions for the entire Reserve.

Furthermore a trend in carbon content was statistically supported by Mull to Amphi or Moder forms: Amphi was found as the richest in organic carbon—OC in the West wing of the Alpine chain (Bonifacio et al. 2011), while Moder was in first place in the centre (Sartori et al. 2007; Garlato et al. 2009a; Ascher et al. 2012) and East (Garlato et al. 2009b) of the chain; all over, the Mull form (average of a large range of values) was poorer in OC than the other forms, even if, on the Dolomite Alps in the Veneto Region, Mull form with the highest storage capacity has been found (Faggian et al. 2012). In Mediterranean area on the contrary, Mull had the highest stock of OC, near to Amphi and decidedly more (2.5×) than Moder (Andreetta et al. 2011; De Nicola et al. 2014).

Despite the limited area of CPR and the similar macro and mesoclimatic conditions there is a large variability inside the vegetation types, humus forms and soil capability to storage organic carbon.

4.1 Deciduous oak forest

The deciduous forest characterized by *Quercus cerris*—EQF has the widest cover area, with a large variability from warmer to more humid stands (Testi et al. 2006, 2013), that can probably explain the largest range of humus carbon stock (Table 1) and the two different humus forms classified: Moder, in the more humid stands of EQF, while Amphi in the warmer. Humid conditions, locally created by

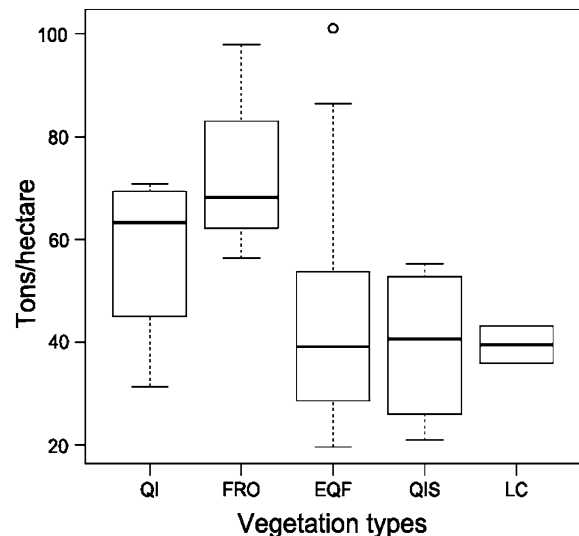


Fig. 1 Changes in carbon stock concentration in humus (OF + OH horizons) and soil (A) horizons in the Mediterranean woodlands of Castelporziano Reserve. The 32 soil samples are categorized and grouped according to the vegetation types: EQF *Quercus cerris*/*Quercus frainetto* deciduous forest, QI *Quercus ilex* evergreen forest, QIS *Quercus suber* evergreen forest, FRO *Fraxinus oxycarpa* humid forest, LC *Laurus nobilis* and *Carpinus betulus* mixed forest. Box plots show mean (bold line), the 25th and 75th percentile (box), 95 % of confidence interval (whiskers), and outliers (empty circle)

temporary pools (De Nicola et al. 2013), increase the acidity of the substrates and the oxygen deficiency (water saturated) that slow down the process of litter biodegradation, limiting the potential growth of soil fauna, which generally avoids acid substrates and water-saturated soils. These conditions favour Moder forms, whilst periodic dryness and hard conditions for biological activity at the soil surface coexisting with water availability in deep soil layers favour Amphi forms. This EQF heterogeneity is responsible for the variability of humus forms, from Moder to Amphi, and subsequently for the largest range of carbon stock: from 19 tons per hectare in Moder to 101 tons per hectare in Amphi forms.

4.2 Evergreen oak forest

The two evergreen woodlands characterized by *Quercus ilex*—QI and *Quercus suber*—QIS have the common trait of broad leaves with leather consistence. This morphological aspect results with a longer process for the decomposition dynamics of the two species leaves (Fioretto et al. 2007). These two vegetation types cover nearly one-third of the Reserve and represent the typical Mediterranean sclerophyllous vegetation. As the EQF woodlands, also the sclerophyllous evergreen forest QI and QIS have the two humus forms: Moder and Amphi prevail in QIS on acid substrates, while Amphi prevails in QI. In addition, a lower

concentration of carbon was found in the QIS—39 tons per hectare—due to the presence of the Moder form with the slowest process of biodegradation (Table 2).

4.3 Deciduous humid forest

The two remaining forest types are FRO characterized by *Fraxinus oxycarpa* and LC characterized by *Laurus nobilis* and *Carpinus betulus*. These vegetation types have an extension of 142 and 50 hectares, respectively, representing the smallest woodland formations of the CPR. Both of these two forest types have Mull humus form. Despite its size FRO has the highest carbon stock with 74 (± 17) tons per hectare—like on the Dolomite Alps (Faggian et al. 2012) and Mediterranean area (Andreotta et al. 2011)—whereas LC has the least stock of only 38 (± 4) tons per hectare like the Alpine chain (Garlato et al. 2009a). The differences in the carbon stock found between these two forest types depend on their different geomorphology and floristic composition.

The woodlands characterized by *Fraxinus oxycarpa* occur in CPR in humid and temporarily flooded areas. High temperatures and more frequent precipitation are favourable to pedofauna and to an efficient turnover of organic matter. Mull forms develop with a high content of organic carbon. The organic carbon is easily fixed favoured both by the presence of mesohygrophile species such as *Populus* spp., *Ulmus minor* and by the flat morphology. The historical management of the CPR as the drainage project, however, reduced the FRO vegetation only in a small part of the Reserve, whereas in normal condition the forest type should cover a wider area with the consequence of a more substantial carbon sequestration. Moreover, from the vegetation point of view FRO represents a residual forest related to specific climatic, hydrological, historical and phytogeographical conditions. These forests have originated at the end of the Quaternary period beginning of the Holocene (Devillers et al. 1991), when a rapid increase of the temperature and humidity began. They are typical for the transitional continental and Mediterranean climate with mild and humid winters.

The forest characterized by *Laurus nobilis* and *Carpinus betulus*—LC grows along the ravines of CPR only in a small portion of the study area where the organic carbon is easily leached. The low level of carbon stock—38 tons per hectare—of this forest type, which occupies the smallest extension in the CPR, is similar to the evergreen forest with *Quercus suber*—QIS, typical xeric vegetation adapted to the Mediterranean dry seasonality. LC represents a mixed deciduous *Carpinus betulus* and evergreen *Laurus nobilis* tree. It is a unique and rare relic of *laurisilva* of warmer and humid climate when broad and evergreen trees species were more abundant on the Italian former land. This

historical vegetation can be observed today, with different species but similar plant function traits in subtropical forests where the coexistence of deciduous and evergreen trees represent the normal forest structure. The origin of the *laurisilva* could explain the reason that even if we have classified a Mull humus form like in FRO, the carbon stock is lowest, close to the subtropical forest (Keith et al. 2009).

We can conclude from this preliminary study that the humus forms have potential impact on the carbon stock in the overall storage of a forest ecosystem and may be indicative for environmental changes since the humus forms evolve together with the whole ecosystem. The present study shows the same results compared to Andreotta et al. (2011) confirming that in a Mediterranean area Mull and Amphi forms can store more organic carbon than the Moder. On this basis, we can reasonably expect that in a Mediterranean forest area, the first 20 cm of soil could store much more organic carbon (nearly twice) if classed as Amphi or Mull than Moder. Graefe (2007) proposed that the Amphi form arrived in Germany only a few years ago and could be used as an indicator of warming climate change. Therefore, it is important to consider and estimate the contribution of humus carbon stock when the carbon stock is evaluated at the regional and national level. The concentration of carbon stock for soil, above and below ground biomass and humus forms will give us complete and reliable information on the total amount of carbon sequestration for the forest ecosystems.

Furthermore, we have compared our results with the Latium Region carbon stock (<http://www3.corpoforestale.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPag./248>), to have a general overview, even if the comparison is not proper due to the low resolution of the vegetation map of the Reserve (Pignatti et al. 2001), and the inclusion in the Latium Region data of *Fagus sylvatica* forest which covers a vast extension of the Region. CPR stocks a mean of 52 tons per hectare; the Region has 84.7 tons per hectare. These data should encourage a better management of CPR forest vegetation to increase the carbon stock in the different humus forms. In particular, reduction of wild fauna disturbance can reduce the surface of warm deciduous *Quercus cerris* forest with *Erica arborea* increasing the extension of the moister *Quercus cerris* stands and consequently the carbon stock. More importantly the relic vegetation of FRO with *Fraxinus oxycarpa* can expand its presence if drainage management of CPR will be accurately studied, expanding the flooding surface of forest. This conservation advice will not lead only into the direction of increase the carbon stock with positive feedback on warming climate mitigation at regional level, but even more is the importance of expanding a vegetation type which represents a relic of humid lowland forests. Being of high conservation value this Habitat has been included in

sites of the European Ecological Network NATURA 2000. This vegetation type is in general critically endangered and has fragmented distributions; it is recorded in CPR and only in few other sites in central Italy (Pignatti 1998). Therefore, it is important to pay more attention on it integrating the action of European project of vegetation and landscape conservation.

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