UNIVERSIDAD DE JAÉN

Departamento de Biología Animal,
Biología Vegetal y Ecología

TESIS DOCTORAL

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Soil organic carbon sequestration in olive groves of Andalusia: effect of the managements on soil organic carbon dynamics

Secuestro de carbono orgánico en suelos de olivar andaluz: efecto de los manejos en la dinámica del carbono orgánico en el suelo

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Memoria presentada por
JOSÉ LUIS VICENTE VICENTE
para optar al Grado de Doctor por la Universidad de Jaén
"Mención Doctorado Internacional"

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Fdo. José Luis Vicente Vicente
Jaén, a 9 de Noviembre de 2016

D. ROBERTO GARCÍA-RUIZ, Catedrático de Ecología

CERTIFICA:

Que la presente memoria titulada **"Soil organic carbon sequestration in olive groves of Andalusia: effect of the managements on soil organic carbon dynamics"**; **"Secuestro de carbono orgánico en suelos de olivar andaluz: efecto de los manejos en la dinámica del carbono orgánico en el suelo"**, ha sido realizada bajo mi dirección. Y considerando que representa trabajo de Tesis, autorizo su presentación y defensa para optar al grado de Doctor en Ciencia y Tecnología de la Tierra y el Medio Ambiente, con Mención de Doctor Internacional.

Jaén, 9 de noviembre de 2016

Fdo. Dr. Roberto García-Ruiz

JAÉN, NOVIEMBRE DE 2016

DIRECTOR

This Doctoral Thesis has been directed by Dr. Roberto García-Ruiz, professor of Ecology of the Department of Animal Biology, Vegetal Biology and Ecology of the University of Jaén (Spain)

DIRECTOR

Esta Tesis Doctoral ha sido dirigida por Dr. Roberto García Ruiz, catedrático de Ecología del Departamento de Biología Animal, Biología Vegetal y Ecología de la Universidad de Jaén (España)

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En estos momentos también me acuerdo de algunos profesores de la Facultad de Ciencias Agrarias y Ambientales de la Universidad de Salamanca, donde tuve la inmensa suerte de poder estudiar mi carrera. Con Miguel Lizana aprendí que el respeto a los animales nace del conocimiento profundo sobre ellos. Nunca podré olvidar su increíble pasión por los animales, la cual lograba transmitirnos a todas las personas que asistíamos a cualquiera de sus asignaturas. Gracias a él muchos íbamos con entusiasmo a las clases (¡incluso a los documentales que nos ponía a las dos de la tarde!). Con José Abel Flores Villarejo tuve conciencia real del problema del Cambio Climático, de cómo todo en este planeta está interrelacionado (jamás había imaginado que la Paleoclimatología y la Oceanografía estuvieran tan ligadas), de lo increíblemente bonito que puede llegar a ser hacer Ciencia (y de cómo se puede ser uno de los mejores oceanógrafos del mundo en una ciudad tan de interior como Salamanca). Finalmente, me apena profundamente no poder dar las gracias en persona al profesor Enrique Clemente. Sus clases de Ordenacion del Territorio eran toda una verdadera introducción al mundo de la problemática socioambiental. Parte de tus enseñanzas se quedan plasmadas en algunas de las portadas de este trabajo (sé que te hubieran encantado y que te habrías reído, conservando siempre esa cara permanente de buena persona). Esta Tesis va dedicada especialmente a ti.

Las portadas de esta Tesis han sido fruto del trabajo de un artista, de Miguel Ángel Ojeda García, un grandísimo artista que ha sabido plasmar a la perfección lo que quería expresar en cada una de las imágenes. El esfuerzo para entender las explicaciones de mis emails (con lo caótico que soy con esas cosas), y poder plasmarlo todo en una imagen, todo eso solo lo puede hacer alguien que tiene unas capacidades extraordinarias. Ójala ese don que tienes sea algún día valorado como se merece por la sociedad. Muchísimas gracias, Miguel Ángel, porque tu trabajo ha sido inmejorable y le ha dado a esta Tesis el carácter artístico-reivindicativo que tanto deseaba.

Durante esta Tesis he realizado dos estancias. La primera de ellas la realicé en Aberdeen (Escocia). I would like to thank Pete Smith and Marta Dondini for their support during my stay. Pete is one of the best researches I have ever met. But he is even a better person. His humility, perseverance and dedication are absolutely amazing. However, his best trait is that he believes in what he does. That is the best merit in a scientist. Thank you very very much, Pete.

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Uno nunca puede ni debe olvidar sus orígenes, aunque seamos ciudadanos del mundo. Soy y seguiré siendo de Masueco de la Ribera (Salamanca), de Los Arribes del Duero, zona olvidada completamente por las autoridades políticas de este país. Sin embargo, seguimos, y seguiremos luchando, como los olivares que aún perviven allí, para reivindicar tener las mismas oportunidades que en otras partes del Estado. Mi otra mitad, pertenece a Armenteros (Salamanca), en las estribaciones del Sistema Central, zona igualmente olvidada por los gestores políticos. A pesar del olvido, algunos no olvidamos, y de ese olvido nacen las fuerzas para luchar contra las injusticias.

Y qué decir de Andalucía. Tengo tantísimas cosas que agradecer… Aquí, en Andalucía es donde realmente he crecido como persona, he encontrado mi lugar, he conocido a la gente más interesante, he aprendido a vivir de un modo diferente. Mis compañeras y, sin embargo, amigas almerienses, María y Cristina tuve que ir a Panamá para descubrirlas. Ese grandísimo y maravilloso viaje (quitando alguna que otra cosilla rara que pasamos…) que jamás olvidaré. Con vosotras pasé días con una tribu indígena del Caribe, atravesé la selva panameña, me llené de picaduras de mosquito… Con vosotras disfruté en Almería, en Cabo de Gata, y con vosotras espero pasar muchos momentos más en la vida (incluidas las maravillosas tapillas almerienses y esos bares de los ochenta).

En Andalucía he descubierto los movimientos sociales, la lucha en la calle, la protesta, la humildad de quien no tiene nada, la sonrisa y el humor aunque no se llegue a final de mes. He podido desarrollar mis capacidades comunicativas en Uniradio Jaén. Mi especial cariño y agradecimiento a su director Julio Ángel Olivares, (¡qué grande es!). Cuántas risas, momentos increíbles y otros algo tensos he vivido en los micros de Uniradio Jaén. Ojalá algún día Julio reciba el homenaje que se merece, porque su inmenso trabajo es imposible de igualar por cualquier otra persona. Y si de movimientos sociales hablo, tengo que nombrar a mis compañeras y compañeros del 15M Granada. De vosotras sí que he aprendido. Gracias a vosotras creo en la autogestión, en el poder de la gente de la calle, en que es posible hacer grandes cosas con manos pequeñas, en que las mujeres son la salvación de esta humanidad que a veces parece que se dirige hacia el abismo. Sin mujeres, sin feminismo, no hay posibilidad de salvación.

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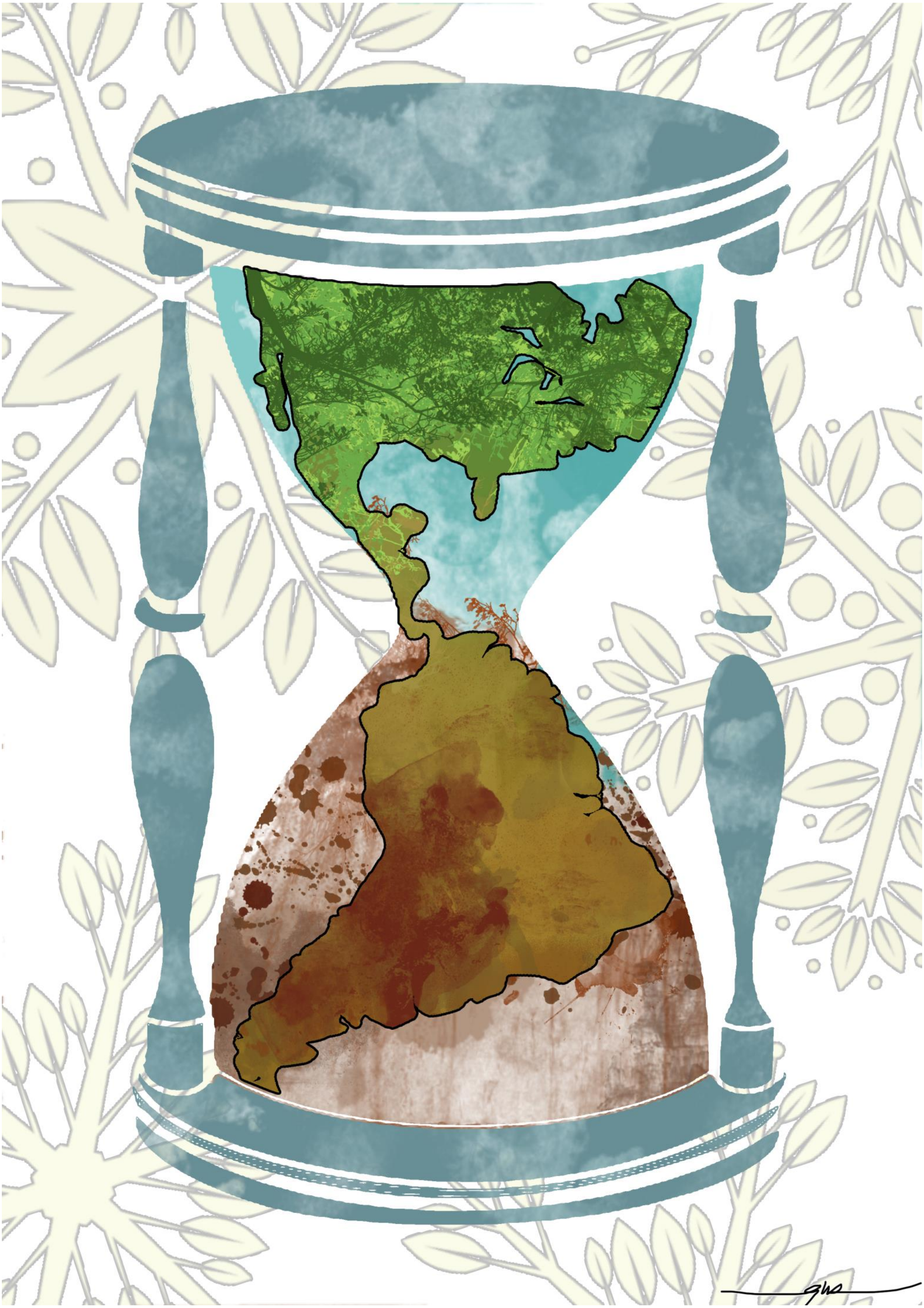
"Hacer buenas preguntas ayuda a que no sea contagiosa,
la idiotez es colectiva cuando nadie se cuestiona las cosas".
Calle 13. "Los Idiotas", álbum Multi-Viral (2014).

A todas y todos los trabajadores del campo



ABSTRACT

Currently, Global Warming is one of the greatest threat the humanity has to face. Agriculture is estimated to contribute about 15 – 20 % of greenhouse gas emissions, of which CO_2 is one of the most important. Andalusia (Southern Spain) is a region where agriculture plays a key role on the social, cultural and economic life. More than 40 % of the total crop surface of Andalusia are olive groves and is the dominant landscape in many areas. Currently, soils of the majority of the olive groves are bare and managed combining tillage with the application of pre- and post-emergence herbicides. Nutrients are incorporated by applying inorganic fertilizers. Even in high-slope plots the conventional tillage is widespread. All these practices have led to a dramatic decrease on soil organic carbon content, and values under 1 % of soil organic carbon content are very common. In addition, the lack of a soil cover have led to high soil losses due to water erosion, especially in areas with high slopes, feeding a vicious circle which leads to a heavy soil organic carbon depletion. Therefore, it is not surprising the scientific efforts aimed to reestablish the level of soil organic carbon content. A suitable solution consists of the implementation of sustainable managements so that olive grove soils can increase the soil organic carbon content. This increase on soil organic carbon content is usually called "carbon sequestration" or "soil organic carbon sequestration". In this Thesis, some sustainable managements (or "recommended management practices") have been assessed in terms of soil organic carbon sequestration, with especial emphasis on unseeded vegetation cover in the inter-row area of olive groves (i.e. weed-cover management). The effects of the presence of a weed-cover, in addition to other managementes which include the application of composted olive mill pomace and the shreded pruning residues, on soil organic carbon content, soil organic carbon dynamics, soil respiration and on reducing organic carbon losses as a consequence of erosion processes were assessed under lab and field conditions. Thus, soil organic carbon content under a weed-cover management was typically two times in comparison to that of the soils under a conventional tillage. This increase was not equally distributed among depth, as it was much higher in the top 5 cm. This increase in the soil organic carbon content was due to the incorporation on the soil surface of the weed-cover biomass. However, biomass production varied a lot. Thus, the incoming organic carbon through the aerial biomass in ten different weed-covered plots ranged in a single year 0.2 – 1.0 t C ha^{-1}. Furthermore, the increase in the soil organic carbon content was due especially to an increase in the unprotected and physically

protected soil organic carbon pools. The protective capacity of the weed cover was clear, and after one hydrological year, the weed-cover management decreased soil organic carbon losses by erosion by 70 Kg C ha^{-1} in a plot with a mean slope of 11 %. This decrease was mainly due to a decrease of the physically protected pool losses. Soil organic carbon content was not only affected by the management, but also by some mineralogical and geochemical properties (e.g. pH, cabonates content, quartz or texture). Higher soil organic carbon content was found for olive grove soils with basic features (about two times that found for acid soils). This increase was especially due to an increase in the unprotected and physically protected pools. Interestingly, the amount of the biochemically protected pool was significantlty higher in siliceous soils. This might be related to the effect of the lower pH on clay and soil organic mater negative charges. The saturation hypothesis of the different soil organic carbon pools was tested. Results suggest that only the chemically protected pools (within soil microaggregates and in the fine fraction) would fit a saturation dynamic, being the saturation level for these fractions about 30 and 50 % in the non-covered and weed-covered soils, respectively. Nevertheless, this saturation dynamic might be visible only when wide ranges of total soil organi carbon are assessed (i.e. about 100 mg C g^{-1} soil). Results of lab and field studies were completed with those obtained in a meta-analysis and after running a model. In the meta-analysis (51 references and 144 comparisons) annual soil organic carbon accumulation rate due to the presence of a vegetation cover, and compared to the conventional tillage, in olive groves was about 1.1 t C ha^{-1}. It was higher than the mean value resulting from the application of organic amendments (e.g. composted olive mill pomace) or tree residues (e.g. pruning debris) were higher (5.4 t C ha^{-1} yr^{-1}), but depended strongly on the amount of the input. The application of the RothC model feed with lab data showed higher soil organic carbon sequestration rates during first 10 years after changing to a sustainable management (between 2 and 3 times than that found for the first 50 years), and a clear trend in decreasing the soil organic carbon sequestration efficiency throughout time. The different scenarios resulting after applying the model also showed that the soil organic carbon sequestration would be higher if others management practices, such as the application of olive pruning debris and composted olive mill pomace, are combined with the weed-cover management, thus obtaining values of soil organic carbon sequestration rates between 1.1 – 1.3 and 0.8 – 1.1 t C ha^{-1} yr^{-1}, respectively. These values mean an accumulation of soil organic carbon after 100 years between 30.4 – 39.9 and 25.5 – 36.0 t C ha^{-1}, respectively. However, composted olive mill pomace and biomass from the vegetation cover showed different decomposition dynamics. Thus, in a lab experiment, after 313 days, the percentage of the cumulative respired C-CO_2 of the total organic carbon added after the application olive mill pomace was less than 10 %, whereas the weed-cover biomass ranged 30 – 60 %, suggesting that composted olive mill pomace is mainly formed by recalcitrant substances. On the other hand, soil respiration was also measured in field conditions in weed-covered and non-covered plots, finding up to 9 times higher respiration in soils under weed-cover management in spring. Nevertheless, the carbon assimilation might be much higher in the weed-covered soils due to the photosynthetic activity of the spontaneous vegetation cover during the growing period. In summary, results of this work show that the implementation of a weed-cover management in olive groves in Andalusia leads to a significant increase in the soil organic carbon content, especially in the unprotected and physically protected pools and to a decrease of organic carbon losses, especially the physically protected organic carbon, because of the erosion in olive groves with a relative high slope. Furthermore, results also show that a combination of the weed-cover with olive mill pomace or olive pruning debris would be a suitable management in terms of soil organic carbon sequestration. The low current soil organic carbon saturation level in olive groves makes that these managements could be implemented with high organic carbon sequestration efficiencies.

RESUMEN

Actualmente, el Cambio Climático constituye el mayor reto al que la humanidad tiene que enfrentarse. Se estima que la agricultura contribuye entre un 15 – 20 % al total de estos gases, de los cuales el CO_2 es el más importante. Andalucía (sur de España) es una región donde la agricultura juega un papel clave en el ámbito económico, social y cultural. Más de un 40 % del total de superficie cultivada corresponde a olivar. Actualmente, la mayoría de olivares se encuentran bajo un manejo convencional, combinando el labrado con la aplicación de herbicidas de pre- y post-emergencia. Los nutrientes son incorporados a través de la aplicación de fertilizantes inorgánicos. Incluso en áreas de gran pendiente el manejo convencional se encuentra ampliamente extendido. Todas estas prácticas han conllevado un importante descenso en el contenido en carbono orgánico en el suelo y, así, es muy frecuente encontrar suelos de olivar con contenidos en carbono orgánico por debajo del 1 %. Paralelamente, al mismo tiempo, la falta de una cubierta vegetal conlleva mayores pérdidas de suelo por erosión hídrica, especialmente en zonas de elevada pendiente, alimentando un círculo vicioso que conlleva un importante descenso en el contenido en carbono orgánico en el suelo. Así, no es sorprendente el hecho de que los esfuerzos de los investigadores se centren en restablecer el nivel de contenido en carbono orgánico en el suelo. Así pues, una solución apropiada consiste en la implementación de manejos sostenibles con el fin de que los suelos de olivar puedan incrementar su contenido en carbono orgánico. Este incremento en el contenido en carbono orgánico en el suelo es conocido como "secuestro de carbono" o "secuestro de carbono orgánico en el suelo". En esta Tesis se han evaluado en términos de secuestro de carbono algunos manejos apropiados (o "prácticas de manejo recomendadas"), prestando especial énfasis al mantenimiento de la cubierta vegetal espontánea especialmente en la entrecalle del olivar (también llamado a lo largo de este trabajo como "weed-cover management"). Así, se evaluaron en condiciones de laboratorio y campo los efectos de la presencia de la cubierta vegetal, junto con otros manejos que incluyen la aplicación de alpeorujo compostado o restos de poda triturados, en el contenido en carbon orgánico en el suelo, dinámica de las fracciones de carbono orgánico, respiración del suelo y reduccion de pérdidas de carbono orgánico por erosión. Así, el contenido en carbono orgánico en suelos con cubierta vegetal espontánea fue alrededor de dos veces superior al obtenido en suelos sometidos a manejo convencional. Este incremento no se distribuyó de igual forma entre las distintas profundidades, sino que fue mucho más elevado en los

primeros 5 cm de suelo. Este incremento en el contenido en carbono orgánico en el suelo se debió a la incorporación al suelo de la biomasa de la cubierta vegetal. Sin embargo, la producción de biomasa varió ampliamente. Así, el carbono orgánico que entró a través de la biomasa aérea en diez parecelas distintas con cubierta vegetal varió en un solo año entre 0.2 – 1 t C ha^{-1}. Además, este incremento en el contenido en carbono orgánico se debió especialmente al incremento en las fracciones no protegida y físicamente protegida. La capacidad protectora de la cubierta vegetal fue clara y, tras un año hidrológico, el manejo con cubierta redujo las pérdidas de carbono orgánico en el suelo en 70 Kg C ha^{-1} en parcelas con pediente media del 11 %. Este descenso fue debido especialmente a un descenso en las pérdidas de carbono orgánico físicamente protegido. El contenido en carbono orgánico no solo se vio afectado por el manejo, sino también por algunas propiedades mineralógicas y geoquímicas (ej. pH, contenido en carbonatos y cuarzo, o la textura). Los suelos carbonatados acumularon mayor contenido en carbono orgánico (aproximadamente el doble que los suelos silíceos). Este incremento se debió sobre todo a un aumento en las fracciones no protegida y físicamente protegida. Por contra, la fracción bioquímicamente protegida mostró valores más elevados en los suelos siliceos. Esto podría ser debido al efecto del pH sobre las cargas negativas de las arcillas y la propia materia orgánica. Por otra parte, se evaluó también la hipótesis de saturación de las distintas fracciones de carbono orgánico. Los resultados sugieren que solamente la fracción químicamente protegida (dentro de los microagregados y en la fracción fina del suelo) seguiría una dinámica de saturación, siendo el nivel de saturación para estas fracciones de alrededor de 30 y 50 % en los suelos sin cubierta y con cubierta vegetal, respectivamente. No obstante, esta dinámica de saturación sería únicamente visible cuando se tienen en cuenta elevados rangos de contenido en carbono orgánico total (alrededor de 100 mg C g^{-1} suelo). Los resultados de laboratorio y campo se complementaron con los obtenidos tras la realización de un meta-análisis y la aplicación de un modelo. En el meta-análisis (51 referencias y 144 comparaciones), se obtuvo una tasa media anual de secuestro de carbono en olivares con cubierta vegetal de 1.1 t C ha^{-1}, en comparación con olivares sometidos a manejo convencional. Los valores tras la aplicación de abonos orgánicos (ej. alpeorujo compostado) o residuos de los árboles (ej. restos de poda) fueron mayores (5.4 t C ha^{-1} $año^{-1}$), pero dependieron fuertemente de la cantidad del input que se aplicó. Los resultados del modelo RothC mostraron mayores tasas de secuestro de carbono durante los 10 primeros años tras el cambio a un manejo sostenible (entre 2 y 3 veces más que el calculado para los primeros 50 años), y una clara tendencia a reducirse la eficiencia en el secuestro de carbono con el tiempo. Los diferentes escenarios resultantes de la aplicación del modelo mostraron que el secuestro de carbono orgánico sería mayor en el caso de que otros manejos fueran combinados con el mantenimiento de la cubierta vegetal, como la aplicación de restos de poda o alpeorujo compostado, obteniendo valores de secuestro de carbono de entre 1.1 – 1.3 y 0.8 – 1.1 t C ha^{-1} $año^{-1}$, respectivamente. Esto se traduciría en un secuestro de carbono en 100 años de entre 30.4 – 39.9 y 25.5 – 36.0 t C ha^{-1}, respectivamente. Sin embargo, el alpeorujo compostado y la biomasa de la cubierta vegetal mostraron diferentes dinámicas de descomposición. Así, en un experimento de laboratorio, tras 313 días, el porcentaje de C-CO_2 respirado acumulado tras este período respecto al total de carbono añadido fue de menos de 10 % en el alpeorujo compostado, mientras que en el caso de la biomasa de la cubierta varió entre 30 – 60 %, sugiriendo que el alpeorujo compostado está principalmente formado por sustancias de carácter recalcitrante. Por otra parte, la respiración del suelo medida en condiciones de campo en parcelas con cubierta y sin cubierta vegetal fue hasta 9 veces mayor en la parcela con cubierta en primavera. Sin embargo, la asimilación de carbono debida a la actividad fotosintética sería mucho mayor en los suelos con cubierta durante el período de crecimiento de ésta. En resumen, los resultados de este trabajo muestran que la implementación de la cubierta vegetal en olivares en Andalucía conlleva un incremento en el contenido en carbono orgánico en el suelo, especialmente en las fracciones no protegida y físicamente protegida y una reducción en las pérdidas de carbono orgánico en el suelo (especialmente del carbono orgánico protegido) por erosión en olivares con relativa elevada pendiente. Además, los resultados muestran también que una combinación del manejo con cubierta con la aplicación de alpeorujo compostado o restos de poda sería una solución apropiada desde el punto de vista del secuestro de carbono. El actual bajo nivel de saturación de carbono orgánico permite que estos manejos puedan ser implementados con altas tasas de eficiencia en el secuestro de carbono.

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Romperemos las nubes negras.
Que nos engañan, que nos acechan.
Abriremos un mundo nuevo, sin fusiles ni venenos.
Enrique Morente

CHAPTER I

Introduction. Climate change mitigation and the role of the agriculture: Soil organic matter and carbon dynamics. Theoretical framework

1. Soil organic carbon in agricultural soils and climate change mitigation

1.1 Presence and evolution of atmospheric CO_2

The mean global temperature of the Earth has been increased by 0.85 °C (0.65 to 1.06 °C) since 1880 to 2012 as a consequence of the increase in the greenhouse gas emissions (GGE) (IPCC, 2013). In addition to the well verified multi-decadal warming, global mean surface temperature shows strong decadal and interannual variability (IPCC, 2013). For example, the rate of warming over the past 15 years (1998 – 2012) shows a mean increase per decade of 0.05 (-0.05 – 0.15) °C, which is half of the decadal rate calculated since 1951, which averaged 0.12 °C (IPCC, 2013). There are many consequences of this increase in the average global temperature, most of them are interrelated and with a very difficult prediction (e.g. increase in the sea level, decrease in the agricultural productivity in low latitudes, increase of droughts and floods events, increase in the strength of the waves with a higher coastal erosion, higher presence of diseases like diarrhoea or cardiorespiratory diseases) (IPCC, 2007). These and other observed effects are mainly due to the increase in the troposphere's temperature as a consequence of the increase in the GGE concentrations.

Of all greenhouse gases, CO_2 is the most abundant in the atmosphere and showed the highest relative annual increase over time (WMO, 2011). The annual concentration in the atmosphere was about 397 ppm in 2014. It represents about a 40% higher than the levels of the preindustrial era (IPCC 2013)

There are many scientific proofs demonstrating that part of the increase in atmospheric CO_2 is due to fossil fuel use. Indeed, during 2015[1] about 35.7 (±1.8) Gt[2] of CO_2 was emitted due to fossil fuel consumption and due to the industry which represents about 59% of that emissions in 1990 (figure 1).

[1] The projection of CO_2 emissions from fossil fuel and industry for 2015 shows a slight decrease (0,6 %) in comparison to that quantified in 2014 (35.9 Gt CO_2) (Le Queré *et al.*, 2015)

[2] 1 GtC= 3.664 billion tonnes CO_2 = 3.664 GtCO_2

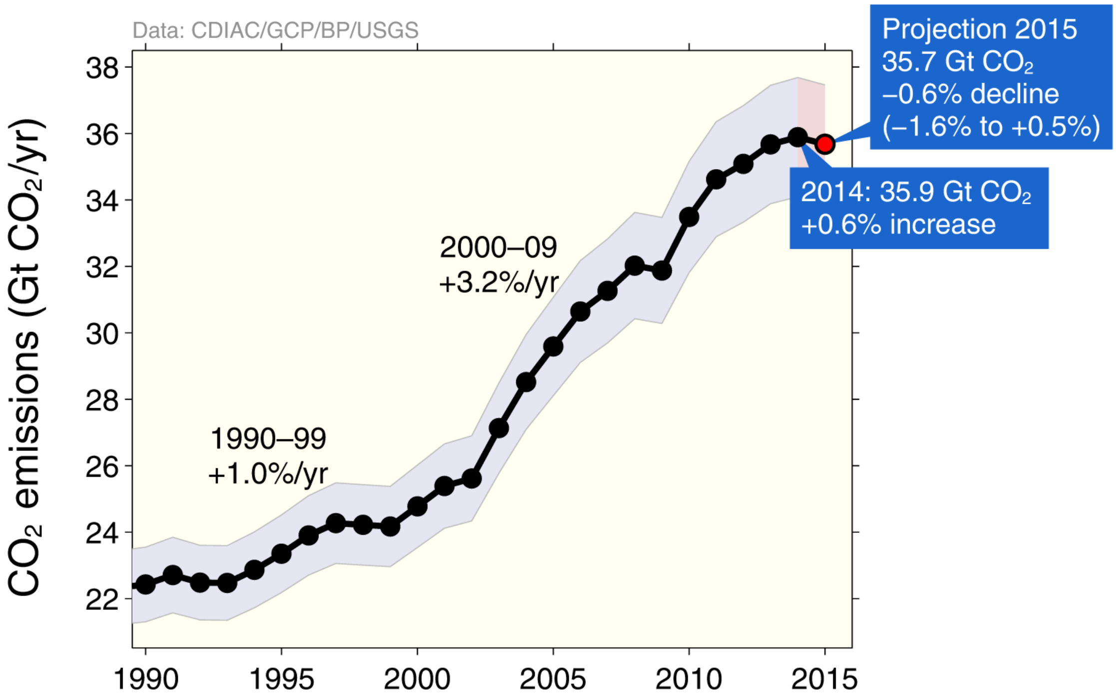


Figure 1. Evolution of the CO_2 emissions (Gt CO_2 yr^{-1}) from 1990 until 2015. Notes the strong increase during the last decade, but a slight decrease during the last two years. Le Quéré *et al.* (2015).

Together with these CO_2 emissions, there are others manmade contributions such as these due to land use management, which account for about 10% of the total CO_2 emissions. Nevertheless, only 44% of the land use derived emissions are transformed in CO_2 concentrations. The rest of the CO_2 emissions are absorbed by terrestrial vegetation (30%) and oceans (26%), since vegetation and oceans are acting as a CO_2 sinks (Le Quéré *et al.*, 2015).

1.2 The C among the different "spheres"

The distribution of the C in the Earth is not uniform, as it depends on the "sphere". Thus, in the atmosphere the amount of C is about 760 Pg[3], in fossil fuels is estimated to be storaged about 4130 Pg (3510 as coal, 230 Pg as oil, 140 Pg of gas and 250 Pg in peat). The oceans stock the highest amount of C, about 38400 Pg (670 in the upper layer, 36730 Pg in deeper layers both as inorganic C, and 1000 Pg as organic C). The terrestrial

[3] 1 Gt = 1 billion tonnes = 1×10^{15}g = 1 Petagram (Pg).

vegetation contains about 560 Pg, whereas in the pedologic sphere the figure amounts about 2500 Pg (1550 Pg C as organic C, and 950 Pg as inorganic forms) (Lal, 2008). These inorganic and organic C stocks exchange in such a way that one can be a sink or a source of C. Figure 2 shows an overview of the different C fluxes between the biomass, soil, ocean and the athmosphere. Consequently, even a small annual percent change in the amount of C stored or released from SOC stocks could easily affect the net change in atmospheric-CO_2 (Smith, 2012).

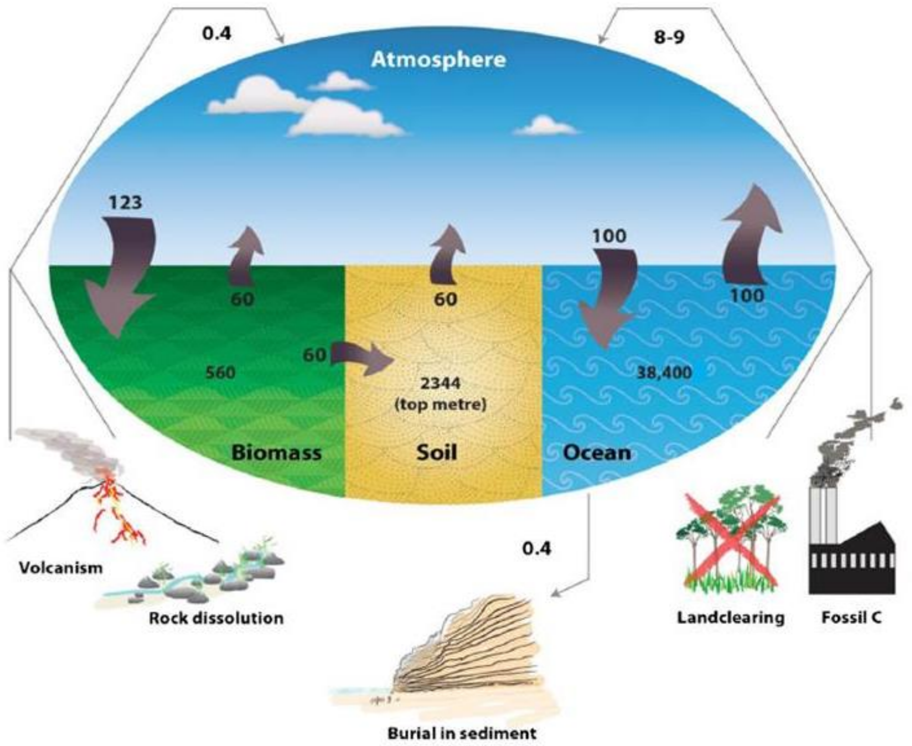


Figure 2. Natural and anthropogenic annual C fluxes (Pg C yr^{-1}) among the different reservoirs and spheres (figure taken from Stockmann *et al.*, 2013).

1.3 Role of agriculture as source of CO_2 and CO_2 after the implementation of environmental-friendly management practices

Agroecosystems have a very high potential for SOC accumulation. SOC concentration is near the steady state at the very long term in natural ecosystems; e.g. inputs and outputs of organic C are similar and thus SOC concentration hardly changes. This is not the case in agroecosystems. After cultivation, SOC in agroecosystems tended to decrease and about 20 years after cultivation between 20 – 30% of the SOC is released to the atmosphere in temperate regions. This value increases until 50 – 75% in tropical regions (Dumanski and Lal, 2004) and in Mediterranean regions percentages are between both.

The decrease in SOC content leads to different but inter-connected environmental and social consequences. Lal (2004a) highlighted that “an optimum level of SOC stock is needed to: hold water and nutrients, decrease risks of erosion and degradation, improve soil structure and tilth, and provide energy to soil microorganisms”. The SOC acts as a biomembrane (it filters and degrades pollutants, decreases sedimentation in rivers and hypoxia in coastal ecosystems) and is a large sink for atmospheric CO_2. Increase in SOC stock leads to an increase in crop yield, especially in soils with very low SOC content. These authors also remarks that “the vicious cycle of declining productivity-depleting SOC stock-lower yields will have to be broken (figure 3) by improving soil quality through SOC sequestration in order to free much of humanity from perpetual poverty, malnutrition, hunger, and substandard living”.

There is a considerable potential to accumulate SOC through the adoption of environmental-friendly management practices transforming a net source of C to a net sink. Dumanski and Lal (2004b) demonstrated that after implementing recommended management practices (RMPs) the potential for SOC increase in agricultural area of United States would be about a 24% of the commitment of GGE of the Kyoto Protocol.

The potential for annual SOC sequestration worldwide is about between 0.4 – 1.2 Pg C yr^{-1}, and most of this potential corresponds to agricultural soils (between 0.4 – 0.8 Pg C yr^{-1}; Lal, 2004b) – with similar, but at the lower end, estimates from IPCC (Smith *et al.*, 2008; 2014) – after the adoption of specific RMPs. Taking into account that the increase of the CO_2 in the atmosphere is about 4.3 Pg C yr^{-1} (Le Quéré *et al.*, 2012; Ciais *et al.*, 2013), if management practices leading to a SOC accumulation in the agriculture were adopted the agriculture would pass from a source to a sink of CO_2, thus reducing the increase of the CO_2 emissions by about 3.5 – 3.9 Pg C yr^{-1}, assuming that the oceans and vegetation will continue absorbing CO_2 at the same rate as currently. In other words, the present atmospheric CO_2 levels would be reduced by 9 and 19% if cropland is transformed from a source to a sink of C. This estimation is similar to that made by Lal (2004b), who calculated an approximated contribution of between 5 and 15%, and slightly lower than the 21 – 24 % of the global anthropogenic GGE estimated for agriculture and land use (Smith *et al.*, 2014; Tubiello *et al.*, 2015).

Figure 3. The vicious cycle of depletion in soil organic matter-decline in crop yield-food insecurity-soil and environmental degradation can be broken by improving soil fertility through the enhancement of the soil organic matter pool, which requires use of sustainable agricultural technologies for water and nutrients management, including no-till farming, composts and mulching, leguminous cover crops, water harvesting, agroforestry, and integrated farming systems, along with judicious of chemicals. This strategy can break the tyranny of hunger. Caption and image obtained from Lal (2004a).

The implementation of these RMPs has two main benefits. On the one hand, it contributes to the increase of soil fertility and long term agricultural sustainability, mainly due the multiple and synergist effects of soil organic matter (SOM) on soil fertility. On the other hand, it contributes to mitigate the multiple effects on climate change of the CO_2 emissions to the atmosphere.

1.4 The Kyoto Protocol, the Paris Agreement and the importance of monitoring CO_2 emissions

Currently, we are in an intermediate period between finishing the Kyoto Protocol and the beginning of the Paris agreement of 2015 (it will start, officially, in 2020, although in October 2016 more than 55 countries emitting more than the 55% of the GGE ratified the Paris Agreement, thus being in force already at this moment). The Kyoto Protocol was extended in 2012 by the Doha Climate Gateway (COP 18, 2012). In this conference the 194 countries agreed with the prorogation of the commitments of the Kyoto Protocol until 2020. This agreement in its article number 3 shows (UN, 1997):

- *The net changes in greenhouse gas emissions by sources and removals by sinks resulting from direct human-induced land-use change and forestry activities, limited to afforestation, reforestation and deforestation since 1990, measured as verifiable changes in carbon stocks in each commitment period, shall be used to meet the commitments under this Article of each Party included in Annex I. The greenhouse gas emissions by sources and removals by sinks associated with those activities shall be reported in a transparent and verifiable manner and reviewed in accordance with Articles 7 and 8 (article 3.3)*

- *"Each Party included in Annex I shall provide, for consideration by the Subsidiary Body for Scientific and Technological Advice, data to establish its level of carbon stocks in 1990 and to enable an estimate to be made of its changes in carbon stocks in subsequent years" (part of article 3.4)*

Recently, the French Government launched the "4/1000 Initiative: Soils for Food Security and Climate" (http://4p1000.org/understand), aimed to ensure that agriculture plays its role in combating climate change in order to achieve the long-term objective of limiting the temperature increase to +1.5/2°C (Lima-Paris Action Agenda 2015). In particular, the initiative proposes to increase organic matter contents and encourage carbon sequestration in soils, through the application of appropriate farming and forestry practices (e.g. permanent soil cover, use of organic products, diversified cropping systems, agroforestry).

The Paris agreement approved in the COP 21 (2015) (http://www.cop21paris.org/) does not specify anything about greenhouse gas emissions in agriculture. Nevertheless, in the Chapter II of the Adoption of the Paris Agreement (Intended Nationally Determined Contributions, i.e. INDCs) (articles from 12 to 21) it requests countries to develop their contributions of greenhouse gas emissions. It is supposed that agricultural and livestock emissions are included in these contributions. In this agreement, it is necessary to highlight the following article (UN, 2015) (see also articles 3, 4, 5, 6 and 7 of the Paris Agreement):

- *Reiterates its invitation to all Parties that have not yet done so to communicate to the secretariat their intended nationally determined contributions towards achieving the objective of the Convention as set out in its Article 2 as soon as possible and well in advance of the twenty second session of the Conference of the Parties (November 2016) and in a manner that facilitates the clarity, transparency and understanding of the intended nationally determined contributions (article II.13 of the Adoption of the Paris Agreement).*

Therefore, Regional, National, European and other International authorities agree on the need to quantify the potential C sequestration of agricultural soils throughout implementing RMPs. And this quantification should be due as soon as possible in order to quantify the *contributions* of each country in the context of the Paris Agreement, and therefore try to not to increase the Earth's temperature over the 2 ºC by 2050 (Article 2 of the Paris Agreement).

1.5 Transparency and verifiability of the measurements

Both agreements, Kyoto and Paris, highlight the necessity of quantifying the CO_2 sequestration due to general and specific land use and management changes. Also, these estimations must be transparent and verified. This means that is necessary to verify the data obtained with empiric data or independent estimations (Smith, 2004a).

There are two methods to measure the SOC losses/accumulation: i) methods based on measuring changes in the SOC stocks after a specific or a combination of management practices, and ii) methods based on measuring in and out C fluxes between the ecosystem and the atmosphere (IPCC, 2000; Smith, 2004a). Both methods are considered as *direct* methods (Post *et al.*, 2001).

The process of measuring changes in SOC stocks with laboratory analysis of the SOC content can take weeks, months of years after a management practice is implemented. The second group of direct methods are based on measuring the CO_2 and/or CH_4 fluxes and they can be carried out independently on the first type of methods (Smith, 2004a; Moncrieff *et al.*, 1997). However, it is not possible to use only this second group of methods as a verification of SOC losses/accumulation, since they represent global changes but they do not distinguish between the different fractions of the SOC or from the vegetation cover.

However, there are some problems to carry out these methods. For instance, for the implementation of the second group of methods, Post *et al.* (2001) remarked that two basic exchanges between organic C and CO_2 could be distinguished: those affecting the vegetation and those affecting the soil. Therefore, it is necessary to remove in some way the emissions from the vegetation to calculate the soil emissions. The easiest way to do that is to measure those emissions affecting the aerial part of the vegetation, so knowing these ones and the net flux (soil + vegetation) those affecting the soil can be estimated. Another problem of the verification is the resolution of the measurements. Garten and Wullschleger (1999) estimated that the minimum detectable change in the SOC was about 1 t C ha^{-1} (after 5 years in arable crop), which representing about 2 – 3% of the total SOC content. This is true when an adequate number of samples are taken (>100), whereas if a reasonable number of samples (e.g. 16 samples) are sampled the minimum detectable change could reach at 5 t C ha^{-1} and, therefore, the results might not be reliable.

Thus, the higher the number of samples are taken the higher reliability of the results is. However, the higher is the number of analysis the higher the costs in economic and time terms are. These can be higher than the costs of implementation managements and techniques of the SOC sequestration, so the method would not be adequate (figure 4). The objective is to minimize costs with the purpose of maximize the return but at the same time obtaining reliable results.

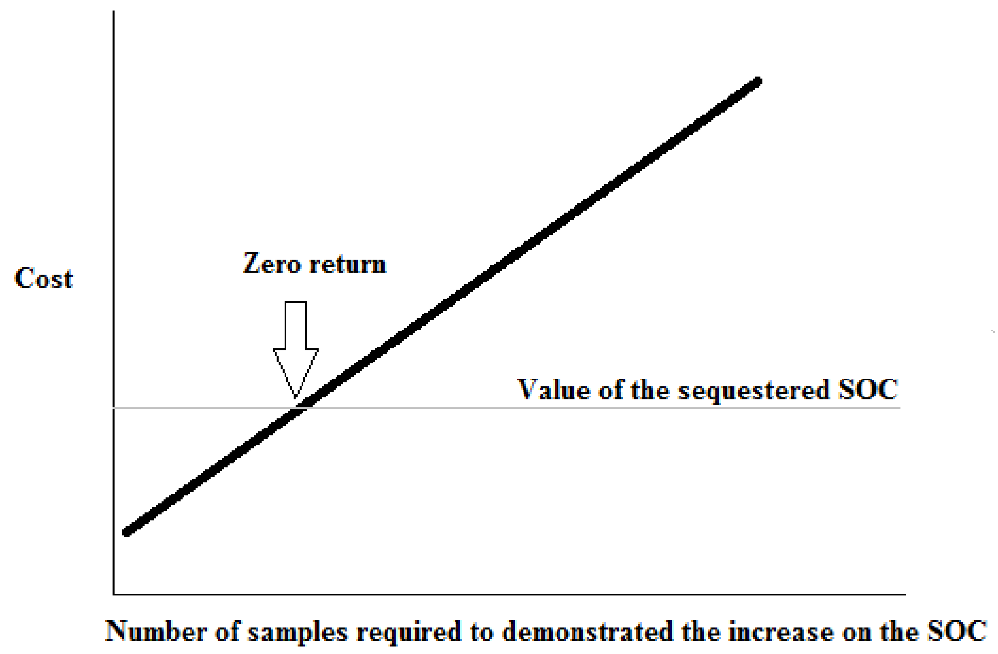


Figure 4. Compensation between the value of the sequestered SOC and the cost of the measurement and verification of the sequestered SOC. The union point between number of samples and cost with the value of the sequestered SOC is the zero return point from which starts to be higher the cost of sampling than the benefits reported because of the sequestered SOC. Adaptation of Smith (2004a).

For that reason it is necessary an intermediate solution between taking a low and a high number of samples. For that reason *indirect* methods are necessary (Post *et al.*, 2001). By modelling the results obtained from "benchmark points" and with an adequate resolution, it is possible to estimate the SOC sequestered for each reference zone (zones with similar pedoclimatic conditions) (figure 5).

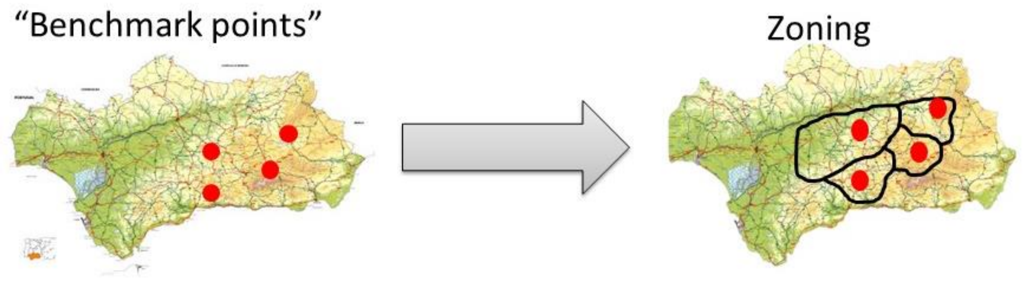


Figure 5. Example of a zoning from some benchmark points.

1.6 Complying the Kyoto Protocol and the Paris Agreement. The possibility of a future CO_2 financial instrument for Andalusian olive groves

There are few studies aimed to quantify SOC accumulation in specific crops at Regional scale. According to the 3.3 and 3.4 articles of the Kyoto Protocol commented above (and the general articles of the Paris Agreement, from article 3 to 7) a quantification of the SOC changes in Andalusian olive groves is needed due to the importance of this crop at this regional scale. Currently, the agriculture is a source of CO_2 emissions to the atmosphere, so it is necessary to analyse the influence of the changes of the managements of the olive groves on SOC accumulation so that it can pass from a source to a CO_2 sink.

If environmental-friendly leading to SOC sequestration managements in olive groves are implemented, then the decrease in the CO_2 emissions due to the adoption of this managements might be very important. If it is possible to quantify (giving an estimation) the CO_2 that is prevented to be emitted to the atmosphere this quantification would be included in the Spanish "contributions" of the Paris Agreement, or be used as an evidence of the environmental positive effects of the sustainable managements in olive groves in the context of the Common Agricultural Policy (CAP). CAP will be revised in 2017 and in 2020 will start a new period. At this time in Spain CAP does not generally include benefits for farmers implementing managements leading to SOC sequestration. Therefore, one of the objectives of this Thesis is to calculate values of SOC sequestration in order to be used in the future negotiations of the CAP.

On the other hand, and due to the existence of a CO_2 market, called *carbon emission trading* (European Emission Allowance, i.e. EUA) the SOC sequestration can be economically quantified, and farmers in the future would be benefited by the implementation of this kind of financial mechanisms.

2. Characterization of the SOC and its relevance on soil function

2.1 SOM is a conglomerate of substances of a very difficult identification

The SOM has a complex and heterogeneous composition and, with no doubt, it is at the same time one of the less understood soil components but also one of the most studied. The highest proportion of SOM consists of a conglomerate of organic molecules with a recalcitrant nature called *humus*. Despite currently it is being developed an active research about humus chemistry and different models based on phenols and lignin have been proposed, the chemical nature of this large proportion of the SOM is highly variable and is still unknown. SOM is made of a highly heterogeneous group of compounds with a chemical nature fully elucidate. Therefore, SOM can hardly be measured as a specific group of chemical substances, and usually their characterization is based on measuring some indicator. For that purpose, two types of models have been developed (Wershaw, 2004): (1) those of the humic polymers and (2) those representing SOM as molecular aggregates. The first one suggests that the molecules forming the natural organic matter (NOM) are polymers which have been synthesized from reactions of secondary synthesis from plant material degradation products. These polymers present a unique chemical structure which is different than the precursor's polymers. From these models it has been suggested wide schemes of humus fractionation according to its acidity, colour and solubility (e.g. Stevenson, 1994). Even some molecular formulas of humic sub-fractions have been suggested under the assumption that these are formed by pure compounds (Wershaw, 2004). In the case of the models representing SOM as molecular aggregates (Wershaw, 1994; Piccolo, 2001) the advances in analytical chemistry have played a key role on the advance of the NOM models. The oxidative degradation of the humic substances coupled to the gas chromatography has led to an aggregate model where the detected aromatic phenolic acids are linked by hydrogen bridges, forming molecular aggregates. These models are the most accepted ones (Wershaw, 2004). Based on these models, it has been purposed for different compartments to characterize the NOM. In soils, the proposed compartments of NOM are: (1) partially degraded vegetal residues, (2) biomass from the microorganisms, (3) organic matter linked to the mineral particles, (4) pyrolytic C (i.e. biochar), (5) organic precipitates and, otherwise (in liquid state) (6) dissolved organic matter (DOM) in the soil solution. Each of one of these compartments would be formed by supramolecular aggregates (figure 6).

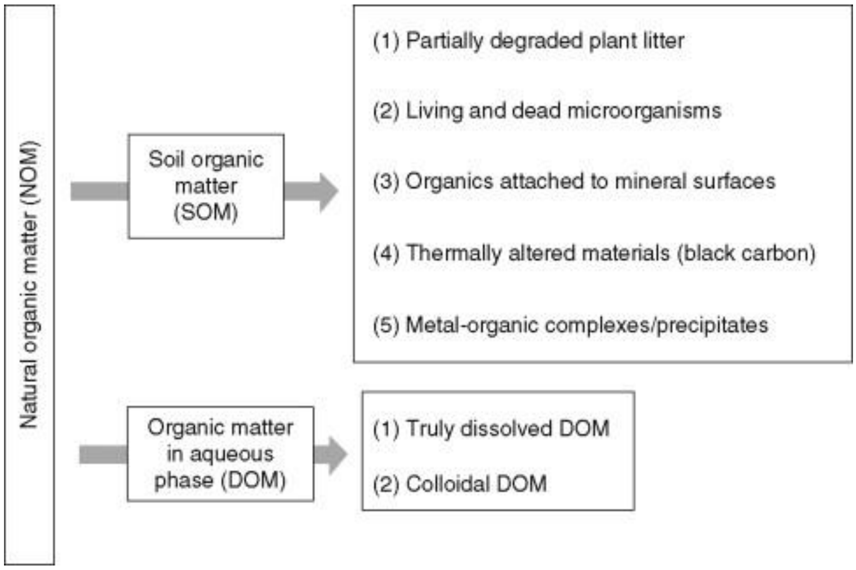


Figure 6. Conceptual model of the different fractions of the SOM based on the "aggregate molecular" model (Wershaw, 2004)

Wershaw (2004) purposed that the NOM is formed by molecular aggregates from the degradation products of the vegetal tissues. Thus, it can be considered that the humification process takes place in three phases: (1) degradation of the substances from the plant residues, (2) reassembly or complexation of the degradation products in NOM and (3) degradation of the NOM formed in the phase 2.

Nevertheless, the main problem of the supramolecular aggregate model are that these compounds cannot be considered unique, simple and identifiable entities, but they are complex substances formed by a mix of aliphatic acids, ethers, esters and alcohols, aromatic fragments derived from the lignin, polysaccharides and polypeptides (Wershaw, 2004). Although the supramolecular aggregate model better represents the NOM it only gives a general idea about the structure of these aggregates in the soil extract. Thus, a soil extract is formed of aggregates from the different compartments which have a difficult characterization (Wershaw, 2004).

2.2 SOM and its relevance on soil function

SOM content is considered a key indicator to study the quality of a soil, from both agricultural (fertility, production and economy) and environmental (e.g. SOC sequestration and climate change) points of view (Bradly and Weil, 2008). SOM is one of the main variables determining the biological activity and functioning of the soil. The quantity, diversity and activity of micro and meso fauna and soil microorganisms are directly related to the SOM content, which directly influence soil physico-chemical properties, providing structural stability. The increase in SOM content leads also to an increase on the soil infiltration rate and water availability. These features give to the soil higher resistance to the erosion. An increase on the SOM content also leads the dynamics and the bioavailability of the main nutrients for plants (FAO, 2001).

Table 1 summarised some of the direct and indirect effects of SOM on the phisical, chemical and biological soil properties (Wander, 2004). Despite the well-known effects of SOM on soil fertility, quality and health, the specific contributions of the different “types” of SOM to soil functions is not well known and the knowledge have not been increased significantly during the last 50 years, maintaining fundamentally its descriptive nature.

Therefore, although the structure and composition of the SOM is not well known yet, it is known their positive effects on soil properties. Additionally, the retention, accumulation or SOC sequestration associated to the SOM is also a benefit to the environment since it contributes to the mitigation of the CO_2 emissions to the atmosphere.

However, the SOC is not homogeneously distributed but it appears in different fractions and according to the specific fraction it will be more or less susceptible to be sequestered and, therefore, to increase the turnover rate. This is the main reason why there is a need to study deeply the main features of specific organic C linked to specific SOM fractions.

Table 1. Main contributions of SOM on physical, chemical and biological soil functions. Adaptation from Wander (2004).

Summary by Waksman (1938)	Summary by Stevenson (1994)
Physical functions	
It modifies the colour, texture, structure, moisture retention capacity and soil aeration.	Colour, water retention, helps to prevent soil contraction and soil drying. Combined with clay minerals it improves the moisture retention capacity, gives structural stability and allows gas interchange.
Chemical functions	
Solubility of minerals, formation of compounds with elements like Fe which give higher possibilities for plant growing, increase soil buffering properties.	Formation of Chelates which improve nutrient availability, soil buffer properties and increasing the CEC
Biological functions	
A source of energy for microorganisms, making the soil a better place for plant growth. Contribute to a slow but continuous nutrient flux for plant growth.	The mineralization is a source of nutrients which can be linked to xenobiotics influenced by the bioavailability and effectiveness of pesticides.

2.3 Soil organic carbon fractions

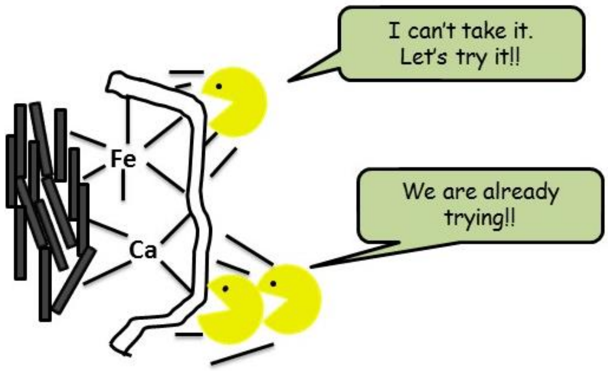
SOM is made of approximately a 58% of C (Mann, 1986). Without any doubt SOM is mainly made of SOC. Considering the high diversity of the chemical nature of the substances containing organic C, the practical impossibility of identifying and quantifying these compounds is a reality. Therefore, to obtain soil organic C substances differing in the potential for being retained, protected or sequestrated, it is necessary to use physical and/or chemical processes to isolate organic substances with similar behaviour. Wander (2004) classified the different SOM fractions according to the methodology applied to their separation and isolation. Table 2 shows some examples of physical and chemical methods used to isolate SOC fractions with a brief description of their general nature and role on SOC sequestration.

Table 2. SOM fractions, kinetics, function and compounds associated to each of the fractions. Letters in brackets identify the compounds with each SOM type commonly used: biologically active matter (B), physically active (F) and chemically active or inactive (Q). Adaptation from Wander (2004).

SOM fractions, theoretical kinetics and function	SOM fractions defined by analytic process
Labile or active SOM	
Average lifetime from days to years	**Microbial biomass**
	Labile SOM with chloroform (B) Labile SOM with microwave irradiation (B) Ammonium compound s (B, F) Phospholipids (B)
	Labile substrates
Plant material of a recent origin or incorporated to the life components of the SOM Material with high nutritive or energetic value The physical status makes that the SOM incorporated probably take part on reactions with chemical or biological nature	Mineralizable C or N, estimated by incubation (B) Substrate activity induced (B) Soluble, extractable with hot water or dissolved salts (Q, B) Easily oxidizable with permanganate or other oxidants (Q, B)
	Residues which chemical formula can be described, inherited from the living organisms
The physical role of the material placed on the soil surface and the components promoting the macroaggregate formation is transitory	Litter, plant fragments or residues (B, F) Particulate organic matter non-associated to aggregates (B, F) Polysaccharides, carbohydrates (Q, F)
Slow or intermediate SOM	
Average lifetime from years to decades	**Partially decomposed residues**
The physical protection, physical status or localization helps to separate this fraction from the other two fractions	Amino compounds, glicoproteins (B, F) POM protected into the aggregates (B, F) Some hydrolysable Acid/basic humic materials (B, F) Mobile humic acids (B, F)
Recalcitrant, passive, stable and inert SOM	
Average life from decades to centuries	**Refractary compounds of unknown origin**
	Aliphatic macromolecules (lipids, waxes, suberines) (Q) Charcoal (Q). Sporopollenins (Q). Lignins (Q).
Recalcitrant due to the biochemical features and/or mineral associations	**Some humic substances**
	High molecular weight, condensed SOM (Q, F) Humine (Q). Non-hydrolysable SOM (Q) Fine silt, SOM associated to clay and fine silt (Q, F)

According to the capacity to be retained at the long term in the soil throughout stabilization or protective mechanisms, Six *et al.* (2002) proposed a classification of the organic substances in protected and unprotected SOC. The unprotected organic C is associated to the fresh plant residues, which are mainly on the soil surface although it has also been identified deeper in the soil. Soil organic C can be protected by means of different protective mechanisms and according to that, protected soil organic carbon can be classified as: chemically, physically and biochemically protected. The physically protected C is that present within soil microaggregates, whereas the chemically protected is that associated to silt and clay by chemical mineral links. The last one, the biochemically protected depends on the biochemical features of the original plant material and on the complexation and condensation reactions taking place during SOM decomposition. The protected SOC fractions are safeguarded from the action of soil microorganisms and their relative concentration in a given soil are deeply related to the prevalence of a mechanisms of stabilization/protection.

Chemical stabilization: SOC linked to silt and clay



Silt and clay are particles with a < 53 µm of diameter formed by organo-mineral complex. Hassink (1997) found a significant linear relationship between the SOC concentration, especially that organic C associated to particles of < 53 µm (silt and clay), and the silt + clay content of a soil for a group of natural and managed terrestrial ecosystems. This relationship suggests that silt and clay particles of soil protect the organic C associated to them from the decomposition mechanisms by soil microorganisms. It is possible to classify two types of protection mechanisms linked to silt and clay: (i) those linked to chemical interactions betweem organic matter and clay and silt particles, and (ii) those improving soil structure, which provide not only chemical, but also a physical protection. Despite the second mechanism is interpreted as a physical protection (see following section), it also implies a chemical stabilization. Feller and Beare (1997) found a linear and direct relationship between soil aggregate stability and SOM content. Currently, it is unclear the role of the clay+silt in the increase on the soil aggregate stability. Therefore, directly or indirectly, soil clay+silt contents affect the formation of stable aggregates in soils and, thus, the chemically protected organic C within soil microaggregates. Thus, there is a fraction of organic C which is chemically protected within the physical protection of microaggregates.

The relationship between soil organic C chemically protected and soil silt + clay content is complex. Six *et al.* (2002) suggested that the SOC associated to silt and clay might depend not only on the silt and clay content, but also on other variables, such the diameter of the particles or the type of the ecosystem. The type of clay might affect also on SOC content of this fraction. Six *et al.* (2002), carried out a similar study that that of Hassink (1997), and found that SOC concentration was significantly higher when particle size was up to 50 µm in comparison to when only was up to 20 µm. This is probably due to the presence of aggregates of silt with higher size in the fraction between 20 and 50 µm, which have more organic C per unit of material due to the additional unions of the primary organo-mineral complex within silt aggregates (Tisdall and Oades, 1982). Nevertheless, it might be also due to the presence of particulate organic matter (POM) (Turchenek and Oades, 1979).

Six *et al.* (2002) also observed that by comparing different type of ecosystems (cultivated, grassland and forest), SOC content in particle of sizes between 0 – 20 µm were not significantly different. However, this was not true (cultivated < forest = grassland) in particle-size of 0 – 50 µm. Thus, correlating SOC content with each size and type of ecosystem or type of clay these authors obtained different functions (Tables 3 and 4) which can be useful to estimate the SOC content in other soils.

Table 3. Soil particle size class, ecosystem type, intercept with the Y axes (g C associated to silt and clay Kg^{-1} soil), slope of the curve and regression coefficient. Values are the average and ± stands for the confident interval at 95%. Adaptation from Six *et al.* (2002).

Size class	Ecosystem	Intercept	slope	r^2
0-20 µm	Cultivated	4.38 ± 0.68	0.26 ± 0.01	0.41
	Grassland	2.21 ± 1.94	0.42 ± 0.08	0.44
	Forest	-2.51 ± 0.55	0.63 ± 0.01	0.55
0-50 µm	Cultivated	7.18 ± 3.04	0.2 ± 0.04	0.54
	Grassland	16.33 ± 4.69	0.32 ± 0.07	0.35
	Forest	16.24 ± 6.01	0.24 ± 0.08	0.35

Table 4. Particle size class, type of clay (1:1 or 2:1), intercept with the Y axes (g C associated to silt and clay Kg^{-1} soil), slope of the curve and regression coefficient. Values are the average and ± stands for the confident interval at 95%. Adaptation from Six *et al.* (2002).

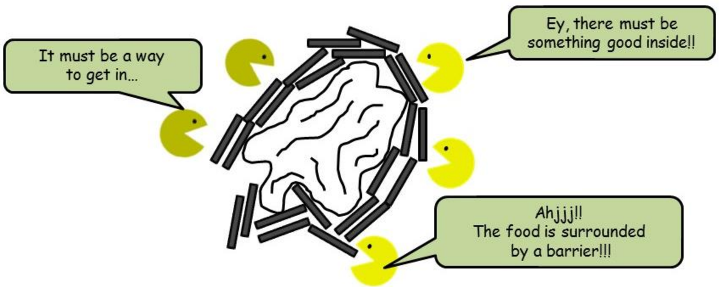
Size class	Type of clay	Intercept	Slope	r^2
0-20 µm	1:1	1.22 ± 0.37	0.30 ± 0.01	0.74
	2:1	3.86 ± 0.49	0.41 ± 0.01	0.39
0-50 µm	1:1	5.5 ± 5.93	0.26 ± 0.13	0.38
	2:1	14.76 ± 2.37	0.21 ± 0.03	0.07

The amount of chemically protected C also differed due to the type of clay particles (1:1 versus 2:1) being typically higher in 2:1, especially when particles sizes were lower than 20 µm size. The differences between the organic C associated to silt and clay due to the clay type might due to the different cation exchange capacity (CEC) of each clay type (Greenland, 1965). Additionally, secondary minerals of Fe and Al oxides which typically are more abundant in soils with higher presence of 1:1 clay type, have a reduced adsorption capacity on the surface of clays.

Therefore, the relationship between clay+silt content and soil organic carbon in the clay+silt fraction is complex, and this complexity is even higher because the predominant environmental conditions might exert a role in the predominant type of clay. For instance, in tropical and sub-tropical regions most of the clay particles are dominated by 1:1 clays, leading to a higher decomposition rate and, thus, a lower organic C stabilization. Therefore, different stabilization mechanisms might take place and some of these mechanisms could be related to the environmental conditions, affecting in different ways leading that the net final effect is still unknown.

Summarizing, there is a direct relationship between the percentage of silt and clay in a soil and the organic C associated to these particles size (up to a limit; assessed in the Chapter VI). In soils of managed ecosystems there is a trend of decrease the organic C associated to silt and clay in comparison to the natural terrestrial ecosystems like forests and grasslands, mainly due to the human impact on them. On the other hand, the organic C associated to silt and clay depends not only on the soil silt and clay content, but also on the particle-size class and type of clay. Therefore, mineralogy (and thus, type of soil) plays a key role to the organic C chemical stabilization due to silt and clay. In this context, there is an important lack of information and studies obtaining quantitative data about SOC sequestration in soils with contrasted mineralogy. It is necessary, therefore, carrying out investigations focused on this aspect. Chapter VI assessed this fact.

Physical protection: protected organic C within soil microaggregates



This type of protection affects the organic C which is located within soil aggregates. From others studies, Six *et al.* (2002) attributed this protection mechanism to: (1) the differential compartmentalization of the subtract and the microbial biomass (Killham *et al.*, 1993); (2) the reduced oxygen diffusion in the macro, and especially in the soil microaggregate, leading to a reduced microbial activity inside the aggregates (Sollins *et al.*, 1996), and (3) the differential compartmentalization of the microbial biomass and the microbial herbivores (Hattori, 1988). This mechanism is described from evidences including, for instance, those established by Barlett and Doner (1988) and Priesak and Kisser-Priesak (1993). These authors found a clear decrease in the microbial activity from the surface inbound soil aggregates, likely due to the physical inaccessibility (due to the reduced pore size) of the soil microorganisms to the source of organic carbon which is inside soil aggregates (Killham *et al.*, 1993).

The protective function of soil macroaggregates (particles with size > 250 µm) over the organic C seems to be negligible in comparison to the physical protection exerted by the microaggregates (53 – 250 µm) (Beare *et al.*, 1994; Elliott, 1986). Nevertheless, the importance is that macroaggregates are a reservoir of microaggregates.

There are two types of POM: (1) that of higher size which are not within soil microaggregates (gross, cPOM) and (2) that lower than < 250 µm, which is located within soil microaggregates (fine, iPOM hereafter) (figure 7). (Six *et al.*, 2000). The cPOM would not be protected, whereas the iPOM would be part of the protected pool (physically protected, within microaggregates).

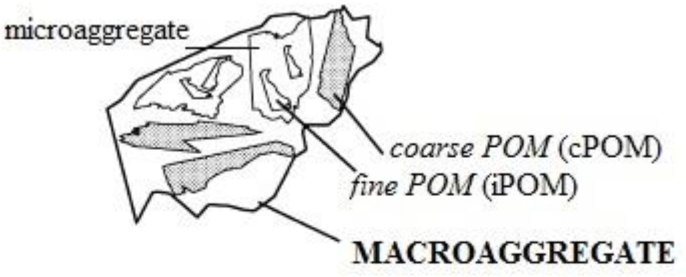


Figure 7. Spatial distribution model of the gross (cPOM) and fine (iPOM) particulate organic matter and the microaggregate within a soil macroaggregate. Adaptation from Six *et al.* (2000).

The source of the iPOM is the cPOM (t1 to t2, figure 8). This fact is supported by the higher age of the iPOM in comparison to that of the gross, and for the progressive reduction of the size which takes place during cPOM decomposition (Six *et al*., 2000). Thus, the ratio iPOM/ cPOM can be used to assess the effect of the different management practices on aggregate formation and destruction rates, and therefore on SOC dynamics. These effects were studied by Six *et al.* (2000), who observed in four agricultural soils differing in the tillage regime (tillage versus no till) that the ratio fine iPOM/ cPOM was two times higher under no till. In addition, these author found that in non-tilled soils microaggregates accounted for about 47% of the weight of the total macroaggregate weight, whereas in soils under conventional tillage this percentage was about half. Furthermore, the age of the organic C in the soil microaggregates of the non-tilled soils was lower than that of the soils under conventional tillage.

Throughout time (t2 to t3; figure 8), new microaggregates are establishing within macroaggregates. These microaggregates are formed from the iPOM, which is incrusted into the clay particles and different microbial products, using the cPOM as a matrix. Thus, the old cPOM would be essential for the formation of new microaggregates.

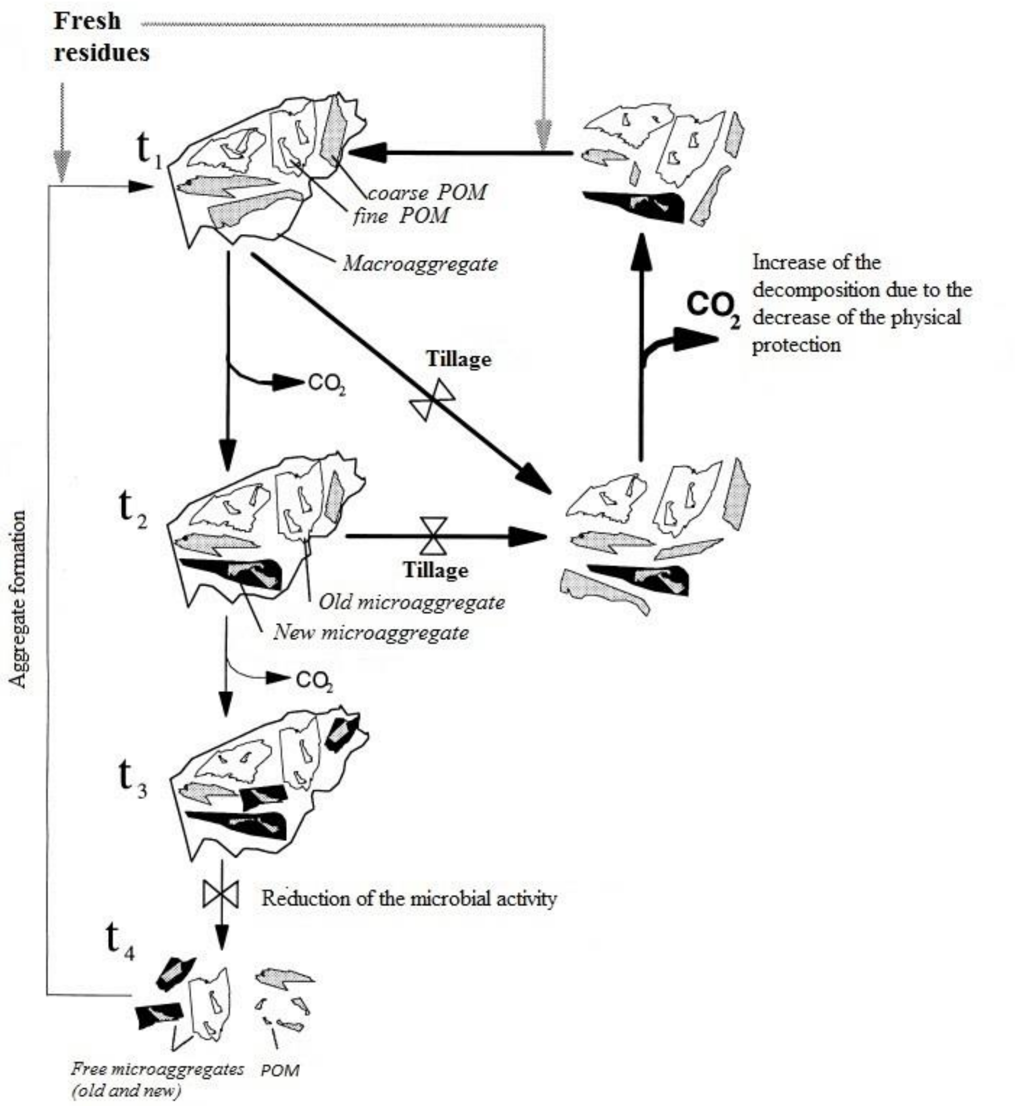


Figure 8. Theoretical model showing the effect of the tillage on the soil macro and microaggregate breakdown and, therefore, on the physical stabilization of the SOC. Adaptation of Six *et al.* (2000).

In the same study it was observed that the organic C within microaggregates of non-tilled soils was approximately three times higher than that of the tilled ones. On contrary, in the space between microaggregates, the organic C was two times higher in the tilled soils than in the non-tilled, but with very low values to those obtained within microaggregates. These data suggest that the amount of the organic C in the soil macroaggregates under a conventional tillage was lower than that of the non-tilled ones.

Thus, the management practices, especially tillage, have a clear impact of the potential for physical protection of the SOC, as soil particle aggregation is reduced by tillage. This was pointed out a decade ago by Six *et al.* (2004) who reviewed the mechanisms of soil aggregates formation in relation to SOM dynamic. They found 5 key factors in the soil aggregate formation which are inter-related in a complex way (figure 9):

1. Soil fauna, especially earthworms and termites.
2. Microorganisms
3. Roots
4. Linking inorganic agents (oxides and Ca)
5. Environmental variables (freeze-thaw cycles, humidity-drought and fire)

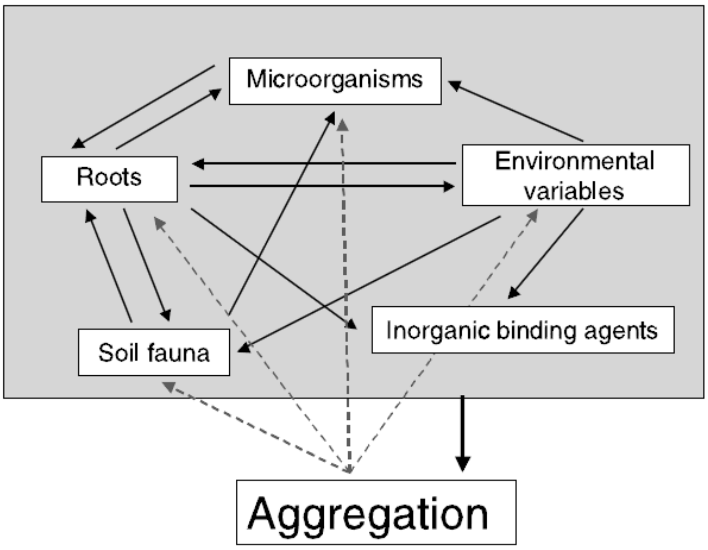
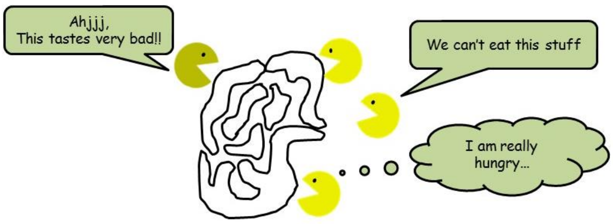


Figure 9. Complexity of the interactions between the main 5 factors affecting the soil aggregate formation (Six *et al.*, 2004).

However, despite the knowledge on the main factors affecting soil particles aggregation (a big part of this knowledge is even before the 50's), there is a lack of information on the specific role of each of these factor, due to: i) the multiple interactions and feedbacks between soil fauna, microorganisms, roots, inorganic linking agents and environmental factors, ii) the different scales and dimensions where each of the mechanisms are acting, and iii) the soil heterogeneity from a point of view of a three-dimensional pore system.

Thus, it is necessary more studies focused on the three-dimensional dynamics of soil particle aggregation and on investigating the synergic effects of the different agents involved in the process.

Biochemical stabilization: biochemically protected SOC



This stabilization mechanism is, without any doubt, the less known stabilization type. This mechanism depends on the origin and composition of the organic matter source (Six *et al.*, 2002; Cadish and Giller, 1997). The SOM biochemical stabilization is due to the complex chemical composition of the organic matter, which can be an inherent property of the nature of the material or can be acquired during the decomposition processes through condensation and complexation reactions of the organic matter subjected to decomposition.

There are clear indications on the recalcitrant nature of the biochemically protected soil organic carbon. Leavitt *et al.* (1996) and Trumbore (1993) found a relationship between the biochemically protected SOC and non-hydrolysable SOC in soils subjected to the combined action of an oxidant source and sulphuric acid.

However, there are not consensuses on the degree of protection exerted by the biochemical features. Thus, according to v. Lützow *et al.* (2006) all the organic substances available for the soil microorganisms are susceptible to be mineralized. According to them, these compounds can have some recalcitrant features and in some cases a higher number of different microorganisms groups are needed to decompose these organic substances (these authors call this recalcitrance as “secondary recalcitrance”), but if these compounds are not associated to some hydrophobic substances this organic C is susceptible to be used by soil microorganisms.

Therefore, it is likely that the biochemically protected SOC might only be “protected” from microbial decomposition in the short term, but at longer period of time, this pool of organic carbon is finally mineralized by the combined action of different groups of microorganisms. Therefore, the biochemically protected SOC could be considered as a sequestered SOC pool but only at the short-to-medium term, as long as these organic substances were not also associated to other hydrophobic substances which avoid the microorganisms’ action.

Unprotected SOC

The unprotected SOC is that SOC pool non-protected by physical, chemical or biochemical mechanisms and, therefore, it is accessible to microorganisms and, thus, susceptible to be easily decomposed.

The unprotected SOC pool is basically composed by two main fractions: the light fraction (LF) (< 250 µm, but only that fine free POM, i.e. not located within soil microaggregates) and the cPOM (> 250 µm) (Six *et al.*, 2002).

The main characteristics of these two fractions are shown in Table 5.

Table 5. General characteristics of the light fraction (LF) of the organic matter and the particulate organic matter (POM) conforming the unprotected organic C. Six *et al.* (2002)

	Characteristics
1.	Consists of plant residues in various stages of decomposition
2.	Presence of charcoal
3.	Mannose+Galactose/Arabinose+Xylose ratio is low
4.	High O-alkyl content
5.	High C/N ratio
6.	Low net mineralization potential
7.	Labile SOM pool
8.	High lignin content (Vanillyl, Syringyl, Cinnamyl content high; Phenylpropenoic acid/benzoic acid ratio high)
9.	Microbial biomass and microbial debris are associated with LF

As Six *et al.* (2002) highlight, the LF and POM fractions are mainly made of plant residues; but also they contain seed residues and microorganisms like hyphae and fungal spores, and charcoal (Besnard *et al.*, 1996; Cambardella and Elliott, 1992). Nevertheless, despite these fractions are characterized mainly by having a plant origin, these usually present a partial decomposition state in which microorganisms play a key role. Several studies have shown that these two fractions are relatively easily decomposable (e.g. Cambardella and Elliott, 1992; Six *et al.*, 1999; Solomon *et al.*, 2000) mainly because they are not protected against microorganisms attack.

There are, however, marked differences among the unprotected organic carbon pools, mainly between the LF and POM, but also with that plant residues recently incorporated into the soil. Depending on the biochemical features of the plant residues which provide recalcitrance (e.g. lignin and polyphenols), these residues remain more or less unaltered at the short term. This pool is what v. Lützow *et al.* (2006) called “primary recalcitrance” and it is likely that this fraction enter the biochemically protected organic carbon pool. However there are not evidences of the long term resistance to the degradation of this fraction unless other stabilization mechanisms take place (v. Lützow *et al.*, 2006).

2.4 The SOC fractions of different degree of protection are linked

In a given soil, the SOC fractions of different degrees of protection are linked (figure 11). The main source for the different protected fractions is the unprotected organic carbon.

The organic C enters the soil from the aboveground, but also from belowground, parts of the plants as plant residues or from the roots. This organic carbon enriches the unprotected organic C pool, and is subjected to decomposition generating the coarse POM (cPOM) and the fine size LF, which are the main components of the unprotected SOC. The cPOM and LF fractions of SOC can be moved to any of the three protected pools: chemically, physically and biochemically protected. It will be biochemically protected pool if the plant residues have biochemical features that prevent them from decomposition. Other types of SOC are considered as biochemically protected if it is transformed towards recalcitrance SOC by complexation and condensation reactions. The partially decomposed cPOM and LF might react with silt and clay soil minerals and being chemically protected. Lastly, if the time passes without major soil structure alteration and perturbations, the organic C can take part of macroaggregate formation and become trapped within microaggregates and become physically protected. In the microaggregates, depending on mineralogical nature of the soil, the organic C can be also linked to silt and clay minerals and become physico-chemical protected. Some microorganisms could accede to a part of this organic C and using them as a source of matter or energy, transforming it and generating throughout complexation and condensation reactions the organic C which is biochemically protected. Therefore, it is not surprising the existence of an organic carbon fraction which is physically-biochemically protected within the microaggregates.

In either via of organic carbon protection, a part of the SOC is mineralized, and then emitted to the atmosphere as CO_2. In terms of reducing CO_2 emissions from agroecosystems, an adequate management should minimize the SOC fractions which can be mineralized as CO_2.

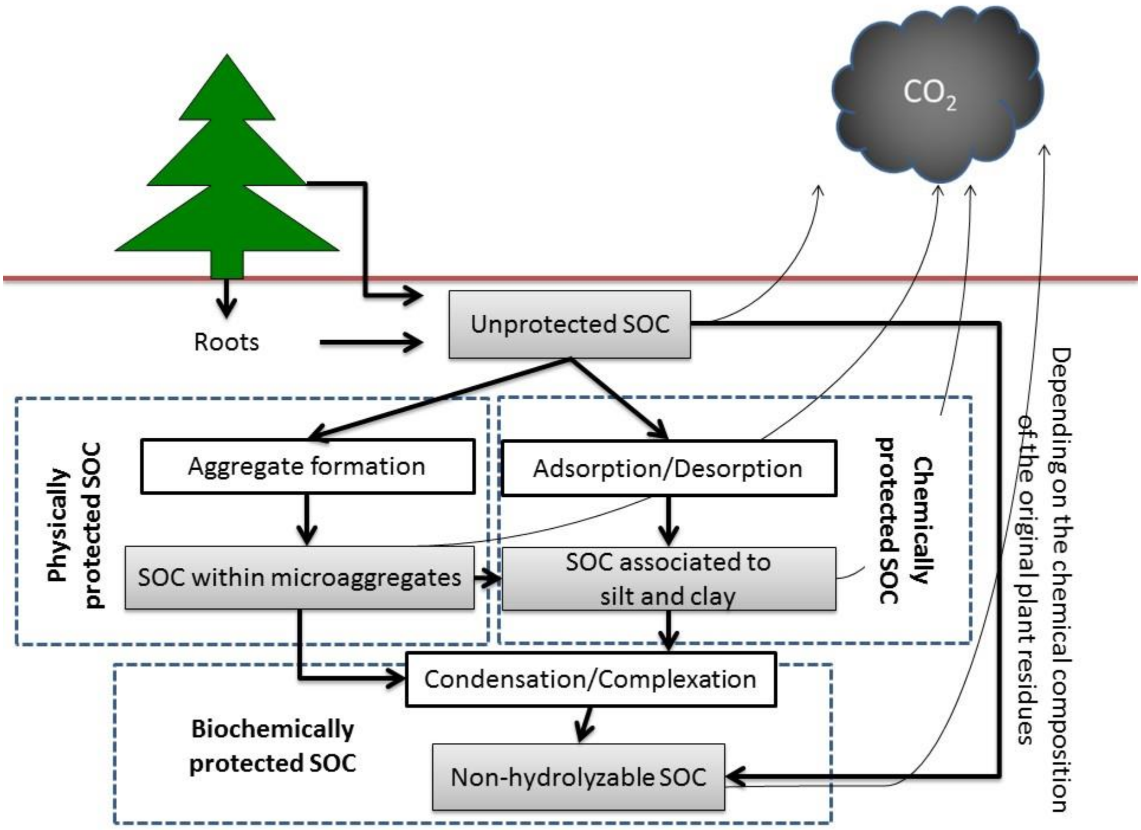


Figure 11. Dynamics of the different SOC fractions. Adaptation from Six *et al.* (2002).

According to that commented above, there is a preferential stabilization of the SOC to the fraction of the SOC within microaggregates, being this one, therefore an indicator of the organic C sequestered by the soil. In other words, considering all the fractions susceptible of acquiring some protection grade the fraction with the highest stability is that of the SOC within microaggregates. Therefore, a correct agricultural management in terms of SOC sequestration would be that leading to preserve and to promote the soil aggregate stability. These fractions can be isolated and quantified. In Chapter II the method is deeply described.

2.5 The influence of the different management practices clearly affect the SOC fractions dynamics

Considering all the SOC fractions and the mechanisms involved in each of the protection methods it is possible to summarize the influence of the management practices on SOC fractions dynamics.

The unprotected pool would be affected by the amount of the incoming organic C as fresh organic matter. Thus, with higher amounts of incoming fresh organic matter the amount of the unprotected fraction would be higher. In olive groves, the presence of a spontaneous resident vegetation cover would increase the amount of the incoming fresh organic matter.

The physically protected pool would be affected by the tillage practices. A frequent tillage would lead to a lower presence of macroaggregates, thus reducing the microaggregates formation. On the other hand, the amount of the incoming organic C also affects the amount of the physically protected C. Higher organic C entering to the soil would increase the amount of the physically protected pool (at least in soils with low-medium total SOC content).

As the unprotected and physically protected pool, the chemically protected SOC in the fine fraction would be higher with higher amounts of the incoming organic C to the soil. However, the chemically protected pool would depend also on the silt + clay content and other mineralogical properties (presence of cations, pH…). Nevertheless, this fact would be relevant only when this fraction is already saturated because of the high levels of SOC or the low content on silt + clay. However, tillage practices could affect the amount of this fraction, since tillage reduces the microaggregate formation and, therefore, the organic C would be linked to silt + clay but outside the microaggregates (i.e. in the fine fraction of < 53 µm, not in the intermediate fraction of 53 – 250 µm).

The biochemically protected pool in the fine fraction would have a similar dynamic than the chemically protected pool in the fine fraction. The only difference is the specific mechanisms that may affect the amount of this fraction. Thus, the amount of this fraction would be higher also when: i) the incoming organic C has higher proportion of recalcitrant compounds (primary recalcitrance), and ii) there is a predominance of condensation and complexation reactions in the soil (secondary recalcitrance). Therefore, some managements leading entering to the soil higher proportion of recalcitrant substances (e.g. composted inputs) would lead to higher amounts of the biochemically protected pool. This fact would also affect the biochemically protected pool within soil microaggregates.

Therefore, tillage and other managements leading to increase or decrease the amount and type of the incoming organic C may affect the dynamics of the SOC pools in Andalusian olive grove soils. Nevertheless, this occurs from a theoretical point of view, so it is necessary to carry out several experiments in order to verify these hypothesis.

3. SOC sequestration: concepts and theoretical dynamics. Hypotheses.

3.1 Concept

The concept of *sequestration* has been used by scientists to refer to the long-term retention of organic C into the soil. However, at a geological point of view, soil C, even that stabilised, continually cycles between the lithosphere, hydrosphere and the atmosphere. C sequestration has been used in this Thesis to refer to that carbon which is stored into the soil at the long term (e.g. human time scales).

During the last decades there have been much discussion on the term C sequestration. For example, Powlson *et al.* (2008) suggested that this term is used only for those situations where the additional C is retained by the soil and separate from the other parts of the ecosystem. In other words, is not only a CO_2 which has been transferred from the atmosphere to the soil, but also this transfer must imply a net SOC accumulation. Powlson *et al.* (2011) highlighted that a given soil sequesters C only in those situations in which there is a contribution to the climate change mitigation, and not when there are movements of SOC between the different fractions (protected and unprotected SOC), compartments or between different areas. In summary, the SOC sequestration refers to the organic C storage with very high resident time. Nevertheless, this is an empiric definition, since it must be based on observations and manipulative experiences directed to establish the resident time.

However, the word *sequestration* is being used widespread, since frequently it is complicated to assess whether the accumulation of SOC is net or it is simply a transitory movement among different reservoirs. This fact leads to some confusion in the terminology used to define a compartment which stocks (or sequesters) C. The challenge is to distinguish between a *stock*, a *sink* and a *source*. A stock is a compartment where the organic C is stored. When the stock accumulates SOC, then that stock is a sink and the CO_2 balance of that stock is positive (e.g. the magnitude of the inflow organic C is higher than that of the outflow). Contrary, when the SOC level of the stock decreases, then it is considered as a source of CO_2 towards atmosphere, hydrosphere or lithosphere, and the SOC balance is negative (inflows are lower than outflows) (figure 12).

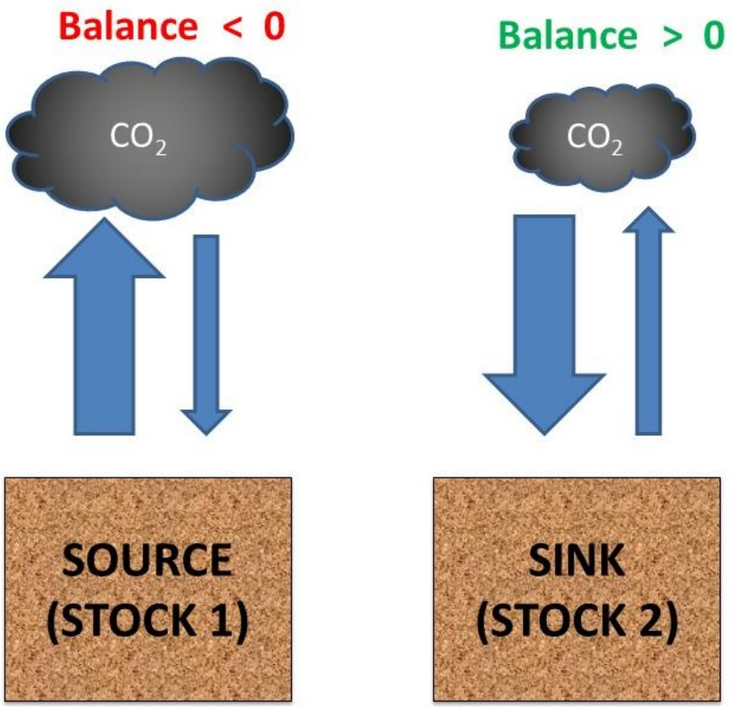


Figure 12. Schematic representation of the C balance in two stocks, one corresponding to a CO_2 source (left) and the other one to a CO_2 sink (right).

The most common units in which SOC is quantified are:

1. Percentage of SOC.
2. SOC concentration (g C Kg^{-1} soil or mg C g^{-1} soil)
3. Amount per soil surface unit for a given depth (Mg C ha^{-1} or t C ha^{-1}, and g C m^{-2}) (i.e. SOC stock)

Usually soil C stock is expressed as the amount of C per area unit, since it gives information about the amount of the SOC stored. Moreover, it is an adequate way to compare ecosystems or agroecosystems with very different pedologic properties (e.g. bulk density), since bulk density is already included in the SOC stock calculation.

To asses for the organic C fluxes between the different compartments or fractions, it is commonly use the mass of transferred C per time and mass, volume or area unit. For that purpose, different units are used depending on the scale) of the assessed area; from Mg C ha^{-1} yr^{-1} or t C ha^{-1} yr^{-1} at a plot or ecosystem scale to Pg C yr^{-1} or Gt C yr^{-1} when the scale is much higher (e.g. at planetary scale).

3.2 Is there a limit in the capacity of the soil to sequester organic C?

In order to estimate the long-term effects of the agricultural management practices on the SOC dynamics it is necessary to know whether there is a limit on the capacity of a soil to protect and stabilize organic C or, on contrary, the organic C reservoirs can increase indefinitely when increasing the incoming organic C. This is not trivial, because the majority of the current models designed to predict the organic C dynamics generally use first-order kinetics and predict linearity between the level of the annual incoming organic C and the SOC content at the equilibrium. This linearity means that the efficiency of the SOC accumulation (ΔSOC/Δincoming organic C) is constant and independent on the SOC level and, therefore, the SOC levels at the steady state will increase continuously without any limit while increasing the incoming organic C.

Some long-term studies in agroecosystems at equilibrium (at the steady state) have shown that by increasing the incoming organic C levels, SOC increases (e.g. Paustian *et al.*, 1997), apparently validating this linear model. However, there are other studies showing that the SOC levels do not change, or slightly change, from a given annual rate of organic C input. This lack of long term response in SOC content when annual entry of organic carbon is relatively high, suggests a saturation in the amount of organic carbon that agroecosytems (and natural ecosystems) may sequester.

Why there must be a maximum capacity of SOC sequestration?

The hypothesis on the existence of a saturation relationship between the annual organic carbon inputs and SOC content, and therefore on the existence of a limit to sequester organic carbon, was first proposed by Hassink (1997) but reformulated by Six *et al.* (2002). These authors reasoned that that limit should be related to the limited capacity of a given soil to protect and, therefore, to stabilize organic carbon. Indeed, these authors proposed the existence of limits in the capacity of a soil to chemically and physically protect SOC.

1. Protective capacity (physically and chemically protected SOC)
2. Protection level (physically + chemically + biochemically protected SOC)
3. Saturation level (physically + chemically + biochemically protected + unprotected SOC)

As it was shown in the previously, the amount of chemically protected SOC is directly related to the silt and clay concentration in the soil (tables 3 and 4). Since the amount of silt and clay in the soil is limited, thus the amount of the organic C which can be chemically protected by these soil particles will also be limited. Therefore, there must be a limit to chemically protect organic carbon.

Physically protected organic carbon is that protected within soil microaggregates. There is a limit in the capacity of a soil to create microaggregates and it is highly dependent on soil texture, environment conditions, soil biota functioning and organic matter, and thus on management practices. Therefore, there must be a limit to physically protect organic carbon.

Regarding the biochemically protected SOC, the amount of this fraction depends on the biochemical features of the original organic matter and subsequent transformation, mainly via condensation and complexation reactions. Thus, it is not clear that a biochemically protection saturation level might exist, since this fraction might not depend on the soil physical properties.

Therefore, if there is a limit in the capacity of a soil to protect physically, chemically and biochemically, then the capacity to sequester organic C is finite and a soil become saturated in organic C. Figure 13 shows the rationale under this hypothesis.

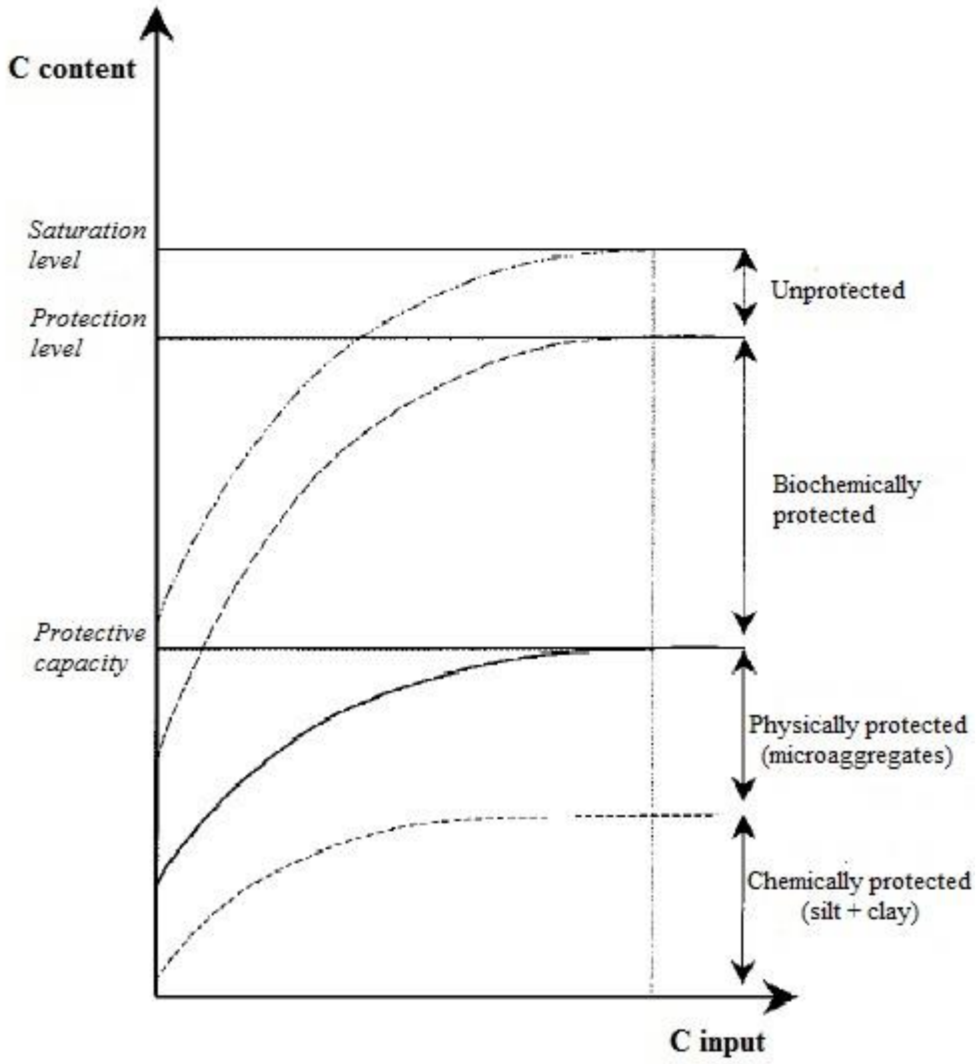


Figure 13. SOC fractions dynamics and pool size are defined by their stabilization mechanisms. Adaptation from Six *et al.* (2002)

Accumulation rate of soil organic carbon may be predicted by the following equation (Eq. 1):

$$\frac{dC_t}{dt} = I\left(1 - \frac{C_t}{C_m}\right) - kC_t \qquad (1)$$

where dC_t/dt is the rate of SOC change. This rate depends on the input organic C (I) in a given time (t) and the SOC losses through decomposition (e.g. soil respiration), which follows a first order kinetic which depend on the SOC (Ct) and a constant (k). The SOC changes rate depends also on the C_m, which corresponds to the maximum amount of C which can be stabilized by soil physico-chemical and biochemical mechanisms (*protective capacity*). As already mentioned, the protective capacity depends on the silt and clay content of the soil (Hassink, 1997; Six *et al.*, 2002), but also on the capacity of the soil to produce microaggregates. As it was shown in the previoulsy, (Tables 3 and 4), the amount of SOC linked to silt and clay can be estimated by using the empirical functions found by Six *et al.* (2002) (Eq. 2), considering the particle size and type of clay (1:1 or 2:1):

$$C_m = slope \times (\% \, sil + clay) + intercept \quad (2)$$

The rate of soil organic carbon accumulation changes according to the differences between the SOC and the level of SOC in which SOC saturation is reached, decreasing when current SOC is close to the maximum SOC level. In other words, SOC accumulation rate decreases when the saturation deficit (sd) decreases, which represents the difference between current SOC and the saturation level, is low (Eq. 3).

$$sd = 1 - \frac{C_t}{C_m} \quad (3)$$

Thus, at the steady state, the relationship between the incoming organic C $(I*)$ and the SOC concentration (C_t*), follows a saturation type curve (figure 14) (Eq. 4).

$$C_t^* = \frac{I^*}{k + \frac{I^*}{C_m}} \quad (4)$$

In agricultural soils the saturation deficit is typically high, mainly because the SOC level is relatively low due to: i) the soil management, which enhances SOC losses, and ii) to the low annual inputs of organic C. Therefore, I hypothesised that the potential for C sequestration in Andalusian olive grove soils may be very high.

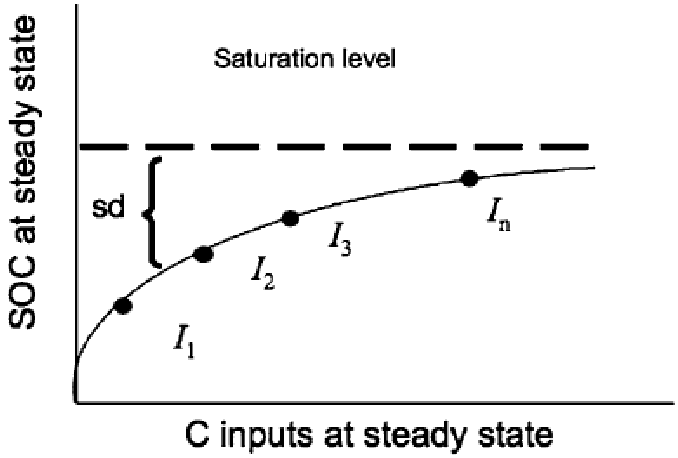


Figure 14. Saturation relationship between the organic C inputs and the SOC levels at the steady state. Note that when the higher the saturation deficit (sd) is, the lower the percentage of the incoming organic C is accumulated in the soil. Note also that the behaviour is almost linear at low levels of organic C inputs. Stewart *et al.* (2007).

3.3. Do all the SOC fractions best fit a saturation model?

Some long-term (assuming steady-state) studies which clearly suggest that C sequestration in the soil under a range of C input is better modelled using a C saturation model rather than a lineal model have been published. For instance, Stewart *et al.* (2007) compiled the most recent SOC contents and average C inputs levels (as crop residues and organic amendments) from a data set complied from 14 long-term agricultural sites around the World. By fitting both models they found that the saturation model best fits the combine data of 10 out of the 14 sites, especially those with an ample range in C input levels, and for four individual sites, those with a narrow range of C input levels, were best-fit with the linear model. Campbell *et al.* (1991) in a 30-year old study reported that SOC in soils already rich in SOC did not increase despite an increase in the organic carbon load. Similarly, in a Canadian barley (*Hordeum vulgare* L.) field high in organic matter, SOC concentrations remained unchanged even when the C input increased due to 15 yr of N inputs and straw additions (Solberg *et al.*, 1997). After 11 years, Nyborg *et al.* (1995) found less stabilization of new C in a Typic Cryoborol (Ellerslie, Alberta) with a greater C content (86.7 Mg C ha^{-1}, 0 – 15 cm) compared to a paired site at Breton, Alberta (Typic Cryoboralf) (32.2 Mg C ha^{-1}, 0 – 15 cm) under straw addition and N-fertilization treatments. Finally, Chung *et al.* (2008) showed that increasing soil C input (gradient in plant productivity and consequently C inputs into the soil) did increase the SOC following a saturation model in a 13-year in field experiment.

These studies suggest that the capacity of soils to stabilize extra C is dependent on how much C is already in the soil rather than the level of C input (Hassink, 1996; Six *et al.*, 2000). If this is the case, most of the SOM models that assume a linear increase in SOC levels with greater C input may not be adequate for predicting changes in SOC levels when C input levels increase, especially in high-C soils.

Therefore, it seems that sometimes the C saturation models are suitable, but in other cases a linear function best fits the SOC fraction dynamic.

Under the assumption of a saturation type curve between the annual input of organic carbon and SOC accumulation, the following must be verified:

1. If the saturation deficit affects the rate of SOC stabilization, therefore, the proportion of the incoming organic C which is stabilized (protected) would be higher in those soils with higher saturation deficit.

2. The efficiency of the SOC stabilization (e.g. amount of organic carbon stabilised relative to that applied) would be greater at lower rate of incoming organic C.

Stewart *et al.* (2009) observed in an experiment carried out in laboratory controlled conditions that after 2.5 years, the lower the saturation deficit was the lower the accumulation of the incoming organic C was. However, this was not true for all SOC fractions, but only in the chemically and biochemically protected pools. On the other hand, and regarding the second hypothesis, only the chemically protected organic carbon pool stabilized higher added residue C with lower amount of the organic C input. The remaining fractions showed no general trend in C accumulation with addition level.

Therefore, chemically and biochemically protected pools accumulated significantly higher SOC with higher saturation deficit. The results of the chemically protected pool are in line with those results obtained by Stewart *et al.* (2008a) who observed how the chemically protected pool best fits a saturation model. However, in the case of the biochemically protected pool results were not clear, since in some cases it followed also a linear model. In this same study, the stabilized organic C in the physically protected pool did not show relationship with the SOC saturation deficit.

The unprotected SOC is directly related to the incoming fresh organic matter and it does not relate with the saturation deficit, since it presents an independent behaviour of the protection mechanisms affecting the mineral fractions. The POM within microaggregates (iPOM) is also non-dependent of the saturation deficit. This fact must be considered, since if the saturation limit of SOC is matched for the mineral fraction (protective capacity) it is still possible to continue providing organic C to the soil and accumulating it not only in the unprotected fraction but also as POM within microaggregates. This fact is again in line with the results obtained by Stewart *et al.* (2008a), who showed that the linear model best explains the behaviour of the unprotected and iPOM fractions. Furthermore, according to these authors, the remaining sub-fractions of the physically protected pool followed a saturation behaviour.

Therefore, those SOC fractions which organic C content is related to the saturation deficit will fit a C saturation function, whereas on contrary, those fractions which SOC content is not related to the saturation deficit will fit a linear behaviour lacking a saturation limit (figure 15).

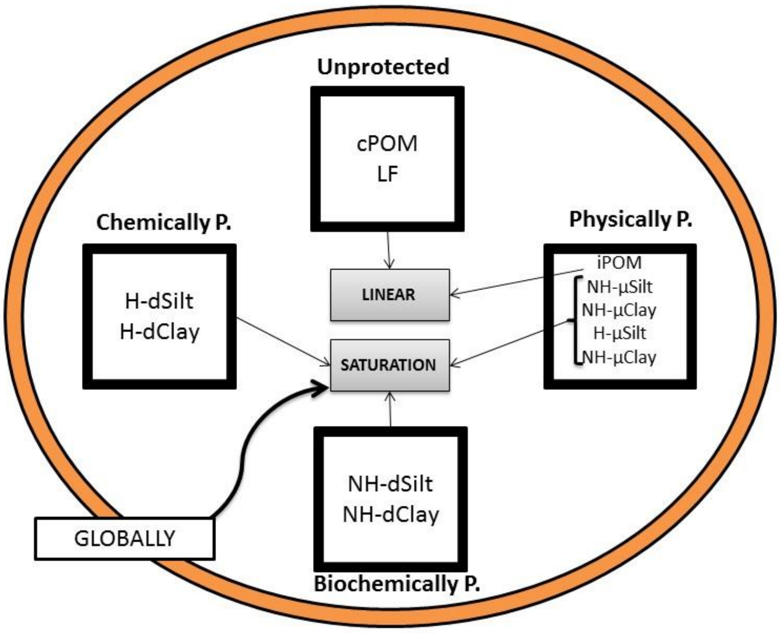


Figure 15. Conceptual model of the different SOC fractions and their behaviour (linear/saturation). Information obtained from Stewart *et al.* (2008a) and Stewart *et al.* (2009).

3.4 Influence of the management practices on the SOC saturation level

Although with some exceptions, there are strong evidences that the hypotheses proposed by Stewart *et al.* (2009), who suggested a relationship between the saturation deficit and the SOC stabilization, is right. If so, degraded soil (e.g. higher saturation deficit) may have a great capacity to accumulate SOC. Therefore, the capacity to sequester organic carbon in the soils of a great proportion of cropland in general, and a significant area of the Andalusian olive groves, is high.

Nevertheless, the capacity to sequester SOC of an agricultural soil depends on the management practices, so it would be possible to vary the maximum capacity of SOC sequestration. As mentioned above, the maximum capacity of a soil to accumulate SOC depends mainly on the soil properties. Nevertheless, this level is only reachable in natural ecosystems and not in agricultural soils because they are under regular perturbations which affects their soil properties, thus reducing the organic C potentially sequestrable. For that reason, Stewart *et al.* (2007) proposed a new concept: *effective stabilization capacity*. According to these authors the same soil with similar amounts of incoming organic C and under different management practices will have different effective stabilization capacity, although their saturation level is similar (figure 16).

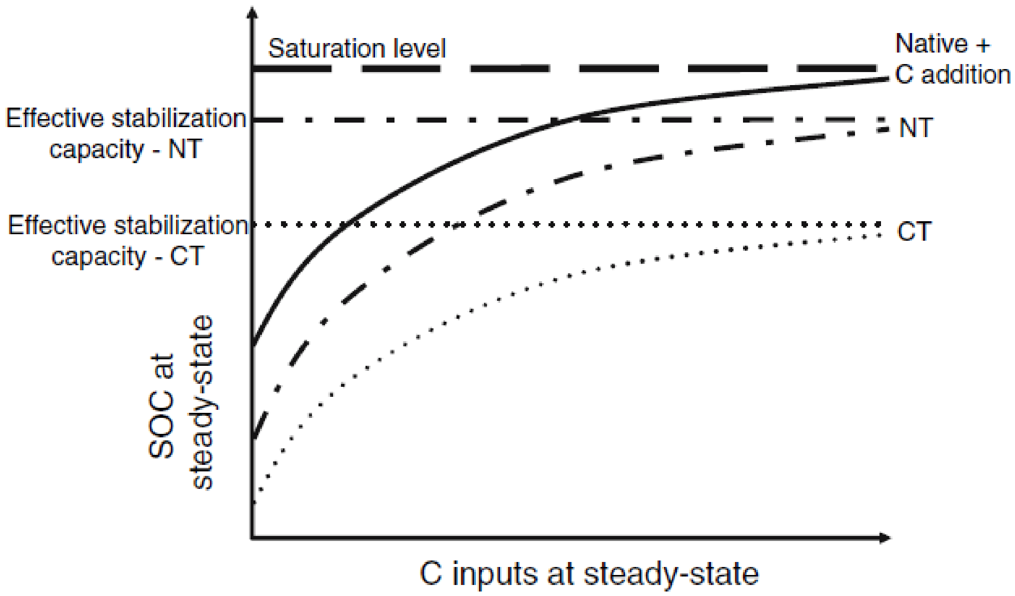


Figure 16. SOC accumulation dynamics under different managements (NT = no tillage; CT = conventional tillage). Note that soils have the same saturation limit but each management has its own effective stabilization capacity. Stewart *et al.* (2007)

Whereas the saturation concept is not recent this is not the case for the effective stabilization capacity concept. For the agroecosystems in general, and olive groves in particular, to change from a C source to a C sink after the implementation of specific management practices it is necessary to set up the differences existing between the SOC at the saturation level and that of the effective stabilization capacity for each of the management practices. Thus, it would be possible to assess what management practices are the best to maximize SOC sequestration and which of them would correspond to those practices where the effective stabilization capacity is near to the saturation limit

3.5 The maximum attainable level, a realistic objective for agricultural soils

The effective stabilization capacity is that what Ingram and Fernandes (2001) named *maximum attainable level*. According to these authors it can be distinguished three levels according to the organic C which can be sequestered by a soil: (1) potential, (2) attainable (3) actual (figure 17).

The *potential* is defined by those physico-chemical factors which mainly affect the maximum limit of SOC accumulation. These factors are those corresponding to the mineralogical composition (silt + clay content) and the soil depth. However, it also affects other factors like stoniness, bulk density and aeration level. Despite the mineralogical composition and depth are intrinsically related to the soil and non-alterable, these three can be modified through the management practices (figure 17).

Secondly, the *attainable* level is established by those factors limiting the organic C inputs to the soil. The net primary production (NPP) is the main driver leading to increase the SOC content. Therefore, any management practice generating an increase on the NPP or an increase on the incoming organic C (in the case of olive groves throughout the implementation of a spontaneous resident plant cover or the incorporation tree pruning residues) will increase the maximum attainable level and, therefore, this will near to the potential. Climate is other driver affecting the organic C inputs to the soil, and this influence is made through two main ways: (1) direct, by increasing of the temperature (increase of the decomposition rate) and/or generating anaerobia conditions (it decreases the decomposition rate); (2) indirect, by affecting the presence and activity of the vegetation, fauna and soil microorganisms. Considering that is not possible and realistic to reach the potential level, in agroecosystems is usually used the maximum attainable level for assessing the best management practices (figure 17).

The *actual* level is defined by those factors responsible for the actual level of SOC. There are five main drivers related to the agricultural management which are responsible for the relatively low levels of SOC. (1) Erosion, which generates SOC losses and the mineral fraction associated to the organic C protection; (2) the increase in the SOM decomposition rate; (3) the decrease of the organic residues inputs (e.g. burning or removal crop residues); (4) the disturbance of the soil biological processes generated by the lower SOC, which limits the formation of organo-mineral stable complexes processes, and (5) the drainage, which facilitates soil aeration and thus SOC oxidation (figure 17).

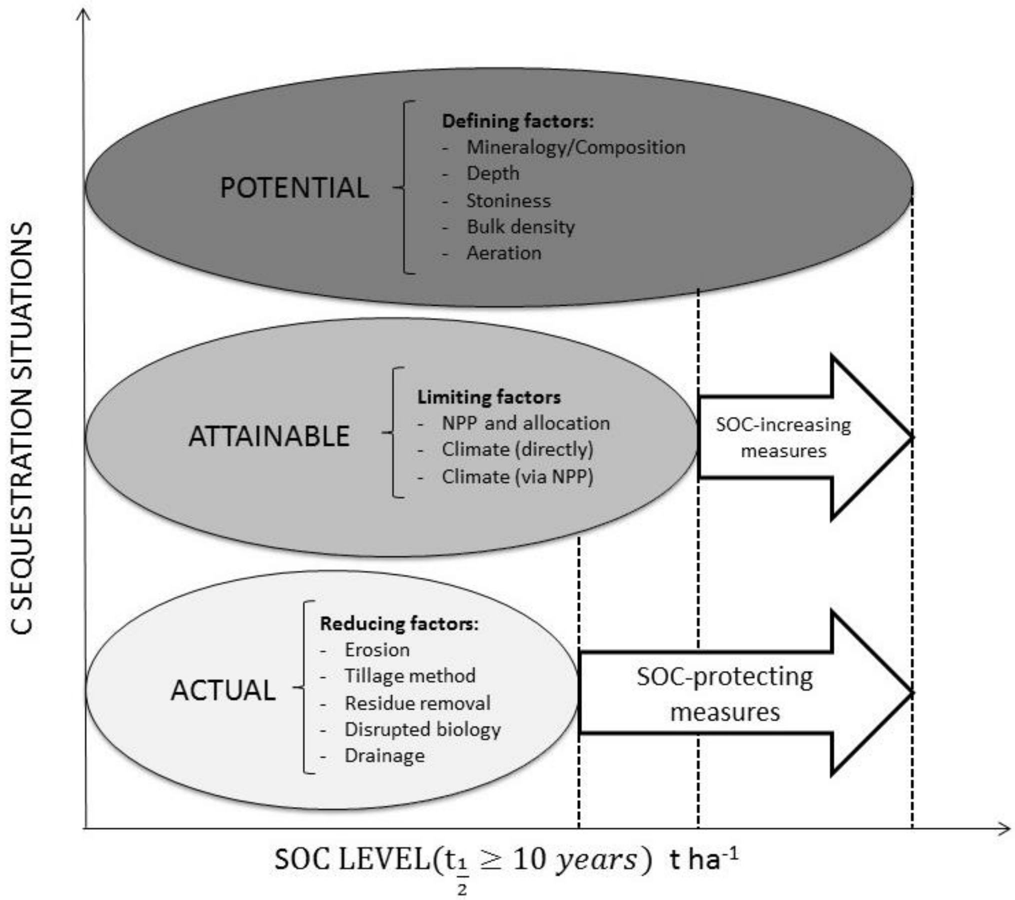


Figure 17. The three different C situations (potential, attainable and actual) in terms of SOC sequestration and the main factors affecting each situation in relation with the SOC levels. Adaptation from Ingram and Fernandes (2001).

4. Main management practices of olive orchards of Andalusia: State of the Art

4.1 Olive oil orchards of Andalusia

Spain has a total surface of olive groves about 2.6 million hectares, of which about a 60% are in Andalusia (1.6 M ha) (MAGRAMA, 2013). In Andalusia, olive groves represent about the 44% of the total crop surface. Jaén is the province with the largest olive grove surface, where the 43% of the province surface is covered by olive groves (MAGRAMA, 2013).

About two thirds of the Andalusian olive groves are non-irrigated, although since the last years the irrigated surface is increasing. Drip irrigation is the most common irrigation type (MAGRAMA, 2013).

These figures show that olive groves have a high economic, social and cultural importance and in the predominant landscape of many areas of Andalusia. Therefore, changes of the management in olive groves not only will have a technical and economical, but also environment impacts (e.g. in the net balance of C), at least at regional scale.

4.2. Types of olive grove farms and main management practices with consequences in SOC

According to tree density, olive groves are classify mainly into 3 categories (Cubero, 2010):

- <u>Traditional olive farms.</u> Trees with two or three feet, large planting framework (10 to 12 m), tree density between 80 and 120 ha^{-1}, usually non-irrigated and with an age of more than 25 years (figure 18).

Figure 18. Olive oil farm with a traditional frame.

- High density. Trees of one foot and usually irrigated. They can be classified into two categories:
    - ✓ Intensive. 200 – 600 trees ha^{-1}, with an inter-row width of less than 6 m and with a life of more than 40 years (figure 19)
    - ✓ Super-intensive (hedgerow plantation). 1000 – 2000 trees ha^{-1}, with an inter-row width of less than 4 m and a life of 12 – 14 as a maximum (figure 19)

Figure 19. Examples of olive oil farms with an intensive (left) and super-intensive (right) frame.

The most common planting frameworks are those belonging to the traditional olive groves, with a low density tree plantation. This feature is also related to the age of the plantation, because it has not been until the last two decades when the intensive and super-intensive plantations have been implemented. Thus, approximately a 70% of the Andalusian olive groves have a planting framework between 80 and 120 trees ha^{-1} (figure 20). Irrigation is relatively common in the high-density frameworks, whereas in olive farms with a traditional planting framework of less than 200 trees ha^{-1} the irrigated area is lower than a 30 %.

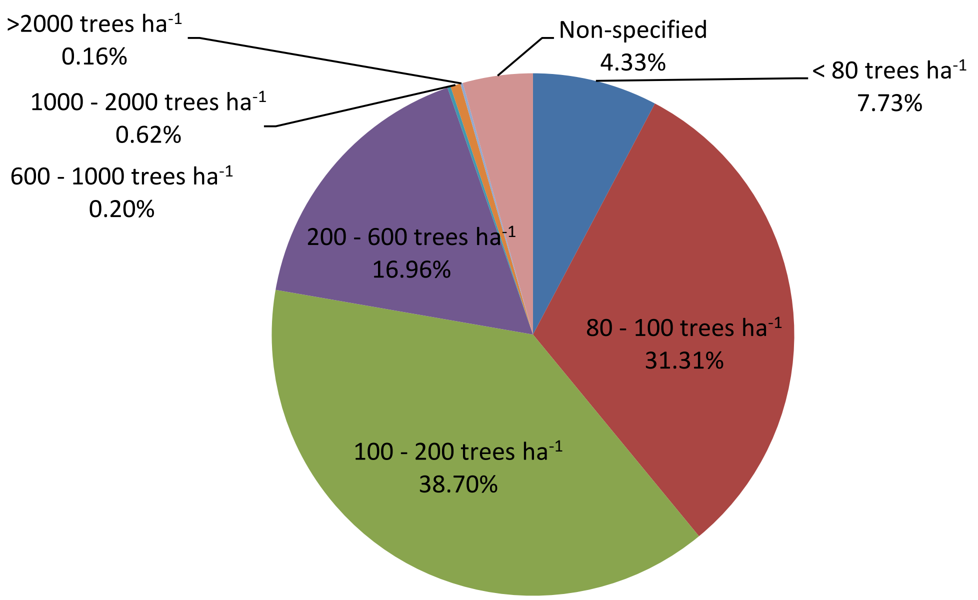


Figure 20. Area (%) of the Andalusian olive grove according to the density intervals (< 80 trees ha^{-1}, 80 – 100 trees ha^{-1}, 100 – 200 trees ha^{-1}, 200 – 600 trees ha^{-1}, 600 – 1000 trees ha^{-1}, 1000 – 2000 trees ha^{-1}, > 2000 trees ha^{-1} and non-specified). Own elaboration. Information obtained from MAGRAMA (2013).

Different tree densities may have a profound impact on the implementation of managements, which affects to the net balance of organic carbon in the soil. Indeed, tree density affects the annual production of biomass of the plant cover and potential for organic carbon inputs due to tree pruning. Some managements may increase SOC losses because of soil erosion and an enhancement of the decomposition rate, whereas others increase the annual input of organic C. However, it is difficult to study the effect of a single management on the SOC turnover, since different managements are usually combined.

Currently, the most common agricultural management practices with implications in the net annual SOC balance are the following:

I. Tillage versus no tillage and spontaneous resident vegetation cover.

II. Application of external organic amendments (manure and composted olive mill pomace)

III. Burning versus shredding the tree pruning remains.

I. Tillage versus no tillage and spontaneous resident vegetation cover.

1. Tillage and net SOC balance

Tillage typically consists of tilling the first 10 – 20 cm of the upper soil layer, between 2 and 5 times a year by ploughing or using a disk harrow, primarily to control the growth of the spontaneous resident vegetation of the inter-row or below the tree canopy of the orchard, but also to improve roots exploration capacity and promote rainwater infiltration. Currently, tillage is the most common soil management practice in olive groves.

This management practice leads to a decrease in SOC mainly due to (figure 21):

i) An increase in soil erosion (especially water erosion), and thus soil organic carbon losses.

ii) An increase of the decomposition rate (SOM mineralization) and soil macroaggregate disruption with a lower microaggregate formation.

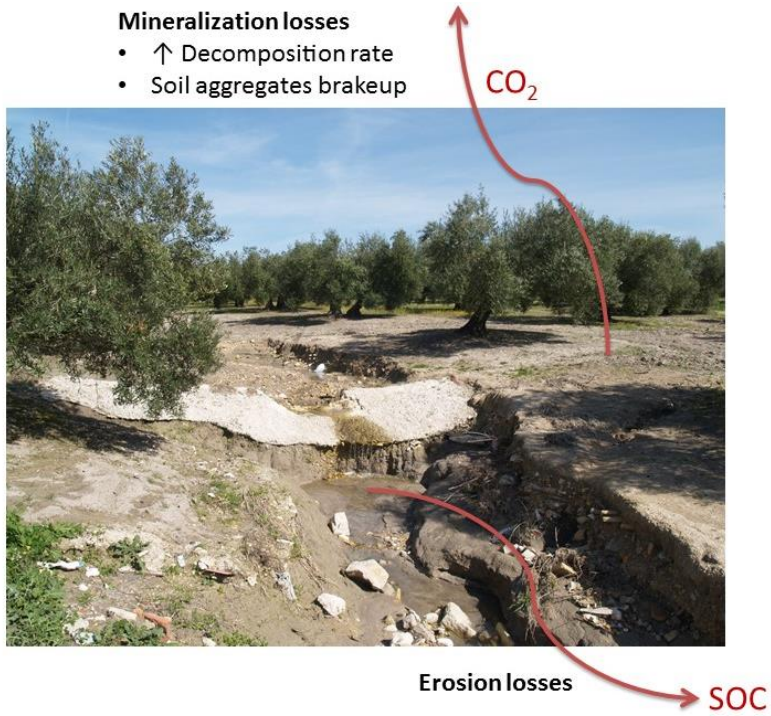


Figure 21. Potential effects of tillage on SOC losses due to an increase in soil organic carbon mineralization, soil aggregates breakup and water erosion**.**

It is well known that soil erosion depends, among others factors, on both climatic and geomorphological conditions. Mediterranean climate is characterized by a high intra and inter year variability. This, together with the fact that many areas of olive cultivation have a slope greater than 8 %, made olive groves susceptible to the water erosion.

The continuous tillage does not allow the presence of a resident vegetation cover and, thus, soil is bare most of the year, increasing soil erosion. For instance, Gómez *et al.* (2004) found that soil losses in an olive grove (slope of 13.4%) under tillage was about 3.3 times higher in comparison with these obtained in the same olive grove but with soil covered by resident vegetation. In another olive grove with lower slope (4%), Gómez *et al.* (2011) found annual soil losses of 2.6 t ha^{-1} in an olive grove under tillage, whereas under resident vegetation cover losses were only 0.17 t ha^{-1}. *However, it is not known neither the SOC fractions which are lost by water erosion nor whether the C of these fractions is lost by mineralization or simply it is moved to another place.*

Tillage also may increase SOC losses due to an enhancement of the SOC decomposition. Indeed, it is well known that one of the main effects of the soil tillage is the soil perturbation and mixing of different soil layers. As a consequence, SOC oxidation is increased and thus, the emission of CO_2 to the atmosphere (figure 22).

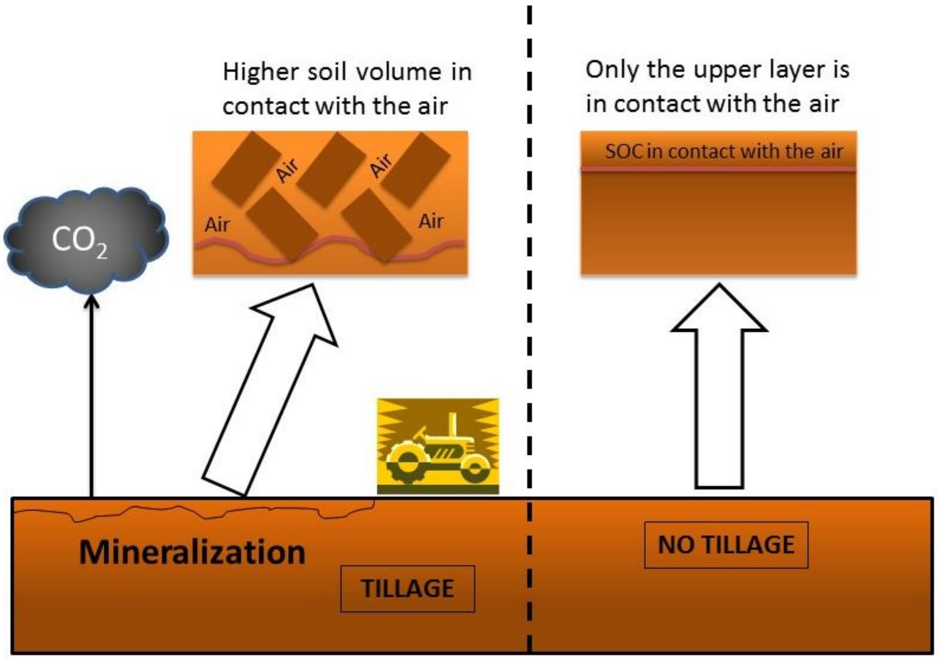


Figure 22. Scheme representing the increase in the mineralization due to tillage.

In addition to the “aeration” of the soil, tillage also has a profound effect on the disruption of soil macroaggregates. As already mentioned, macroaggregates play a key role on SOC physical protection, and in to a lesser extent, on chemical protection. As it was shown previously, soil microaggregates, which generates a physical barrier between microorganisms and SOC within microaggregates, would be formed from soil macroaggregates. Therefore, under tillage it is expected a decreased not only in the pool of soil organic carbon physically and chemically protected, but also in the potential for soil organic carbon protection. Two mechanisms might explain the increased in CO_2 emissions after tillage. First, the direct emission of the CO_2 contained into the soil pores (“degasification” process) (Rochette and Angers, 1999). This is a short-term, short-lasting mechanism. Secondly, the disruption of soil aggregates, which leave without protection the SOC initially physically protected and, therefore, it can be relatively rapid mineralized due to the increase in soil oxygen.

Álvaro-Fuentes *et al.* (2007) observed that the increase in the CO_2 fluxes from soil to the atmosphere after tilling has a duration of about 3 hours, and after this period the CO_2 emissions in the tillage system returned to similar values to those of the no tillage systems. This was likely due to the soil degasification. At the long term, the CO_2 emissions due to macro and microaggregate disruption is, likely, the most important mechanism, but is difficult to evaluate due to the high “bacKground” soil CO_2 emissions.

Therefore, *under tillage lower amount of physically protected organic carbon is expected*. However, *there are no studies designed to evaluate the dynamics of these SOC fractions in olive groves*.

2. <u>No or minimum tillage and SOC balance</u>

An alternative to tillage widely studied and applied in olive grove soils is the no tillage or minimum tillage. This management consist of keeping the soil free of vegetation (e.g. bared) by using pre- and post-emergence herbicides. Nevertheless, in the conventional tillage, herbicides are applied very often.

In the no-tillage systems, SOC losses are diminished mainly because soil aggregates are not disrupted and oxygen diffusion into the soil is limited. However, no-tillage systems might promote water runoff when farm slope is medium to high (figure 23). For instance, Gómez *et al.* (2004) observed in an olive grove with an average slope of 13.4%, that soil losses under no-tillage were more than 2 times higher than under tillage.

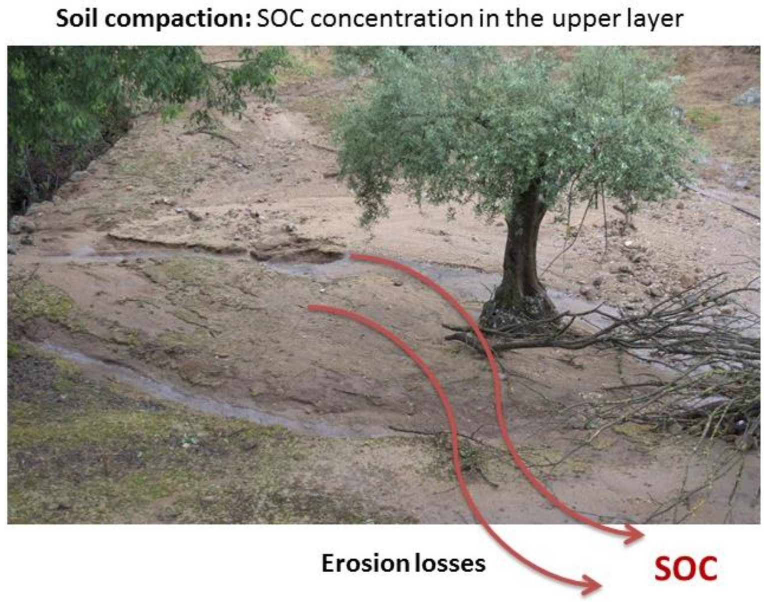


Figure 23. The no-tillage management may leads to a high SOC losses by water runoff in high sloped farms.

Many studies have been designed to evaluate changes on SOC under tillage versus no-tillage. Most of the studies found that SOC content of both managements is, in general, very similar. Nevertheless, depth patterns are markedly different. In no tillage systems SOC is clearly stratified, whereas under tillage SOC is distributed almost homogenously along the arable layer (upper SOC is “diluted” along the arable layer). This may have a significant effect in reducing the SOC which can be lost by mineralization or erosion with take place in the upper layer. On the other hand, under tillage disruption of soil aggregates is enhanced, preventing the accumulation of the SOC within microaggregates and, therefore, keeping the SOC in contact to soil microorganisms thereby facilitating its oxidation. In general, the results of these studies are contradictories highlighting the fact that many other factors such as soil type and geomorphologic features (e.g. orientation, slope, texture...) are determinants of the effects of tillage on SOC pools. Furthermore, more studies are needed to assess for changes in the SOC fractions and potential protective capacity of soils under contrasting tillage regimes.

3. The vegetation cover and changes in the potential for SOC accumulation

Since last two decades the implementation of a spontaneous resident vegetation cover in inter-row area of olive groves is spreading, mainly due to the "condicionalidad" (in Spanish) regulation, which was born to mitigate soil erosion of the olive oil groves at the long term. This Regulation states the obligation for the implementation of a vegetation cover of a minimum of 1 m wide, to receive direct payments under certain conditions (when the slope averaged more than 10 %). Generally, spontaneous vegetation is controlled (clearer) between the end of March middle April, when transpirations might reduce significantly soil water content. Plants are usually controlled by chemical (herbicides) or mechanical (tillage or brush cutter) means. Plant residues are typically left on the soil surface or mixed with the soil, when controlled by tillage. Spontaneous vegetation cover is usually no fertilised.

Currently (2012), about a third of the total olive grove area is covered by a spontaneous resident vegetation. In the other two thirds soil is bared by the combination of the application of herbicides with a minimum tillage. Approximately, about a 15% of the olive groves do not till and the spontaneous vegetation is controlled by pre- and post-emergence herbicides (figure 24) (MAGRAMA, 2013).

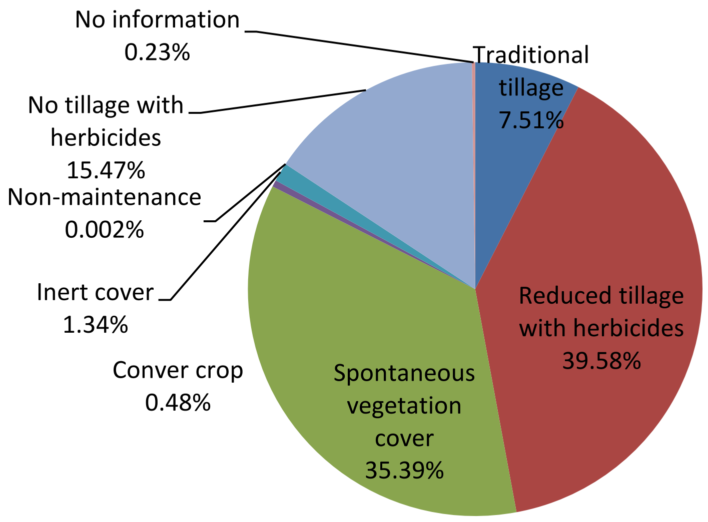


Figure 24. Main soil management of olive groves in Andalusia. More than a half uses herbicides, whereas the spontaneous vegetation cover is implemented on only in one third of the total olive grove area. Own elaboration. Information obtained from MAGRAMA (2013).

There are several alternatives in the implementation of the vegetation cover according to the:

- Species composition of the vegetation cover:
    1. Sown. Low diversity of species but selection of appropriate species. In these cases, plants are sown and usually the selected species are legumes (typically wild legumes) or a combination of legumes and cereals or grasses.
    2. Spontaneous natural vegetation cover. All the vegetation cover is composed of spontaneous growing species.
    3. Mixed. A proportion of the vegetation cover was sown, whereas the other part is formed by spontaneous resident species.

- Proportion of the area covered by the vegetation:
    1. Full cover. Vegetation covers the inter-row and the area under three canopy. The percentage of the soil covered between September-October to the end of March-middle April is near 100 %.
    2. Partial cover. Only soil of the inter-row area is covered (typically a band of between 2 and 4 meters wide).

The vegetation cover in olive groves has two main advantages in terms of SOC sequestration:

1. Reduces significantly soil losses due to erosion in comparison to soils under tillage or no tillage (figure 25). Soil losses due to water erosion in olive oil farms with vegetation cover is expected to be inversely proportional to the area covered. Thus, Durán-Zuazo *et al*. (2009) found in mountainous olive plots in Granada (SE Spain) a significant reduction in runoff (between 94 and 95% lower) and erosion rates (between 59 and 71% lower) in the vegetation-covered plots in comparison to plots without vegetation cover. For an average slope of 20 %, Gómez *et al.* (2003) predicted soil losses between 45 and 30 t ha^{-1} yr^{-1} in plots with a resident vegetation cover with 2 and 4 m of width, respectively (figure 25). Although high, this estimate represents a reduction in soil loss of between 40 to 60 % in

comparison no-tillage with herbicides management. In another study in an olive grove with lower slope (4 %), Gómez *et al.* (2011) measured annual soil losses in vegetation-covered plots of 0.17 t ha^{-1} (figure 25) which was 7 times lower than that obtained under tillage.

2. The yearly application of the biomass residues of the vegetation cover on the soil surface or within the soil implies an increase in the annual input of organic carbon. In addition, the presence of a vegetation cover contributes to improve soil structure features and, thus, facilitate the transformation of labile C into protected C pools.

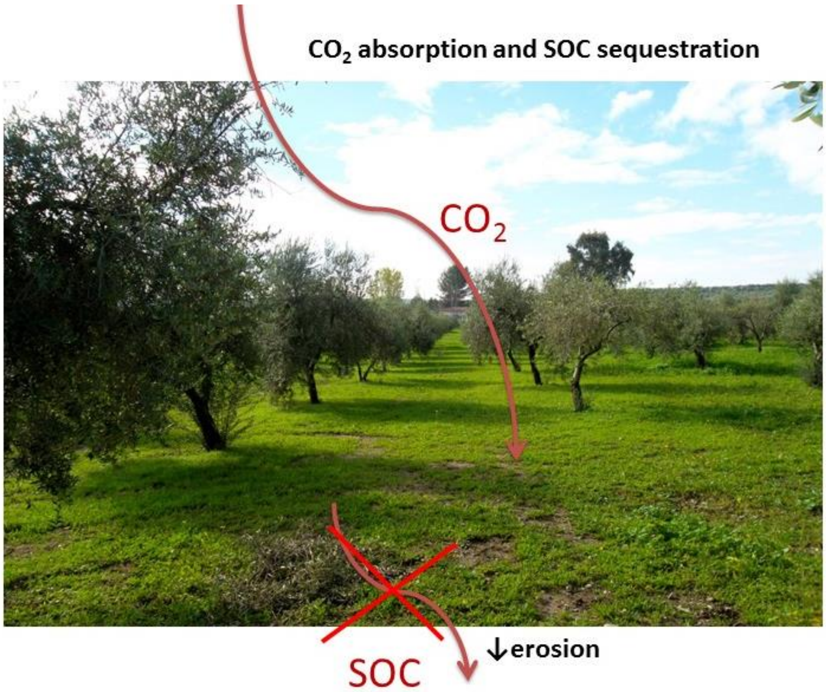


Figure 25. The vegetation cover increases the CO_2 absorption, the incoming organic matter with the above- and belowground biomass, and decreases the SOC lost because of the erosion.

The amount of the organic C input in olive groves from the vegetation cover depends mainly on the annual biomass production, which can show high inter-annual variability, typically of Mediterranean conditions (especially rainfall). Furthermore, there is also a spatial variability due to the differences on geomorphological, landscape and edaphic features. Therefore, it is difficult to provide a range of most probable values of annual biomass production of the vegetation cover.

Nevertheless, not all this organic C is stored in the soil at medium-long term, since a great proportion can be lost to atmosphere as CO_2 during decomposition processes. The control method of the vegetation cover can affect its decomposition rates and, therefore, also the amount of the organic C which is stored in the soil.

As it was mentioned before, the aerial biomass of the vegetation cover can be added to the soil through two ways: i) being mowed and deposited over the soil surface, and ii) being ploughed and, thus, buried into the soil (i.e. controlled by a reduced/minimum tillage).

Although there are some information on the changes in SOC pools of olive oil orchards under a vegetation cover, studies aiming to assess for the effects of the annual application of plant residues on SOC fractions of different levels of protection are lacking.

II. Application of "external" organic amendments (manure and composted olive mill pomace)

Main sources of organic matter in the commercial olive oil orchards are manure (mainly sheep, but other sources such as goat, poultry and horse is also applied) and composted olive mill pomace. Although there are not statistics, the number of olive groves, and thus the area in the whole Andalusia, which apply external sources of organic matter is very low. This is so, mainly due to the scarcity of manure and the difficulties in their application in the field. *Despite the well recognise effects of manure application on SOC in many other cultivation systems, there are very few studies on olive orchards. This is true also for composted olive mill pomace.*

García-Ruiz *et al.* (2012) carried out a study consisting on the annual application of between 4 and 6 t ha^{-1} (wet weight) of composted olive mill pomace in olive orchards, and found that SOC content increased significantly, especially after 16 years of application. Similar results were obtained by Lopez-Piñeiro *et al.* (2011) after applying between 27 to 54 t ha^{-1} yr^{-1} of composted olive mill pomace during 8 years. They found that SOC in the first 25 cm of soil was 15.9 and 43.9 tonnes higher in those plots than in the control, respectively.

III. Application of "internal" organic matter sources from the tree pruning

A rainfed olive grove produces annually between 1.3 and 3 t ha^{-1} yr^{-1} of pruning residues, with a mean organic matter content about 72% (Ramos, 1999). *Therefore, the management of the olive tree pruning can be significant regarding the changes of the SOC pool*

Pruning residues can be managed in two different ways:

- Shredded and applied on the soil surface. This implies an annual input of soil organic carbon. In addition, the application of pruning residues as a mulching has other medium-to-long-term benefits such as: i) modifies the physico-chemical soil properties, increasing those indicators related to soil fertility. In addition, it has been described a lower need for herbicides after the continuous application of pruning debris due, especially, to the lower growth of the spontaneous plant cover (Ordóñez-Fernández *et al.*, 2007), ii) it acts as a physical barrier lowering the soil losses due to erosion. There are few studies assessing the effect of the application of the shredded pruning debris on the SOC accumulation. Repullo *et al.* (2012a) after applying different doses of pruning residues of different size found that: i) SOM increased by between 0.86 to 1.52% , ii) after one year, more than 50% of the added pruning residues-C were released to the atmosphere as CO_2, and iii) in two years the SOC content increased between 5 and 12 t C ha^{-1}. This study clearly shows *the importance of the application of pruning residues on the soil surface on SOC accumulation.*

- Burned, thus generating ashes and *biochar* (Lehman *et al.*, 2006). Biochar is formed by graphene crystals and amorphous aromatic compounds (Bourke *et al.*, 2007). These chemical features leads biochar is a very stable input and, therefore, it can be used to increase SOC content (Lehmann and Joseph, 2009). The heterogeneous composition of the biochar is responsible for its high reactivity, especially in the surface are. These features suggest that the application of biochar might improve the agricultural production (Jeffery *et al.*, 2011).

  However, in a field experiment in olive groves, García-Ruiz *et al.* (personal communication) found that only between the 4 and 5% of the pruning debris become ashes (80%) and biochar (20%). *Therefore, the importance of the biochar production in olive groves is very low*.

Despite there is not statistical information, the majority of farmers usually burn the pruning residues.

5. General and specific objectives

The main objective of this Thesis is to assess the effects of the implementation of sustainable management practices, especially a natural plant cover, in olive orchards on SOC and protected SOC, and to quantify the potential for the SOC sequestration. This main objective is achieved throughout three objectives:

1. Assess the <u>potential of SOC sequestration after changing from conventional to environmental-friendly management practices in olive groves</u>, especially in the management consisting on the implementation of a spontaneous resident vegetation cover in the inter-row area of these orchards.

2. <u>Assess SOC dynamics and its fractions</u> under Mediterranean pedoclimatic conditions.

3. <u>Comply the requirements of the Kyoto Protocol and the Paris Agreement of 2015</u> by quantifying the SOC which can be potentially sequestered (i.e. “contributions” in the Paris Agreement) in Andalusian olive grove soils after implementing environmental-friendly management practices.

In order to implement these three general objectives this Thesis has been divided into seven specific objectives corresponding to seven in field and lab experiments (specific objectives 2, 3 4 and 5 are represented in the figure 26):

1. Assess the influence of the type of organic carbon input, dose of organic carbon and SOC saturation deficit on CO_2 emissions and SOC accumulation in laboratory conditions (Chapter V).

2. Quantify aerial biomass production of the spontaneous resident plant cover in olive groves and its influence on total SOC accumulation and SOC fractions dynamics (Chapters IV and VI).

3. Influence of mineralogy and management (no plant conver vs spontaneous plant cover) on total SOC accumulation and SOC fractions dynamics (Chapters IV and VI).

4. Assessment of the effects of the implementation of a spontaneous resident plant cover on CO_2 emissions in field conditions (Chapters IV and V).

5. Assessment of the influence of a plant cover on the organic carbon fractions which are lost *via* erosion (Chapter VII).

6. Assessment of the SOC response ratio, SOC sequestration and SOC sequestration efficiency in Mediterranean woody crops (Chapter III).

7. Predict the potential for SOC accumulation in olive orchards under specific environmental-friendly management practices (Chapter VIII).

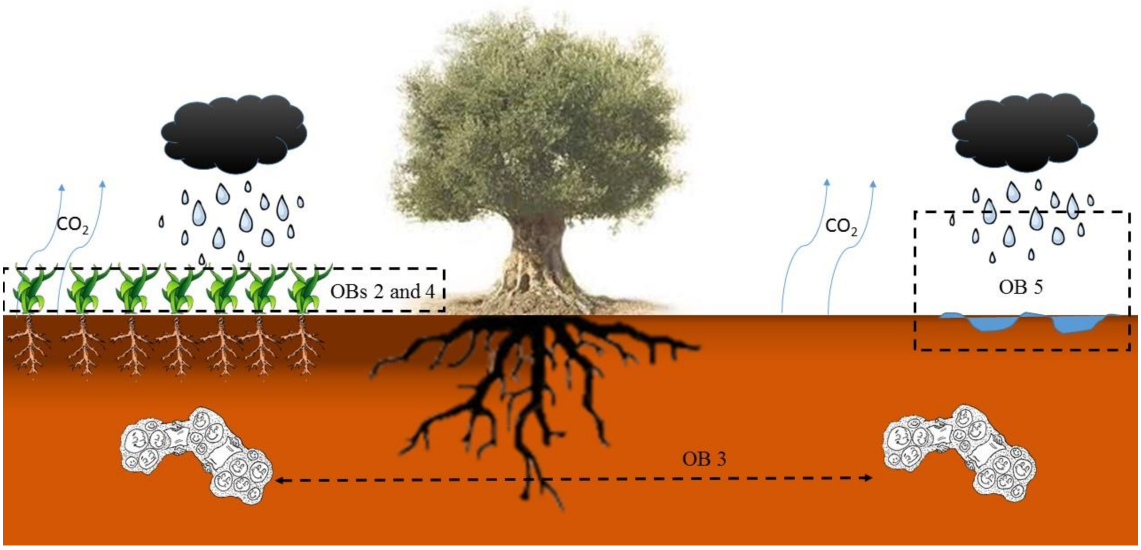
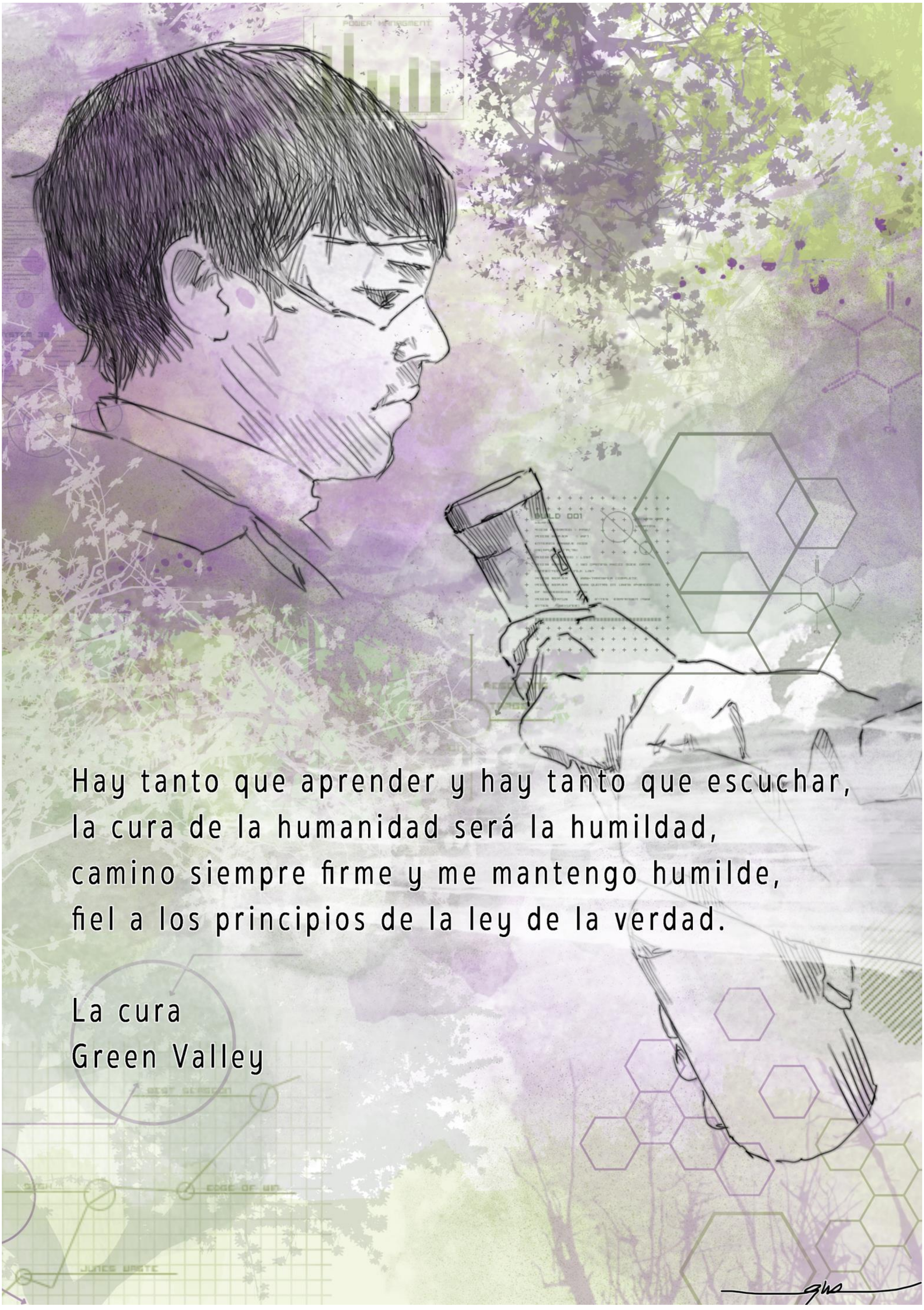


Figure 26. Scheme representing experiments carried out in order to comply the specific objectives number 2, 3, 4 and 5.


Hay tanto que aprender y hay tanto que escuchar,
la cura de la humanidad será la humildad,
camino siempre firme y me mantengo humilde,
fiel a los principios de la ley de la verdad.

La cura
Green Valley

CHAPTER II

General materials and methods

This section only shows those materials and methods which are used in different studies. Other specific material and methods are described in the corresponding chapter and sections.

1. Soil and biomass sampling and pre-processing

1.1 Soil sampling and pre-processing

Soil samples were usually taken by opening 25 x 25 cm pits. The depth depended on the specific study (typically top 5 cm and from 5 to 15 cm). When the soil was hard to be sampled by opening pits, a soil auger (8 cm diameter) was used. The number of soil samples taken in each studied depended on the heterogeneity of the plot, and ranged from 3 to 10, each on them typically composited of 3 to 4. After collection, soil samples were transported in plastic bag during the same day and air dried for between 5 to 10 days.

Once soil samples were dried, they were sieved by a 2 mm-sieve. All the soil analysis were done in the < 2 mm soil. For SOC analyses, soil samples were grounded with a ball mill.

1.2 Biomass sampling and pre-processing

Aboveground biomass of spontaneous natural plant cover was measure just before controlling it by mowing or ploughing in spring (usually in early April). Biomass samples were collected using a framework of 50 x 50 cm by cutting storey. Frameworks were randomly chosen always in the inter-row area of the olive orchards. Since the biomass production is not usually homogeneous distributed among the inter-row area, samples were taken depending on the biomass production heterogeneity. Belowground biomass was not sampled.

After sampling, the collected biomass was transported to the lab into plastic bags in the same day and dried at 60 ºC in the oven for two days. When dry, each sample was weighted to calculate the biomass production (Eq. 1).

$$t\ biomass/_{ha} = {g\ biomass}/_{0.25m^2} \times \frac{1\ t}{10^6 g} \times \frac{10^4 m^2}{1\ ha} \quad (1)$$

After weighting, the biomass was milled (figure 1) for further analysis (e.g. carbon and N analysis). Total organic C content in the aboveground biomass per area unit was calculated by applying the following equation:

$$ {}^{t\,C}/_{ha} = {}^{t\,biomass}/_{ha} \times \frac{Organic\ C\ (\%)}{100} \qquad (2) $$

Figure 1. Images of the aerial biomass mill process (left) and final milled biomass (right)

2. Soil properties analysis

2.1 SOC fractionation

Separation of the various C pools was accomplished by a combination of physical and chemical fractionation techniques in a simple, three-step process developed by Six *et al.* (2002) and modified from Stewart *et al.* (2009). First of all, the soil is sieved by 2 mm. After that, approximately 50 ml of soil are added to 50 g of soil and mixed in order to carry out the wet sieve which consists of two sieving (250 µm and 53 – 250 µm) obtaining three size fractions (> 250 µm, 53 – 250 µm and < 53 µm) (figure 2). Finally, the three fractions are dried at 60ºC and weighted. The > 250 µm, 53 – 250 µm and < 53 µm fractions isolated after the first step corresponded to the coarse non-protected particulate organic matter (cPOM, hereafter), microaggregate (µagg) and easily dispersed silt and clay (dSilt+dClay) fractions, respectively.

Figure 2. Images of the physical fractionation at the beginning (left) and the end (right) of the process.

The next step involves further fractionation of the 53 – 250 µm fraction previously isolated. In this second step a density flotation with 1.85 g cm^{-3} sodium chloride was used to isolate fine non-protected POM (LF). For that purpose, 5 g of the intermediate fraction were put into a Falcon tube and 35 ml of a saturated solution of NaCl were added to the soil. Then, manually the mixture must be shaken 10 times and wait 20 minutes so that the LF moves to the upper layer of the mixture. Then, centrifuge for 60 minutes at 2500 rpm and aspire the supernatant with a pipette, which must be passed by a sieve of 20 µm and washed with deionized water (DW). Finally, the LF must be dried and weighted.

Before the dispersion of the heavy fraction it is necessary to wash the soil in order to remove the NaCl. The soil must be washed with 40 ml of DW and centrifuged for 5 minutes at 2500 rpm. This process must be done two times. After this process, the fraction is ready for the dispersion. It consist of adding 20 ml of Sodium Polyphosphate at 0.5% (5 g/L) with 12 glass beads and shaking for 18 hours. Then, it is filtered by a 53 µm sieve, separating the microaggregate-protected POM (> 53 µm in size, iPOM) from the microaggregate-derived silt- plus clay-size fractions (µSilt+µClay). The two fractions must be washed with DW, dried at 60ºC and weighted.

The following step involves the acid hydrolysis of each of the isolated sil+clay-sized fractions. The silt+clay-size fraction from both the density flotation of the 53 – 250 µm fraction (µSilt+µclay) and the initial dispersion and physical fractionation of the < 53 µm fraction (dSilt+dClay) were subjected to acid hydrolysis as described by Plante *et al.* (2006). Acid hydrolysis consisted of incubating the samples at 95 ºC for 16 h in 25 ml of 6 M HCl. After hydrolysis, the suspension was filtered and washed with deionized water over a glass-fiber filter. Residues were dried at 60 ºC and weighed. These fractions represent the non-hydrolyzable C fractions (H-dSilt+dClay and H-µSilt+µClay). The hydrolysable C fractions (H-dSilt+dClay, H-µSilt+µClay) were determined by difference between the total organic C content of the fractions and the C contents of the non-hydrolyzable fractions.

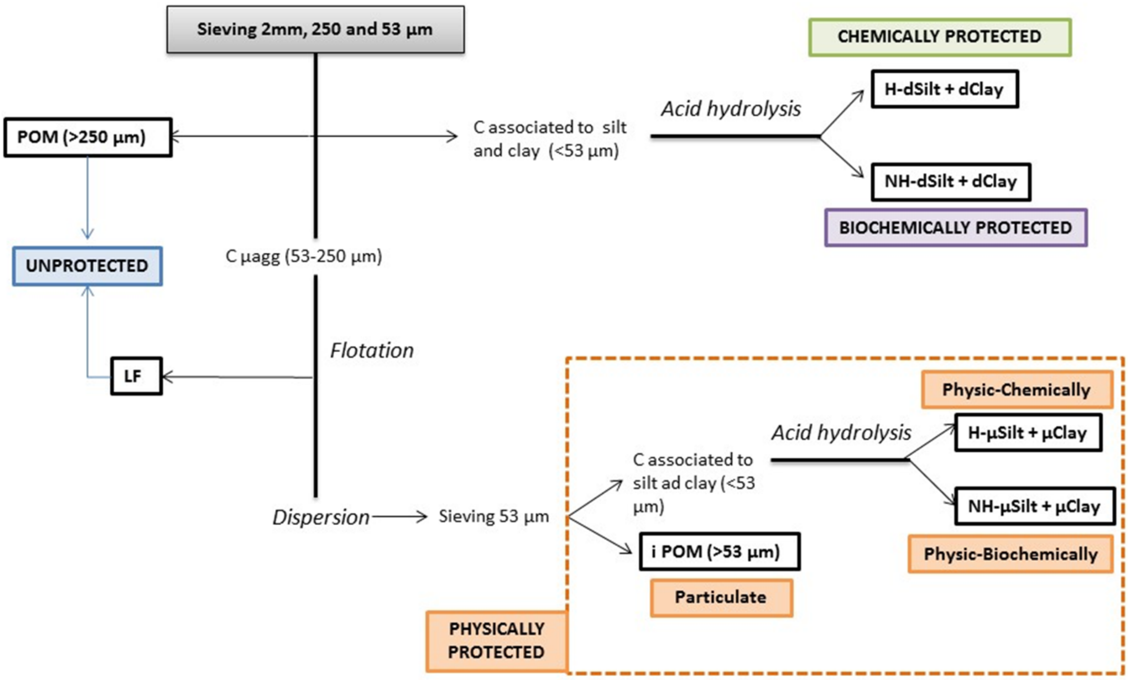


Figure 3. Scheme of the fractionation (Stewart *et al.*, 2009) and relation between pools and stabilization mechanisms (Six *et al.*, 2002). Adaptation.

This three-step process isolates a total of 12 fractions and is based on the assumed link between the isolated fractions and the protection mechanisms involved in the stabilization of organic C within that pool and as described in detail by Six *et al.* (2002) (figure 3).

2.2. Organic carbon analysis

SOC of the whole soil samples, previous grounded with a ball mill, and of each of the soil fractions isolated were determined by digesting the soil samples with a mix of dichromate and sulphuric acid following the method proposed by Anderson and Ingram (1993). For soil samples, between 0.1 (samples with high SOC content) and 0.3 g (samples with low SOC content) of soil were added to a digestion tube, and 1 ml of distilled water (DW) was added. In the case of the analysis for humic substances were used the following quantities: 3 mL of the total extractable carbon (TEC), humic acids (HA) and fulvic acids (FA) for soils with low SOC content; 2 mL of HA and TEC, and 3 mL of FA for soils with intermediate SOC content; 1 mL of TEC and HA, and 3 mL of FA for soils with high SOC content. For the total organic carbon in the plant cover residues, typically 30 mg of plant material were weighted and added into the digestion tubes. Then, 5 mL of potassium dichromate ($Na_2Cr_2O_7$) 1N were added to each tube, followed of 7.5 mL of sulphuric acid (figure 4). Blanks (e.g. tubes without soil or plant material samples) were always prepared following the same procedure. The calibration curve was prepared with dry glucose (typically between 0 to 10 mg of C-glucose). Digestion tubes were introduced into the digester at 155ºC for 30 minutes. After digestion, 10 mL of DW were added to each tube (figure 5), well shaken and transferred to a 50 ml Falcon tube. Then, Falcon tubes were centrifuged (2500 rpm for 5 minutes) to avoid turbidity. The final step consisted of measuring the absorbance (600 nm) the supernatant liquid resulting from the centrifugation (figure 6). Finally, the concentration of the organic C in the sample was calculated through the calibration curve.

Figure 4. Images of the potassium dichromate (left) and sulphuric acid (right) addition.

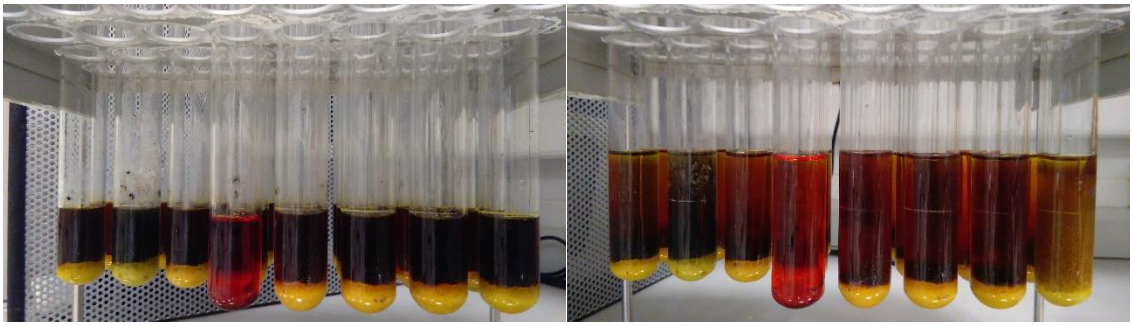


Figure 5. An example of the colour of the samples after digestion process (left) and after addition of 10 ml of deionized water (right).

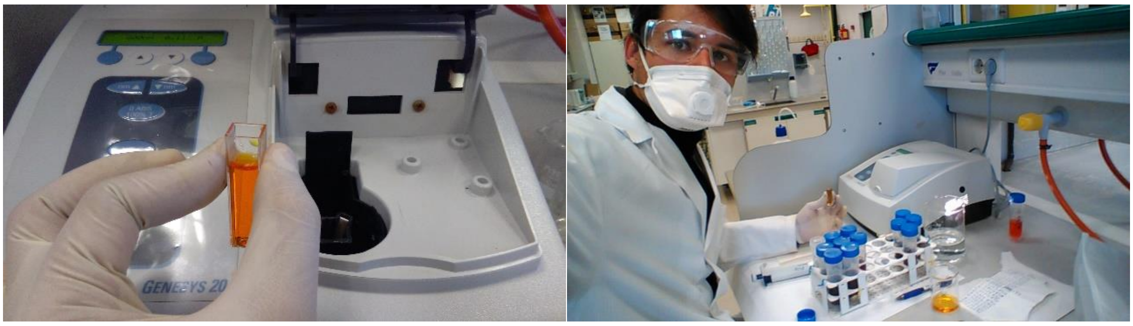


Figure 6. Measurement of the absorbance at 600 nm by spectroscopy UV-VIS

2.3. Physico-chemical soil properties

pH

Soil pH was determined in soil samples in extract with DW (1:10 w:v) using a pHmeter Crison GLP21.

Electrical conductivity

Soil samples were determined for electrical conductivity (EC) in an extract (1:10, w:v) soil and DW by conductivimeter crison 524.

Water holding capacity

The water holding capacity (WHC) was determined in oven-dry soil sample. 20 g of sieved soil (< 2 mm) was placed in a filtrate system and 20 ml of water was added. To accelerate the drained was used a vacuum pump at 253 mmHg (equivalent to 1/3 atm). WHC at 100% was calculated according to:

$$WHC\ (100\%) = \frac{V_r}{W_s}$$

Where;

V_r = Volume of water retained

W_s = Weigh of soil sample

Bulk density

It was assumed bulk density as the ratio between the mass of oven dry soil and the volume of the undisturbed fresh samples. For mineral soil with a coarse fragment content of more than 5 % a core sampler was used for collecting the soil. In the laboratory, the sample was dried (105 ºC); 2 mm sieved and weighted the fine earth and coarse fraction. The bulk density of the fine earth of soils (BDfe) was determined according to the equation of Blake and Hartge (1986):

$$BD_{fe} = \frac{M_{fe}}{V_s - \frac{M_{ce}}{\rho_{cf}}}$$

where:

BD_{fe} = Bulk density of the fine earth

M_{fe} = Mass of the fine earth

V_s = Volume of total samples

M_{ce} = Mass of the coarse fragments

ρ_{cf} = Density of the coarse fragments

Particle size analysis

Particle-size analysis (PSA) is a measurement of the size distribution of individual particles in soil samples. Soil samples were treated with hydrogen peroxide to remove the organic matter. Subsequently, samples were dispersed with sodium hexametaphosphate and shaking during 8 hours. Total sand fraction was separated by wet sieving, followed by dry mechanical sifting sub-fractions. Clay and silt were separated by sedimentation following the pipette method of Robinson (Gee and Bauder, 1986). Particle size classification has been developed as described in Soil Conservation Service (1972).

Soil stable aggregates

The soil content of stable aggregates (SA) was estimated following Lax *et al.* (1994) method. Briefly, a 4 g aliquot of sieved (0.2 – 4 mm) soil was placed on a sieved with a pore size of 0.250 mm and wetted by spry. After 15 min the soil was subjected to an artificial rainfall of 150 ml with energy of about 270 J m-2. The remaining soil on the sieve was placed in a previously weighed capsule (C), dried at 105 ºC and weighted (W_1). Then, the soil was soaked in DW and after 2 hours, it is passed through the same 0.250 mm sieve with the assistance of a small stick to break the remaining aggregates. The remaining residue on the sieve was weighted again (W_2). The percentage of the stable aggregates with regard to the total aggregates was calculated as:

$$\%\,SA = \frac{(W_1 - W_2)}{(4 - W_2 + C)} \times 100$$

Cation exchange capacity

Cation exchange capacity (CEC) was analized according to Rhoades (1982). Soil was saturated with sodium acetate 1N and pH 8.2. Salt excess of soil was removed and the absorbed sodium was displacement with ammonium acetate 1N, finally in this solution was determined the sodium content by photometry.

Carbonates

Carbonate content was analized following the calcimeter method described by Nelson (1982). The carbonate content was determined by reaction with acid, in a closed system, to form CO_2. At constant temperature, the increase in pressure is linearly related to the quantity of carbonate present in the sample.

Organic matter

Organic matter content of soil was determined as loss of ignition (LOI). Samples were placed in the furnace at 550ºC during 5 hours (Nelson and Sommer, 1982). The percentage of organic matter loss of ignition was calculated as:

$$\%LOI = \frac{W_{104℃} - W_{550℃}}{W_{104℃}} \times 100$$

where:

$W_{104℃}$ = Weigh of dry soil at 104 ºC

$W_{550℃}$ = Weigh of dry soil at 550 ºC

*When the organic matter content of the soil was estimated from the soil organic carbon content, the following equation was used Mann (1956):

$$SOM = SOC/0.58$$

Total C and Total N

An aliquot of soil samples were grounded using a ball mill and analized for total C (TC) and N (TN) in a CHN auto-analyzer (Carlo Erba NA200, Milan Italy).

Total phosphorus and potassium content

Total phosphorous and potassium in soil and plant residues samples were analized in an ICP–MS Agilent Series 7500 after an acid digestion (Sommer and Nelson, 1972). Briefly, 200 mg of milled samples were mixed with 5 ml acid solution of perchloric acid (60%) and nitric (60%) in a proportion 3:5 (v/v), and digested on a BD-40 block digester in two phases: 90 minutes at 130 ºC and 75 minutes at 204 ºC.

Micronutrients

The content of Mg, Ca, Zn, K, Fe, Mn and Ni in soil samples were analized in ICP–MS Agilent Series 7500 after an acid digestion as in the case of the total phosphorous and potassium content.

3. Statistical analysis

Differences in the measured variables between and among treatments were typically evaluated by analysis of variance (ANOVA).

In all cases the theoretical assumptions of ANOVA (normality and homoscedasticity of the data) were checked and data were transformed (log (dependent variable + 1)) when necessary. When normality requirements were not satisfied after transformation, the nonparametric Kruskal-Wallis test was applied. Significant differences were assessed by the post-hoc Tukey HSD test (Zar, 1999).

Repeated measures ANOVA was carried out to search for differences among treatments along different samplings. This type of statistical method was applied when time was one of the factors.

Correlation among variables were analized after Pearson correlation coefficient (Sokal and Rolf, 1995).

In some cases, to reduce the dimensionality of all variables a principal component analysis (PCA) was performed. As is well known, the purpose of this analysis is to express the covariance of many variables into fewer composite variables by finding the structure of strong linear correlation between them. The basic results of this analysis the variance explained by each axis collection (eigenvalues) and the linear equations that combine the original variables (eigenvectors) so that each object is projected in the new space management using the eigenvectors and the matrix data. Thus, the coordinates of a given sample in the new ACP axes are a linear combination of its coordinates in the space defined by the set of variables. This combination could be represented by the following expression:

$$Y = u_1 X_1 + u_2 X_2 + u_3 X_3 + \ldots + u_n X_n$$

Where Y would be the coordinate of a given sample in one of the linchpins of the new space defined after the ACP, u1, u2, u3…. One would be the eigenvectors which determine the combination of each variable on the y-axis, X1, X2, X3,…Xn would be the values of each of the variables included in the analysis for the sample in question. The covariation between variables more interesting and emerge stronger in the first axes.

The statistical analysis was carried out using the program STATISTICA (StatSoft, 2001) and IBM SPSS Statistics 20. In the case of the meta-analysis MetaWin software

(Rosenberg *et al.*, 2000) was used. The statistical methodology more specifically used in this work will be quoted prior to the description of each chapter's material and methods.

4. Figures

For the construction of the different graphics and figures different software were used. IBM SPSS Statistics 20 and SigmaPlot 10.0 was mainly used. The Microsoft Office Excel 2013 was used for generating some figures with a less grade of statistical complexity than in the previous case.

Levántate, olivo cano,
dijeron al pie del viento.
Y el olivo alzó una mano
poderosa de cimiento.

Andaluces de Jaén
Miguel Hernández

CHAPTER III

SOC sequestration in woody crops: A meta-analysis

1. Introduction

Forests and grasslands contain high stocks of C and are considered as net sink of C, while croplands often act as net sources of CO_2 due to soil disturbance which enhance soil organic carbon decomposition and to field management involving direct (e.g., diesel fuel for machinery) or indirect (e.g., chemicals) emissions of fossil fuels (Ceschia *et al.*, 2010).

Finding low-cost methods to sequester C in agricultural systems is emerging as a major international policy goal in the context of increasing concerns about global climate change. Among the methods that may reduce agricultural CO_2-derived greenhouse gas emissions, there is the adoption of recommended management practices (RMPs), which involves an accumulation of organic C in the soil without compromising crop production. In agricultural systems, the gain or loss of C over time due to cultivation (e.g. net ecosystem C balance) depends on the amount of C entering (for example through organic amendments or cover crops residues) and on that leaving the system (e.g. harvest of products, soil and plant respiration). In terms of SOC balance, RMPs reduce the oxidation of SOC and increase organic C inputs (Six *et al.*, 2004). A reduction of the SOC oxidation can be achieved by changing the tillage type from conventional tillage (CT) to reduced tillage (RT) or no-tillage (NT). The increase in organic C input on farm can be achieved by the use of a cover crop (CC) in the rotations, or allowing the growth of wild vegetation in the inter-row of perennial orchard-type crops. Off-farm organic inputs can also be used for this purpose, such as manure, compost, or agro-industrial and urban wastes.

Fruit orchards, such as olive groves and almond, and vineyards, are usually cultivated where soil fertility is relatively low and water availability limited, and therefore they are relatively well adapted to Mediterranean climates. These perennial crops represent about 16% of the agricultural land in the Mediterranean area (FAO data, 1998) and are of a great economic importance (Olesen and Bindi, 2002).

In comparison with annual crops (Smaje, 2015), fruit orchards have some structural features allowing them to potentially sequester significant quantities of atmospheric C. Their long life cycle allows them to accumulate C in permanent organs such as trunk, branches, and roots and in the soil (e.g. rhizodeposition). In addition, the massive and deep-rooted systems in these perennial woody crops allow direct transfer of SOC into the subsoil, making it less prone to mineralization. However, some conventional management of these cropping systems might lead to significant losses of SOC. Usually, conventional management involves bare soil in the inter-canopy area of the orchards, through regular tillage and/or pre- and post-emergence herbicides, leading to SOC losses not only because of the higher mineralization rates but also because of higher erosion rates. For example, Gomez *et al.* (2004) measured annual rates of soil losses in a conventional olive grove (4.0 t ha^{-1} yr^{-1}) which is 3.3 times higher than in a comparable plot where the soil was covered with spontaneous resident vegetation (1.2 t ha^{-1} yr^{-1}). Since the Mediterranean climate is characterized by relatively frequent, extreme, short-lasting rainfall events, erosion represents a problem, especially in high slope areas that can be solved – or minimized – by implementing RMPs. The relevance of SOC changes to the net greenhouse gases balance of cropping systems can be very large, particularly in the specific case of Mediterranean woody crops. In a life-cycle assessment study under Mediterranean conditions in Spain, Aguilera *et al.* (2015) found that soil C sequestration in organic olive orchards was equivalent to all other emissions combined, resulting in C-neutral crop production.

Soil C accumulation in these fruit orchards can be achieved relatively easily, both economically and technically, through the adoption of RPMs which include: i) reduced or zero soil tillage, which preserves soil organic matter from mineralization; ii) the frequent presence of herbaceous vegetation in the alleys, which can contribute to the build-up of soil organic matter, and iii) the inputs of external (e.g. manure) and internal (e.g. pruning debris) sources of organic matter. In addition, some fruit orchard crops have relatively low yields with a tendency to partition less C to the fruits than high-yielding ones and, therefore, some of the C fixed by photosynthesis enters the detritus cycle. In addition, improving soil resilience through increased SOC may positively impact the whole fruit tree industry. Increased knowledge of atmosphere-soil C fluxes mechanisms may facilitate interventions capable of enhancing C capture (Marland *et al.*, 2004).

In spite of the strategic role of orchards and vineyards in Mediterranean regions (Olesen and Bindi, 2002), the role of RPMs on C fixation potential has only partially been explored. In recent years, the C budget of fruit tree plantations has received increasing attention with studies conducted in olive (Nardino *et al.*, 2013; Sofo *et al.*, 2005), palm (Navarro *et al.*, 2008), apple (Zanotelli *et al.*, 2015), peach (Sofo *et al.*, 2005), and pear (Zhang *et al.*, 2013). However, unlike other systems such as croplands (Ceschia *et al.*, 2010; Smith, 2004b), grasslands (Derner and Schuman, 2007; O'Mara, 2012) and forests

(Barr *et al.*, 2002; Vogt, 1991), there are no published studies comparing the ability of perennial fruit tree plantations to fix atmospheric C into the soil under RPMs.

Some recent meta-analyses have provided insight on the role of specific or grouped management practices on SOC. For instance, Poeplau and Don (2015) assessed the influence of cover crops on SOC stocks, Tuomisto *et al.* (2012) analized the impacts of the organic farming in Europe on SOC content, nutrient losses, energy requirements or land use, Tian *et al.* (2015) assessed the influence on SOC changes of the addition of different fertilizers and crop residues in paddy soils in China, and Zhao *et al.* (2015) identified the management practices that lead to an increase in the SOC content in China. However, these studies do not distinguish between herbaceous and woody crops, and usually only herbaceous crops are considered.

2. Objectives

The aim of this study is **to assess the influence of specific RMPs on SOC content of three common Mediterranean woody crops** (olive orchards, vineyards and almond orchards), throughout a **meta-analysis**. For that purpose it has been compared SOC in: i) conventionally managed (used as control) without cover crops in the inter-row; ii) Farms with cover crops or spontaneous resident vegetation cover in the inter-row; iii) Farms applying organic amendments, and iv) combining management practices.

3. Methodology of the meta-analysis

3.1 Literature review and data selection

A literature search was conducted for articles reporting comparisons between RMPs and conventional management in three typical Mediterranean woody crops: olive and almond orchards and vineyards. All the studies were carried out in areas under a Mediterranean climate type. Laboratory studies were excluded and only studies under field conditions were selected. We did not distinguish between irrigation and no irrigation, since irrigation in Mediterranean woody crops is usually done under the tree canopy, near the trunk and soil samples are usually taken in the inter-row area. When more than one study included data from the same experiment, the longest study was selected. If the duration of the study was the same in both cases, the study with most information was included in the analysis.

The studies included in the analysis were those cited in Scopus until January of 2016. Two fields where used for the search in the title, abstract or keywords of the article. The first field was the crop type, using the following words: “olive” or “vineyard” or “almond” or “Olea europea” or “Vitis vinifera” or “Prunus dulcis”. For the second field, the search terms “soil organic carbon” and “soil organic matter” were used. Thereafter, we only included in the meta-analysis those studies conducted under Mediterranean climate. We obtained 213 results, resulting in 60 potential articles. This search was completed with other studies cited in Aguilera *et al.* (2013).

3.2 Definition of categories

The types of C input were those summarised in Table 1, namely: (i) None: no external organic C input was applied. The growth of a cover consisting of natural plant cover was prevented by frequent tillage and/or by pre-emergence herbicides. (ii) CC: a cover crop (seeded plant cover) or a cover of spontaneous resident vegetation (unseeded cover) in the inter-row area. Sheep or goat excretion resulting from grazing of these plant covers was included in this category since it is not an external organic input and the input of organic C by this route is typically very low. (iii) CR: crop residues, such pruning residues or olive leaves which were left on the top soil. (iv) OA: an external organic amendment was applied frequently. This external amendment typically consisted of farmyard manure, composted or un-composted olive mill pomace or sewage sludge. A wide range of doses and biochemical properties of these external organic amendments was found.

For the tillage types we used the following categories (Table 1). (i) T: frequent tillage. Tillage consisted of 3 or more annual passes. Very often this is combined with the use of herbicides. This tillage method was common in the conventional (CONV) management. (ii) RT: reduced tillage. This tillage method was common in the studies where wild resident vegetation covered the inter-row area of the farm. Reduced tillage was usually done during the spring to control the vegetation. (iii) NTH: no tillage, and unwanted plants were controlled using pre-emergence herbicides. As a consequence, the soil is permanently bare. As in the case of the T, NTH is common in the CONV management.

(iv) NTM: no tillage and wild resident plants are eliminated by mowing, or using post-emergence herbicides in the spring. (v) NTG: no tillage where unwanted plants were controlled by animal grazing.

The comparisons were classified by management according to Table 1. The management is a result of the tillage and the type of organic C input. Conventional management (CONV) was used as a control group in the majority of the comparisons. CONV management typically includes the use of mineral fertilizers under the T tillage category. The rest of the management practices belong to the RMPs group. Some of these were the same as those proposed by Aguilera *et al.* (2013). (i) CC: a cover crop was implemented in the inter-row area or the orchard or vineyard. In most cases the soils were covered by a community of natural resident vegetation which was allowed to grow, typically between early autumn to middle spring. This plant community was controlled by mowing, grazing, or by applying post-emergence herbicides during the spring. Aboveground plant residues were left on the soil surface or incorporated by tillage. (ii) OA: organic amendments (manure, compost, agroindustry by-products or other residual organic inputs) were applied. Crop residues, such as pruning debris were included in this category. The growth of unwanted plants was prevented by tillage or application of pre-emergence herbicides. (iii) CMP: combined management practices. This is the most environmentally-friendly management category. It includes the existence of plant cover (cover crop or resident vegetation cover) or an inert cover (crop residues), combined with an organic amendment. In some cases, plants were controlled by grazing.

The influence of four variables (Table 2) on the calculated effect sizes was assessed: management, woody crop species, time and climate. Management is a variable which includes: OA, CC, CC + OA (CMP) and none. In this analysis, three typical Mediterranean woody crop species were distinguished: olive orchards (*Olea europaea*), vineyards (*Vitis vinifera*), and almond orchards (*Prunus dulcis*). According to the duration of the study, the studies were classified into 3 categories: (i) Short-term: less than 6 years, (ii) Medium-term: 6 – 10 years, (iii) Long-term: more than 10 years. Finally, 6 different sub-climates of the Mediterranean climate according to Köppen-Geiger classification were also distinguished (Kottek *et al.*, 2006): Csa, Csb, Cfa, Cfb, BWh and BSk (see Table 2 for the description of the different Mediterranean sub-climates).

Unfortunately, the influence of the different Mediterranean sub-climates and the duration of the study were only assessed for the CC management, since for OA and CMP managements, the high variability of the C inputs and the low number of studies with a duration longer than 5 years made the analysis impossible.

Table 1. Management, C input and tillage types considered in the meta-analysis. The management type is the result of combining an organic carbon input and a tillage practice.

Management Type	**Description**	**Observations**	**C Input Type**	**Description**	**Tillage Type**	**Description**
CONV	Conventional management	Used as a control. Management included T or NTH with none C input.	None	No organic C input	T	- Tillage with herbicides - Frequent tillage without herbicides
CC	Cover Crops	Cover crop or natural plant cover, which were eliminated by a combination of NTM and NTG or with a RT	CC	- Cover crop - Natural cover of resident vegetation	RT	Reduced tillage. Usually once in spring and once in autumn
OA	Organic amendment	Annual organic amendment consisting in compost, manure, olive mill waste, sewage sludge or CR. Soil were T or NTH.	CR	Crop residues (e.g. pruning debris)	NTH	No tillage with pre-emergence herbicides
CMP	Combined management practices	CC + OA/CR + RT/NTM/NTG	OA	- Manure - Olive mill waste - Sewage sludge - Other	NTM	- No tillage mowing - No tillage with post-emergence herbicides in spring
					NTG	No tillage with grazing. Implies small amounts of manure

Table 2. Variables with their respective categories studied in the meta-analysis.

Variable	Category
Management	OA (organic amendment: compost, manure, crop residues, sewage sludge, other) CC (cover crops, resident vegetation cover) CC + OA = CMP (combined management practices) None
Specie	Olive orchards Vineyards Almond orchards
Duration	< 6 years 6 – 10 years > 10 years
Depth	< 16 cm 16 – 30 cm > 30 cm
Mediterranean Sub-climates according to Köppen-Geiger classification (main climate, precipitation, temperature)	Csa (Warm temperate, summer dry, hot summer) Csb (Warm temperate, summer dry, warm summer) Cfb (Warm temperate, fully humid, warm summer) Cfa (Warm temperate, fully humid, hot summer) BWh (Arid, desert, hot arid) BSk (Arid, steppe, cold arid)

Table 3. Effect sizes, description and equations used for the calculation of each one.

Effect size	Description	Equation
SOC response ratio	Shows the variation in the SOC content in the RMP relative to the CONV management. If > 1 the RMP increases the SOC, if < 1 it decreases it.	1,2
C sequestration rate	Shows the variation per unit of time (year) in the SOC stock in the whole profile in the RMP relative to the CONV management. If > 0 the RMP increases the SOC stock, if < 0 it decreases it. If > 0 the RMP accumulates SOC, if <0 it decreases SOC	3,4,5
C sequestration efficiency	Shows the percentage of the incoming organic C that is fixed into the soil after the implementation of the RMP.	6

RMP = Recommended management practice; CONV = Conventional management; SOC = Soil organic carbon; C = Carbon

3.3 Data management

An effect is a statistical measure that portrays the degree to which a given event is present in a sample (Cohen, 1969). An effect size is a standard measure which can be calculated from any number of statistical outputs. We assessed 3 effect sizes (Table 3): (i) SOC response ratio, (ii) C sequestration rate, and (iii) efficiency of C sequestration. Data measured in most studies were SOC concentrations (g C Kg^{-1} soil, or mg C g^{-1} soil). When data of the studies were presented only in a figure and not in numeric format, data were extracted from figures using WebPlotDigitizer software (http://arohatgi.info/WebPlotDigitizer) after figure digitalization. When soil organic matter concentration was determined instead of SOC, SOC was calculated using the Mann (1986) relationship (SOC = 0.58 x SOM).

SOC response ratio was calculated applying the Eq. 1:

$$\text{SOC response ratio (RR)} = \frac{SOC_{RMP}}{\text{SOC}_{\text{Control}}} \quad (1)$$

where SOC_{RMP} and $\text{SOC}_{\text{Control}}$ are the SOC concentrations (g C Kg^{-1} soil) measured in the RMP management and in the control (CONV management) farms, respectively. Nevertheless, in order to normalize the sampling distribution, the natural logarithm of the RR was used (Hedges *et al.*, 1999). Thus, the final equation was (Eq.2):

$$\ln(RR) = \ln SOC_{RMP} - \ln SOC_{Control} \quad (2)$$

We assumed a significant response ratio under a specific management when values were significantly different from 1 ($\text{e.g.}\, \text{SOC}_{\text{RMP}} > \text{SOC}_{\text{Control}}$).

To calculate soil C sequestration rate (t C ha^{-1} yr^{-1}) the change in the SOC stock (t C ha^{-1}) was calculated according to Eq. 3.

$$\text{Soil C sequestration rate} = \frac{C_t - C_{t'}}{t} \quad (3)$$

where C_t and $C_{t'}$ represent SOC stocks (t C ha^{-1}) at the end and the beginning of the experiment, respectively, while *t* stands for the duration of the experiment (years). We assumed significant positive C sequestration rate under a specific RMP management relative to CONV management when values were significantly different from zero.

When data of SOC at the beginning of the experiment were not available, values of SOC stocks in the CONV treatment were selected, assuming similar initial C levels in RPM and CONV plots, since the plots used for the comparisons in the different studies had similar pedoclimatic conditions. Some studies provided the data of SOC stocks. However, most studies did not show values of SOC stocks, so these were calculated following the equation (Eq. 4):

$$\text{SOC Stock (t C } ha^{-1}) = \sum_{i=1}^{k} \frac{d_i \rho_i SOC_i}{10} \qquad (4)$$

where d_i, ρ_i and SOC_i are soil depth (metres), bulk density (t m^{-3}) and SOC concentration (g C Kg^{-1} soil) for the different soil layers (from i to k soil layers), respectively. The SOC stock was the sum of the stocks for the k soil layers considered in each study. Since bulk density was not provided in many of the studies, values were estimated using the algorithm used by Aguilera *et al.* (2013), which was modified from Howard *et al.* (1995) but re-parametrized with data from Mediterranean soils (Eq. 5):

$$\rho\ (\text{t } m^{-3}) = 1.84 - 0.443 \log 10\ (\text{SOC (g C } kg^{-1} soil)) \qquad (5)$$

In the case of the studies providing enough information on the amount and characteristics of the organic inputs (both internal and external) – especially for OA and CR inputs – we also calculated the efficiency (E) of soil C sequestration following the equation (Eq. 6):

$$E = \frac{\text{C sequestration rate}}{\text{Annual organic C input}} \text{ x } 100 \qquad (6)$$

3.4 Statistical analysis

We used a meta-analysis technique to assess the influence of RMPs on SOC using data from CONV management as the reference. A meta-analysis is a quantitative research synthesis which analyses the results of a set of analyses (Glass, 1976). The meta-analysis used a methodology similar to that used previously by Aguilera *et al.* (2013). For the meta-analysis, only independent studies were considered. We considered as independent studies those differing in management, duration, pedoclimatic or geomorphology conditions. For the non-independent studies, an average was calculated in order to avoid redundancy of the data and, thus, to transform them into independent values.

A “random-effects model” was used to carry out the meta-analysis. This type of model allows data from a wide range of scenarios to be compared (Borenstein *et al.*, 2009), and assumes that the dispersion of data for a given category is not only due to a sampling error, but also due to other sources of variation which might have an effect on the mean effect size and the dispersion of the data (Borenstein *et al.*, 2009). The dispersion of the data was relatively high in some cases and, therefore, it was difficult to detect significant differences.

The database contains 144 comparisons from 51 references. Nevertheless, not all the references contained all of the necessary data to calculate the effect sizes. Thus, we found 135 comparisons of SOC concentrations, and in 123 the C sequestration rate was shown or was calculated. Finally, in 49 comparisons, the efficiency of C sequestration was calculated. In the case of the efficiency calculations, the majority of the data belonged to the studies which applied an organic amendment or crop residues. The studies which included cover crops did not usually show the amount of the inputs of organic C through plant residues.

Results of effect sizes were weighted in order to give more importance to larger studies (those with higher number of samples). Meta-analysis studies usually use the inverse of the variance of each study to weight the results. However, it was not possible in our case because this information was not provided for most of the studies. Thus, studies were weighted by sample size according to the methodology proposed by Adams (1997) (Eq. 7):

$$w'_i = \frac{N_i^{RMP} N_i^{CONV}}{N_i^{RMP} + N_i^{CONV}} \quad (7)$$

Where *w’* refers to the specific weight of the comparison, and the N^{RMP} and N^{CONV} represent sample sizes in the recommended (RMP) and control (CONV) treatments, respectively.

As a result of a bootstrapping procedure (999 iterations) using MetaWin software (Rosenberg *et al.*, 2000), 95% confidence intervals (CIs) were generated for each weighted mean effect size. Resampling techniques can be important for determining the significance of meta-analytic metrics since data often have small sample sizes and may violate some basic distributional assumptions. Bootstrapping chooses *n* studies from a simple size of *n* and then calculates the statistic, and this process is repeated many times to generate a distribution of possible values. The lowest and highest 2.5% values are chosen to represent the lower and upper 95% bootstrap confidence limits.

4. Results and discussion

4.1 General information

A total of 51 studies were selected resulting in 144 independent comparisons between the RMP and the CONV managements, i.e. about 3 comparisons per study. The number of studies performed in Spain was the highest (33 studies), followed by Italy (7), Greece (2), France (2), Portugal (2), South Africa (2), Syria (1), Turkey (1) and the United States (California) (1). According to the crop type, olive orchards were the most common woody crop (31 studies) studied, followed by vineyards (16) and almond orchards (5). One study included olive orchards and vineyards.

The number of studies devoted to olive groves and vineyards was somewhat related to their areas. Indeed, olive orchards in Spain and Italy cover 2.5 and 1.14 million hectares, respectively. However, this was not the case for almond orchards, at least in Spain and California, where there are about 700,000 ha and 331,000 ha planted, respectively. Therefore, the number of studies on almond orchards was underrepresented in comparison to those on olives and vineyards. This fact might be due to the lower economic importance of the almond products in comparison to olive oil and wine. Most of the studies were published during the last 10 years, peaking during 2012 and 2013 (figure 1).

The duration of the study in 64 out of 144 comparisons was lower than 6 years, whereas in 37 and 22 of them it was between 6 - 10 years and more than 10 years, respectively (some studies do not show data about the duration of the management). The relatively short time frame (typically lower than 4 years) of most of research programs at National and EU levels is likely the responsible for the relatively high proportion of studies which evaluate changes in SOC over the short term. This contrasts with the fact that changes in SOC typically occur at different rates after a change in management practices. Indeed, Poeplau and Don (2015) found that highest rates of SOC accumulation occur during the first few years, and usually decline afterwards until near zero changes when the steady state is reached. Thus the data on SOC accumulation provided in most of the articles of this study might be overestimated if interpolated over time. Clearly, long-term experiments would be highly valuable to fully understand soil C dynamics over long periods.

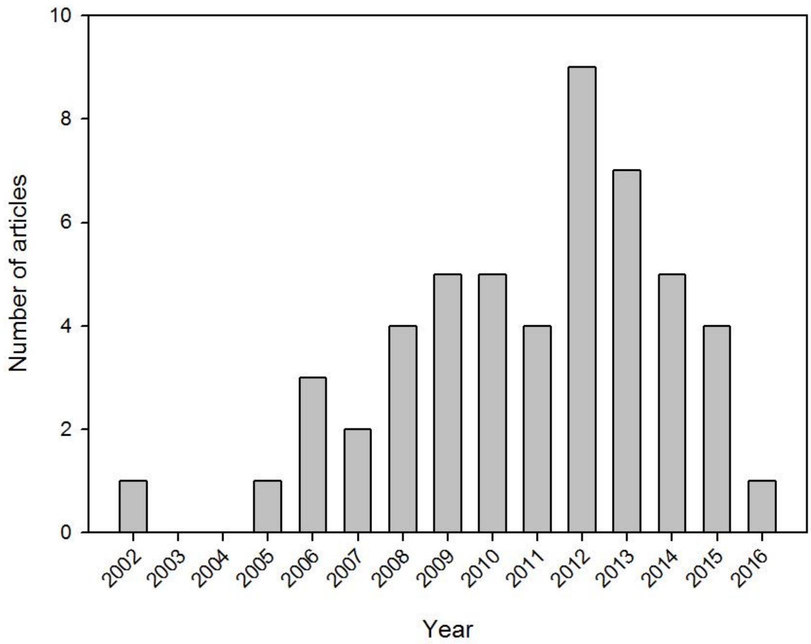


Figure 1. Evolution of the number of publications used in the meta-analysis including enough information about SOC sequestration.

Only studies under a Mediterranean-type climate were selected. However, mean annual rainfall and temperature vary according to the geomorphological properties and other geographical features of the experimental sites. The great majority of the comparisons (95) were undertaken under warm temperate conditions with relatively hot and dry summers (Csa type climate), followed by BWh (14), BSk (11), Cfb (9), Csb (12) and Cfa (3). This was especially true for olive orchards. Nevertheless, studies on vineyards were also done in Csb, Cfb and BWh climate types, whereas for almonds, the studies were also performed under a BSk type climate.

4.2 Influence of management on the effect sizes of soil C sequestration

Response ratios of the three tested managements (CMP, CC and OA) ranged 1.35 - 1.45 and averaged 1.40, and were significantly different from 1.0. There were not large differences in the response ratios of the three managements (figure 2a). The mean lowest value (1.35) was observed in farms under CC, whereas intermediate values were obtained for the CMP (1.40) management, and the highest (1.45) for the farms that received organic amendments.

The similarity in the SOC response ratios among RMP managements contrasts with the relatively large differences in C sequestration rates. This might be related to the differences between bulk densities and soil depths considered among the studies, since the same response ratio does not mean same C sequestration rate when different depths and bulk densities are considered. Thus, the C sequestration rate would be more appropriate than response ratio when assessing the influence of management practices on the changes of SOC in studies which include data from different depths.

Figure 2b shows that annual C sequestration rate averaged 4.07 t C ha^{-1} yr^{-1} under OA management. This figure was about 1.5 times higher than the rate found for CMP (2.62 t C ha^{-1} yr^{-1}) and four fold that under CC (1.03 t C ha^{-1} yr^{-1}), although these mean values were obtained with wide confidence intervals. For the whole set of studies and the three management types, minimum, mean and maximum annual C sequestration rates were -0.5, 3.8 and 6.6 t C ha^{-1} yr^{-1}. The fact that an accumulation of SOC was detected in the majority of the studies means that inputs of organic C and/or the slow-down of SOC losses under RMPs management compensate for SOC losses by organic matter decomposition and soil erosion. The average annual C sequestration rate for the whole set of studies was higher than that described for annual crops. For instance, Aguilera *et al.* (2013), in their meta-analysis involving Mediterranean crops, found a change of only about +8% in SOC content in the cereal rotations in the organic treatments compared to conventional management. The majority of cropping systems are dominated by annual plants that rely on cycles of tillage and planting of seed to ensure sufficient productivity. By comparison, fruit tree orchards, such as olives, almond and vineyards are capable of surviving many seasons requiring less soil disturbance. Perennial cropping systems have been recently proposed as systems that could protect soil C well, and since perennial plants often rely on more extensive root systems to ensure longevity, they likely produce more belowground biomass (Cox *et al.*, 2006).

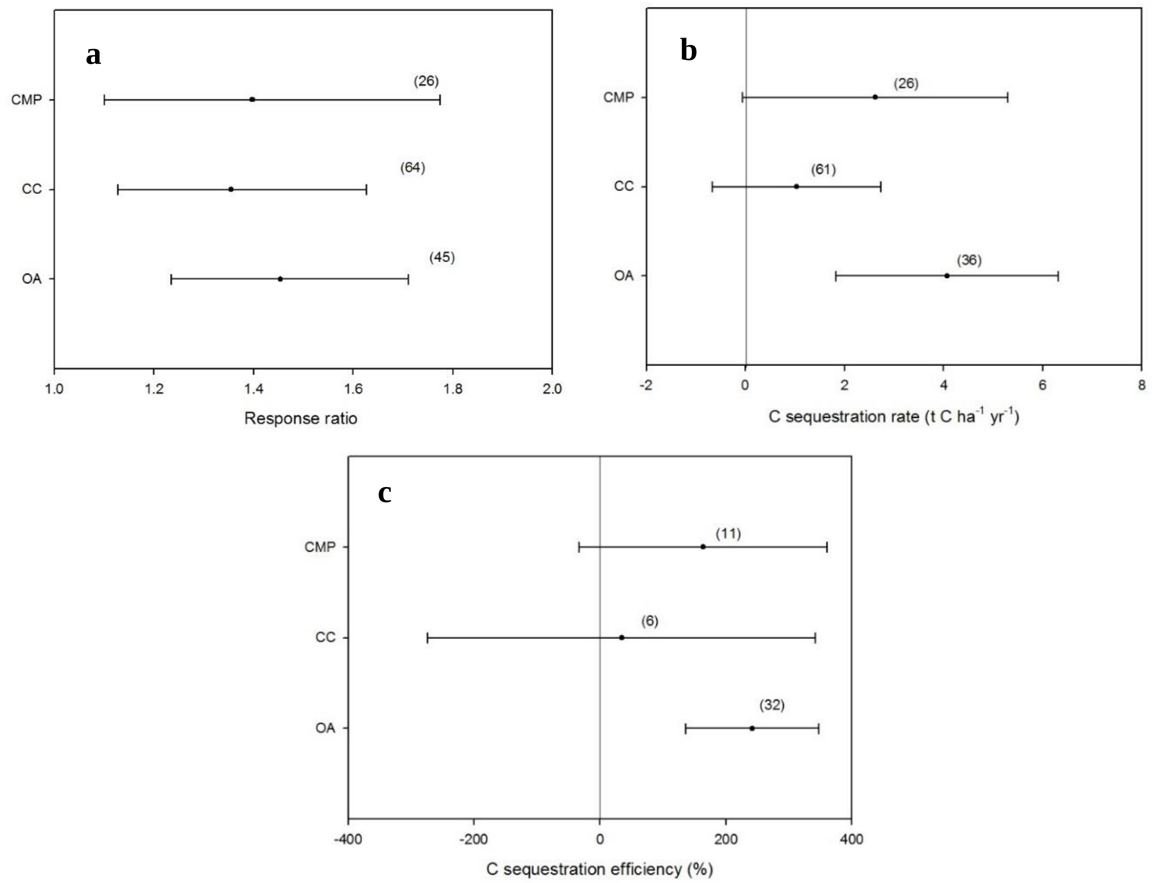


Figure 2. Influence of the three managements (organic amendments, OA; cover crops, CC; and combined management practices, CMP) on natural logarithm of the response ratio (a), C sequestration rate (b) and C sequestration efficiency (c). The zero line represents the limit between a positive and negative response of each size effect. Numbers in brackets represent the number of comparisons used in each category.

The highest SOC sequestration rate in the fruit tree orchards and vineyards was achieved for organic amendment management. For the whole set of studies under the OA management, the mean rate of organic C added was about 1.6 times higher than in CMP management, so the relatively high annual rate of C sequestration under the OA management compared to the other management practices is not surprising. The lower annual rate of organic carbon inputs in CMP compared to OA treated farms was likely due to the fact that farmer think that there is no need to add a high annual dose of organic matter when a cover crop is implemented in the inter-row area.

The fact that organic amendment additions represent direct inputs of organic C into the soil systems, and that these materials are often in forms that are much more recalcitrant than plant fresh residues should, in the absence of additional constraints, translate into moderate to high C sequestration rates. It is important to note that applications of manure are often assumed to increase C sequestration in soils at farm scale, but not at higher spatial scale (e.g. application of manure in one farm means an inefficient transport of organic C from other ecosystems to this farm), but manure is not likely to yield a net sink for C in soils (Smith, 2012), as would be required by the Kyoto protocol and also the Paris Agreement. Therefore, an ideal option would be apply organic C sources coming from the by-products of olive oil, wine and almond industries, thus avoiding CO_2 emissions from long-distance transportation, and from waste management.

The mean annual C sequestration rate reported here for CC was lower than the average of 1.59 t C ha^{-1} yr^{-1} found by the meta-analysis carried out by González-Sánchez *et al.* (2012) from 13 olive farms of Andalusia with CC. By using plant cover in the inter-row of tree orchards, an annual input of C is ensured, and this is true independently of the plant cover control technique. For instance, Castro *et al.* (2008) found a 3-year average annual aboveground biomass input of between 2.6 – 4.0 t ha^{-1} in an olive farm in Jaén (East Andalusia) with unseeded plant cover. The relatively high C sequestration rate under the CC treatment might be due not only to the annual C input of the plant residues, but also due to a decrease in C losses from soil erosion. In this line, Gomez *et al.* (2004) found a reduction in soil losses (and thus of organic matter and C) of about 70 % in an olive farm after the implementation of unseeded plant cover.

In addition, the diversity of unseeded plant cover might have an important impact on soil C accrual by improving the ability of soil microbial communities to rapidly process plant residues and protect them into aggregates. The presence of many different annual plants in unseeded plant cover also introduces a greater diversity of C compounds into the soil, some of which may be more resistant to decomposition (Tiemann *et al.*, 2015). While previous theories stated that microbial processing of residues in soils eventually produced similar C pools and compounds, a recent laboratory experiment found that the initial chemistry of the plant residues and the microbial community had a strong influence on which C compounds are present in the soil (Wickings *et al.*, 2012). The presence of a diversity of plants, then, might ensure that a diversity of C compounds is present in the soil, improving soil C sequestration potential. Thus, strategies which increase productivity of non-commercial biomass without compromising the quantity and quality of the economic products, such as the inter-row seeded or unseeded cover in fruit tree orchards, is desirable to increase the amount of biomass C returned to the soils, which can affect the size, turnover, and vertical distribution of SOC (Franzluebbers *et al.*, 1994). If suited to the climate and the technical and economic viability of the farming operation, then such cropping systems provide an opportunity to produce more biomass C than in a monoculture system, and to thus increase SOC sequestration. Lal *et al.* (1997) reviewed the literature on this topic and concluded that the potential for sequestering C by the application of cover crops residues was about 0.1 – 0.3 t C ha^{-1}, values much lower than those reported in our study for fruit tree orchards. However, the degree of intensification (more tillage events) of soils in these crops systems reviewed by these authors was much higher, likely with more SOC losses.

C sequestration efficiency is commonly expressed by the relationship between annual C input and SOC accumulation rate, which is an indicator of soil C sequestration ability (McLauchlan, 2006). Therefore, information about C sequestration efficiency is useful for seeking management strategies of enhancing the SOC stocks and soil fertility. On average, C sequestration efficiency was over 100% for OA (241%) and CMP (164%), whereas it was as low as 34% under CC management (figure 2c). Variability in soil C sequestration efficiency was ample, especially for CC and CMP managements, and no significant differences between groups were found. C sequestration efficiency is regulated by climate, the quantity and quality of added organic materials, soil organic C and inherent soil properties (Freibauer *et al.*, 2004). These factors might explain the great variability observed in this study, which compiles many studies with wide pedoclimatic variability, and diverse quantities and qualities of the organic C amendments. High soil C efficiency in fruit tree orchards systems was expected, as these are usually cultivated on soils with low organic matter, and a negative linear relationship between C sequestration efficiency and initial SOC content has been reported, mainly because SOC tends to increase faster if initial SOC content is far from its saturation level. C sequestration efficiency of most of the studies used was lower than 50 %. For instance, after 29 years, the C sequestration efficiency after application of pig and cattle manure and wheat straw ranged between 11 – 17 % in a Vertisol cultivated by a soybean-wheat rotation (Hua *et al.*, 2014). Triberti *et al.* (2008) found C sequestration efficiencies between 3.7 and 8.1% in a maize-wheat rotation after applying organic amendments. The unrealistically high C sequestration efficiency in the examined studies of our analysis could be due to four major reasons: (i) Uncertainties in the quantification of annual entry of some of the organic C inputs and lack of quantification for others. These uncertainties are quite common to many long-term field studies. For CC and CMP managements, only aboveground biomass of the unseeded or seeded plant cover were recorded or estimated; in some studies it was quantified on only one occasion. The C input *via* roots of the plant cover might represent a significant input of C which was not taken into account in the examined studies. Guzmán *et al.* (2014) found a root/shoot ratio of 0.8 (about 44% of the organic C in the biomass belongs to the belowground biomass) for cover crops, and also Kuzyakov and Dumanski (2000) and Ludwig *et al.* (2007) estimated an incoming organic C through the rhizodeposition process of 50% of the organic C content of the incoming biomass. (ii) Inaccuracies in the estimation of SOC stock (Aguilera *et al.*, 2013). SOC stocks calculations require the measurement of soil bulk density, and in some of the studies soil bulk density was estimated but not experimentally calculated. Moreover, changes in bulk density lead to changes in sampled soil mass when a fixed sampling depth is used, possibly biasing the results. (iii) Positive feedback between the incoming organic C and the improvement of soil fertility features, which might reduce SOC oxidation and increase SOC protection mechanisms of the native SOC. (iv) RMPs (such as organic amendments, plant or pruning debris cover) tend to decrease soil loss, and therefore SOC, by erosion. For instance, in experimental olive plots with a relative low slope (about 4%), Gómez *et al.* (2011) found soil losses about 2.6 t ha^{-1} yr^{-1} under conventional tillage, whereas for those plots under vegetation cover this value was one order of magnitude lower (0.17 t ha^{-1} yr^{-1}), in a relatively rainy year (845 mm).

Furthermore, when organic materials, such as manure, compost and by-products of the olive oil and wine industries, are added to the soil, at least a share of their organic C is decomposed producing CO_2, while another part is sequestered in the soil. Increase in the SOC pool in the 0–0.3 m depth after long-term use of manure when compared with chemical fertilizers was 10 percent over 100 years in Denmark (Christensen, 1996), 22 percent over 90 years in Germany (Korschens and Muller, 1996), 100 percent over 144 years at Rothamsted, United Kingdom (Jenkinson, 1990) and 44 percent over 21 years in Sweden (Witter *et al.*, 1993). Triberti *et al.* (2008) reported that 29 years after the start of a trial comparing different off-farm organic amendments, the cattle manure gave the quickest organic C stock build-up: 0.26 t organic C ha^{-1} yr^{-1}. In another study, about 25 and 36 % of applied manure and compost C remained in the soil after 4 years of application, indicating greater C sequestration efficiency with composted than non-composted manure (Eghball, 2002). Annual off-farm organic amendments Zhang *et al.*

(2010) encouraged significant SOC increase of about 7–45% after 25–28 years compared with the mineral fertilizer treatments, with a sequestration rate of about 0.70 to 0.88 t ha^{-1} yr^{-1}. Recently, Hua *et al.* (2014) found a linear relationship between off-farm organic C inputs (from 0.5 to 7.0 t ha^{-1} yr^{-1}) and SOC sequestration, although a linear relationship is not always observed (see for example Stewart *et al.*, 2009 and Chung *et al.*, 2009)

4.3 C sequestration rate in the 3 types of fruit tree orchards and under RMPs management

The effects of RMP management on C sequestration were only evaluated on olive orchards and vineyards, due to the lack of sufficient comparative data for almond orchards. The C sequestration rates in olive orchards were as follows: OA>CMP>CC (5.36, 3.33, and 1.10 t C ha^{-1} yr^{-1}, respectively) (figure 3a). The relatively large differences among management types, although with mean values with a wide dispersion which prevented statistical significance from being determined, were not found for vineyards, where C sequestration rates under the different management practices were relatively similar and not significantly different: CC>OA>CMP (0.78, 0.65 and 0.34 t C ha^{-1} yr^{-1}, respectively) (figure 3b).

In all cases, C sequestration rates were the highest for olive orchards, especially for OA and CMP managements. These differences were due to two main factors. Firstly, the mean annual rate of application of organic amendments to olive orchards was more than 25 times higher than that of vineyards. Secondly, the area covered by plant cover in olive orchards is much higher than in a vineyard, and thus aboveground and belowground biomass is expected to be much higher.

In the case of almond orchards, the C sequestration rate was 2.04 t C ha^{-1} yr^{-1} for CC management (n = 6) (figure not shown). For the rest of the management types it was not possible to assess the C sequestration rate due to the low number of available comparisons. This value is about 1.9 times that of the olive orchards and 2.6 times that of vineyards. Nevertheless, more studies should be carried out with cover crops in almond orchards to obtain consistent results.

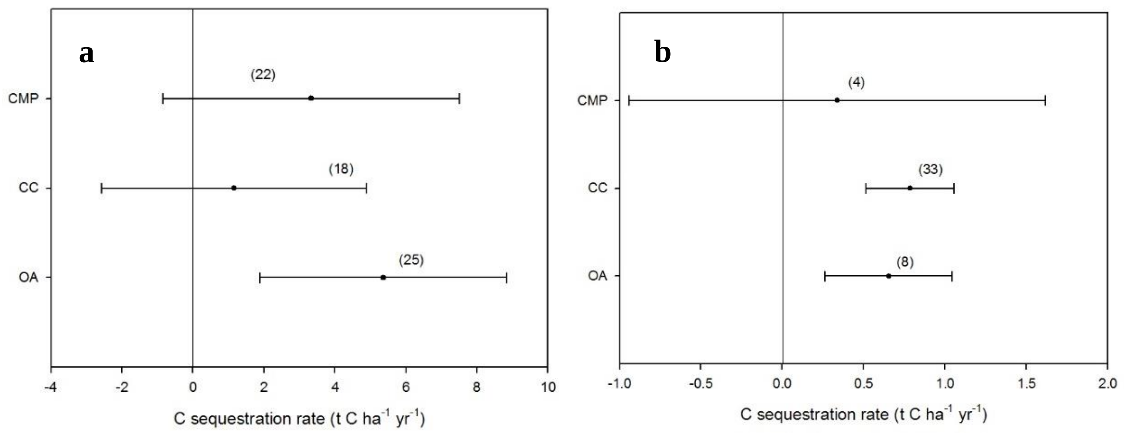


Figure 3. Influence of the three managements (organic amendments, OA; cover crops, CC; and combined management practices, CMP) on C sequestration rate in olive orchards (a) and vineyards (b). The zero line represents the limit between a positive and negative response of the C sequestration rate. Numbers in brackets represent the number of comparisons used in each category.

Smith (2004b) estimated with relatively high uncertainty the potential SOC sequestration for European croplands, mainly for herbaceous crops, according to different managements. For example, in the case of the organic farming the potentially SOC sequestration rate would be between 0 and 0.54 t C ha^{-1} yr^{-1}, for the use of animal manure was about 0.38 t C ha^{-1}, whereas with the use of cereal straw it was about 0.69 t C ha^{-1} yr^{-1}. Zero tillage potential SOC sequestration was about 0.38 t C ha^{-1} yr^{-1}, whereas for reduced tillage, this value was lower. Comparing these results for herbaceous crops with those obtained in our study, for olive orchards the C sequestration rate was about one order of magnitude higher after the use of organic amendments, whereas it was about 1.7 times for vineyards. In the case of SOC sequestration for CC management, the values estimated by Smith (2004b) were in most cases lower than those obtained in this study for olive orchards, vineyards and almond orchards. Triberti *et al.* (2008) found in soils under a maize-wheat rainfed rotation C sequestration, rates between 0.16 and 0.26 t C ha^{-1} yr^{-1} by using residues, slurry and manure. Again, these values in herbaceous cropping systems are lower than those we found in woody cropping systems. The relatively high annual dose of organic matter application in treated woody crops, the implementation of cover crops, where residues are left annually on the soils, and the lower soil perturbations of woody crops, especially in olive orchards, compared to herbaceous crops, might explain the higher C sequestration rate.

4.4 Influence of duration of the experiment on C sequestration rate for CC management

The average of soil C sequestration rates for studies with duration of less than 6 years, between 6 to 10 years and higher than 10 years were significantly higher than zero. On average, soil C sequestration for the studies with a duration of less than 6 years was 1.22 t C ha^{-1} yr^{-1}, a figure which was 1.7 times higher than that observed in studies carried out during 6 to 10 years (0.72 t C ha^{-1} yr^{-1}) (figure 4). Higher C sequestration rates in studies with a duration of less than 6 years were not unexpected, since changes in SOC are projected to be faster just after a change in a management practice, and decline thereafter until a new equilibrium is reached some time later (Smith, 2005b). For instance, West and Post (2002) found that the majority of SOC change in response to a change to no tillage occurred within the first 10 to 15 years following the implementation of this practice, and Rui and Zhang (2010) found that there was a negative correlation between soil C sequestration rate and duration of soil C sequestration. Finally, Poeplau and Don (2015) found an average C sequestration rate of 0.23 t C ha^{-1} yr^{-1} during the first 54 years after a change in the management, but an average of 0.11 t C ha^{-1} y^{-1}, thereafter reaching the new equilibrium (steady state) after 155 years following the adoption of the new management. Thus the soil C sequestration and soil C efficiency reported in this study should be treated with caution, as the experiment duration in about 44 % of the studies of this meta-analysis was lower than 6 years.

Soil C sequestration rates for studies longer than 10 years, tended to be higher, although differences were not significant, than that of studies between 6 to 10 years of duration. However, caution should be applied as the number of studies of a duration of more than 10 years is scarce (n = 7) and with wide confidence intervals due to the high dispersion of the data.

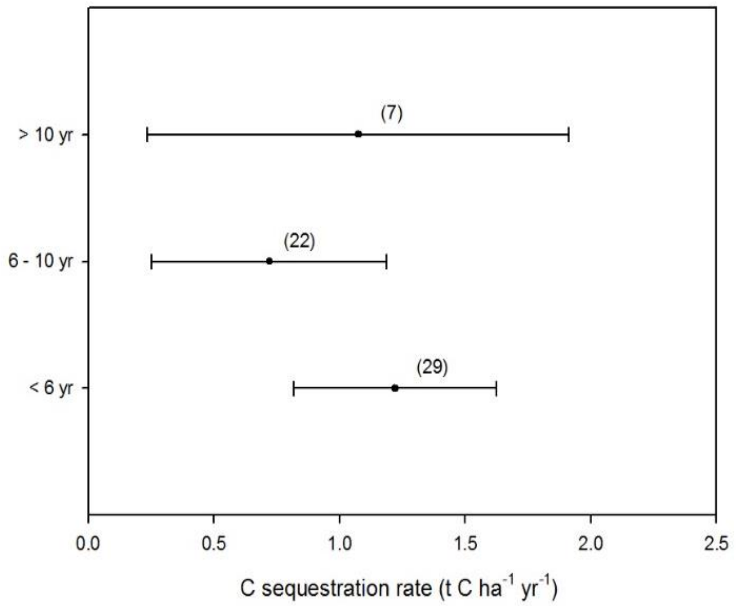


Figure 4. Influence of the time on the natural logarithm of the response ratio (a) and C sequestration rate (b) in the cover crop management (CC) in the three crops. The one line represents the limit between a positive and negative response of the response ratio. Numbers in brackets represent the number of comparisons used in each category.

4.5 Influence of Mediterranean sub-climates on C sequestration rate for CC management

Soil C sequestration rate under CC management varied according to the sub-climates of the studies. Values averaged 1.18, 1.22 and 1.27 t C ha^{-1} yr^{-1} for Cfb, Csb and Csa sub-climates, respectively. Averages of soil C sequestration rates of studies under B-type climates (semiarid to arid) were 0.39 t C ha^{-1} yr^{-1} and 0.53 t C ha^{-1} yr^{-1} for BWh and Bsk, respectively (figure 5), but these were not significantly higher than zero. In general, it is acknowledged that the C sequestration potential of semiarid to arid soils is relatively low, because of water and edaphic limitations such as fertility, and chemical (i.e. sodicity and acidity) and physical constraints (Post *et al.*, 1996). Soil C storage is controlled by a series of hierarchical processes, including C inputs and outputs. For example, the upper limit of C input to the soil is determined by net primary productivity of plants, which is in turn constrained by solar radiation, climate, and limitations in soil water and nutrients. Thus, the lower soil C sequestration measured in olive and almond orchards and vineyards on semiarid to arid climates was likely due to the fact that crop productivity in these dry locations is low, and thus so is the annual rate of organic amendments. In addition, C inputs throughout the above and belowground biomass of the plant cover under these climates is expected to be low, and thus so is the soil C sequestration rate.

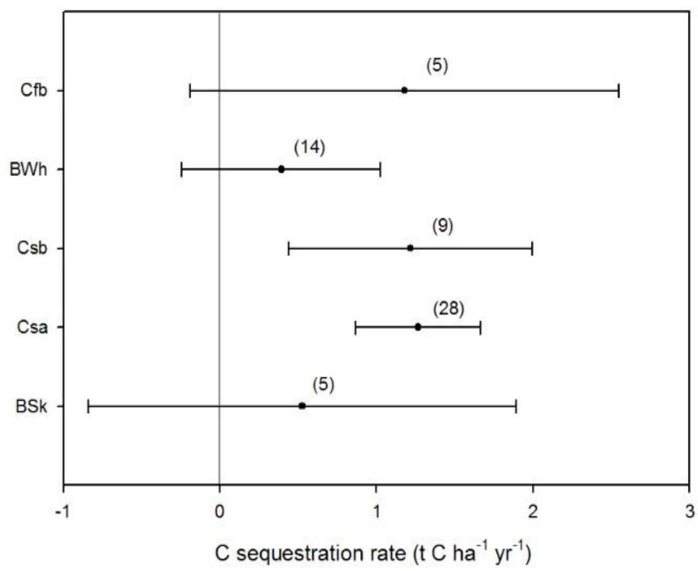


Figure 5. Influence of the climate on C sequestration rate in the cover crop management (CC) in the three crops. The zero line represents the limit between a positive and negative response of the C sequestration rate. Note that Cfa sub-climate is not included in the analysis due to not to have enough number of comparisons. Points represents average values, whereas extremes corresponds to confidence intervals at 95%. Numbers in brackets represent the number of comparisons used in each category. Csa (Warm temperate, summer dry, hot summer), Csb (Warm temperate, summer dry, warm summer), Cfb (Warm temperate, fully humid, warm summer), BWh (Arid, desert, hot arid), BSk (Arid, steppe, cold arid).

5. Conclusions

- Specific recommended management practices (**RPMs**) **increased C sequestration** in Mediterranean olive and almond orchards and vineyards compared to conventionally managed cropping systems.
- For the whole set of studies and the specific RMPs, **annual soil C sequestration rate was almost one order of magnitude higher than that found for annual cropping systems** and other organic-input-related C sequestration management options in agricultural soils. This was likely due to the low organic matter content of the soil (e.g. high SOC deficit) and the relatively low degree of intensification of these perennial cropping systems.

- **The highest soil C sequestration rate was found under organic amendment** management, and it was not unexpected taking into account the relatively high annual doses of organic material applied.

- **Plant cover management, used as green manure**, was also revealed as an important management option to increase SOC stocks in these cropping systems. Particularly, **in olive groves a C sequestration rate of about 1 t C ha^{-1} yr^{-1}** was found. This value was slightly higher than that found for **vineyards** (**0.8 t C ha^{-1} yr^{-1}**) and about half of that found for **almond orchards** (**2 t C ha^{-1} yr^{-1}**).

- **Efficiency of C sequestration was higher than 100% after OA and CMP managements**, indicating that some of the organic C inputs were unaccounted for, and a possible positive feedback effect of the application of these amendments on SOC retention, and on protective mechanisms of the SOC.

- **Soil C sequestration rates were greatest during the first years after the change in management and progressively decreased.**

- **Lower soil C sequestration rates were achieved in semiarid to arid Mediterranean conditions,** likely due to lower off-farm organic amendment application rates and/or productivity of the plant cover.

Appendix A. Additional table showing country, location, sub-climate, woody crop specie, management type, time (years), soil layers considered and maximum depth (cm) sampled for each reference. Note that tillage and organic input types are not included in the appendix since the management type is the consequence of the combination of a tillage and an organic input.

Management types. OA = organic amendments: compost, manure, crop residues, sewage sludge, other; CC = cover crops/seeded cover, natural plant cover/unseeded cover; CMP = CC + OA Combined management practices.

Mediterranean sub-climates: Csa (Warm temperate, summer dry, hot summer), Csb (Warm temperate, summer dry, warm summer), Cfb (Warm temperate, fully humid, warm summer), Cfa (Warm temperate, fully humid, hot summer), BWh (Arid, desert, hot arid), BSk (Arid, steppe, cold arid).

Authors	Country	Location	Climate	Woody crop	Management	Time (years)	Layers	Max depth (cm)
Alguacil *et al.* (2014)	Spain	Valencia	BSk	Olive orchard	OA	8	1	5
Almagro *et al.* (2013)	Spain	Murcia	BSk	Almond orchards	CC	4	1	15
Altieri *et al*. (2008)	Italy	Peruggia	Cfa	Olive orchard	CMP	5	1	20
Álvarez *et al.* (2007)	Spain	Córdoba	Csa	Olive orchard	CC	ND	2	20
Aranda *et al.* (2011)	Spain	Jaén	Csa	Olive orchard	CC	16	2,4	46
Beltrán *et al.* (2006)	Spain	Toledo, Madrid	Csa	Olive orchard	OA	4	2	30
Benítez *et al.* (2006)	Spain	Córdoba, Granada	Csa	Olive orchard	CC	ND	1	20

Calleja-Cervantes *et al.* (2015)	Spain	Navarra	Cfb	Vineyards	OA	13	1	30
Campos-Herrera *et al.* (2010)	Spain	La Rioja	Cfb	Vineyards	OA	ND	1	20
Carbonell-Bojollo *et al.* (2010)	Spain	Córdoba	Csa	Olive orchards	OA	3	3	60
Castro *et al.* (2008)	Spain	Jaén	Csa	Olive orchard	CC	28	5	30
Celette *et al.* (2009)	France	Montpellier	Csa	Vineyards	CC	4	2	60
Coll *et al.* (2011)	France	Languedoc-Roussillon	Csa	Vineyards	OA	17	1	15
Doni *et al.* (2010)	Italy	Pantanello farm	Csa	Almond orchards	OA	0.5	1	15
Fernádez-Romero *et al.* (2016)	Spain	Jaén	Csa	Olive orchards	OA, CMP	8, 10, 14	5	160
Ferri *et al.* (2002)	Italy	Apulia	Csa	Olive orchards	OA	3	1	20
Fourie *et al.* (2012)	South Africa	Breede River Valley	Csb	Vineyards	CC	10	3	60
Fourie *et al.* (2007)	South Africa	Breede River Valley	Bwh	Vineyards	CC	10	3	60

García-Franco *et al.* (2015)	Spain	Murcia	BSk	Almond orchards	CC	4	3	30
García-Ruiz *et al.* (2012)	Spain	Cádiz, Málaga, Jaén	Csa	Olive orchard	OA	3,4,9 and 16	1	10
García-Ruiz *et al.* (2009)	Spain	Jaén	Csa	Olive orchard	CMP	4 and 5	1	10
Gómez *et al.* (2009)	Spain	Córdoba	Csa	Olive orchard	CC	7	2	10
Gucci *et al.* (2012)	Italy	Toscana	Csa	Olive orchard	CC	5 years	2	20
Hernández *et al.* (2005)	Spain	Toledo	BSk	Olive orchard	CC	5 years	1	20
López-Piñeiro *et al.* (2011)	Portugal	Elvas	Csa	Olive orchard	OA	8 years	1	25
López-Piñeiro *et al.* (2008)	Portugal	Elvas	Csa	Olive orchard	OA	5	1	25
Lozano-García and Parras-Alcántara (2013a)	Spain	Córdoba	Csa	Olive orchard	CC	20	3	89

Lozano-García and Parras-Alcántara (2013b)	Spain	Jaén	Csa	Olive orchard	OA	3	4	115
Mahmoud *et al.* (2012)	Syria	Saida village	Bsk	Olive orchard	OA	15	1	5
Marques *et al.* (2010)	Spain	Madrid	Csa	Olive orchard	CC	1.6	1	10
Nasini *et al.* (2013)	Italy	Assisi	Cfa	Olive orchard	CMP	4	2	30
Nieto *et al.*, (2013)	Spain	Granada, Málaga, Córdoba	Csa	Olive orchard	CC	2, 12 and 14	1	30 30
Okur *et al.* (2009)	Turkey	Western Turkey	Csa	Vineyards	CMP	3	1	20
Olego *et al.* (2015)	Spain	Valladolid	Csb	Vineyards	OA	3	1	30
Palese *et al.* (2014)	Italy	Ferrandina-Basilicata	Csa	Olive orchard	CMP	6	2	20
Parras-Alcántara *et al.* (2015)	Spain	Córdoba	Csa	Olive orchard	CMP	20	3	89

Parras-Alcántara and Lozano-García (2014)	Spain	Córdoba	Csa	Olive orchard	CMP	20	3	140
Peregrina *et al.* (2014a)	Spain	La Rioja	Cfb	Vineyards	CC	5	5	45
Peregrina *et al.* (2012)	Spain	La Rioja	Cfb	Vineyards	CC	4	4	25
Peregrina *et al.* (2012)	Spain	La Rioja	Cfb	Vineyards	CC	5	5	45
Peregrina *et al.* (2014b)	Spain	La Rioja	Cfb	Vineyards	CC	3	1	5
Ramos *et al.* (2011)	Spain	Granada	Csa	Almond orchards	CC	5	1	20
Ramos *et al.* (2010)	Spain	Granada	Csa	Almond orchards	CMP	5	1	20
Repullo *et al.* (2012a)	Spain	Córdoba	Csa	Olive orchard	CMP	2	1	20
Repullo *et al.* (2012b)	Spain	Córdoba	Csa	Olive orchard	CC	3	3	20
Ruiz-Colmenero *et al.* (2013)	Spain	Madrid	Csa	Vineyards	CC	4	1	10
Seddaiu *et al.* (2013)	Italy	Sardinia	Csb	Vineryards	CMP	20	1	22

Soriano *et al.* (2012)	Spain	Córdoba	Csa	Olive orchards	CMP	5	2	20
Steenwerth and Belina (2008)	USA	California	Csb	Vineyards	CC	5	1	15
Vavoulidou *et al.* (2006)	Greece	Santorini	Csa	Vineyards	CC, OA	ND	1	30
Vavoulidou *et al.* (2009)	Greece	Attiki, Santorini, Arkodia, Mesinia, Chania	Csa	Vineyards, olive orchards	OA	ND	1	30

Appendix B. References of the meta-analysis

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Verde que te quiero verde.
Grandes estrellas de escarcha,
vienen con el pez de sombra
que abre el camino del alba.
Romance Sonámbulo
Federico G. Lorca

CHAPTER IV

Influence of the spontaneous vegetation cover on total SOC content in olive groves

1. Introduction

One of the main characteristics of the semiarid regions is the relative low SOC content (Lal, 2004c). Olive tree cultivation is distributed mainly in Mediterranean areas, which are characterized by relatively high evapotranspiration and irregular precipitations. Under these conditions the biomass production of the spontaneous resident vegetation cover is assumed to be relatively low and with strong inter-annual variability. Therefore, annual input of organic matter into olive orchards is relatively low, partially explaining the organic carbon poor soils of these woody crops.

As SOC content is affected by agricultural management practices, a change to a management involving an increase in the SOC content will also lead to a higher biomass production. There are many studies which have assessed enhancing the SOC content by changing the management practices from conventional tillage to sustainable managements (e.g, no tillage, reduced tillage) in Mediterranean semiarid conditions in Spain (e.g. López-Fandos and Almendros, 1995; López-Bellido *et al*., 1997; Hernanz *et al*., 2002; Moreno *et al*., 2006; Martin-Rueda *et al*., 2007; Ordóñez-Fernández *et al*., 2007; Virto *et al*., 2007; Álvaro-Fuentes *et al*., 2008).

The results of many studies involving the study of SOC accumulation by changing the management practices are shown in Aguilera *et al*. (2013) research, who concluded, in a meta-analysis with 174 data sets of Mediterranean crop plots, that by combining external or internal organic inputs with managements involving a reduction in the soil structure disturbance it is possible to maximize the SOC levels. They also concluded that the more intense is the cropping system the bigger the differences are between the conventional and the sustainable management.

However, Farina *et al*. (2013) used data from five semiarid sites in Foggia (Italy), Zaragoza (Spain), Córdoba (Spain), Tel Hadya (Syria) and Waite (Australia), and one temperate site at Rothamsted (UK) to validate the RothC model and to make adjustments so that the model best fits the real conditions. They concluded that RothC does not simulate well C dynamics in semiarid soils. They found an “unrealistic high C input needed to fit the modelled data to the measured, especially in systems with a fallow in the crop rotation”. Therefore, there is a lack of information involving SOC content and biomass production.

Due to this behavior of Mediterranean soils, it is necessary to carry out more studies linking SOC dynamics with biomass production, especially in Mediterranean woody crops, where the presence of a spontaneous vegetation cover affects strongly on the SOC cycle.

As it was shown in Chapter I (4.2 section) the weed-cover management in olive groves in Andalusia consists of allowing the growth of a spontaneous resident vegetation cover from September, when the vegetation starts to grow, until the end of March or the beginning of April, when the vegetation has to be controlled, in order to not to compete with the olive trees for water, usually by mowing and leaving the biomass residues on the soil surface. Sometimes, the vegetation can be controlled by a reduced tillage affecting only the upper layer.

The presence of a spontaneous resident vegetation cover in olive groves has been especially studied to assess the decrease on soil losses by erosion (see 4.2 section in Chapter I). Thus, one of the main advantages of the weed-cover management is not only to increase the SOC content throughout the increase in the amount of the organic C content from the biomass, but also the decrease on SOC losses via erosion. This fact is remarkable, since a great proportion of the olive orchards is cultivated under slopes greater than 15%.

However, only few studies have measured annual weed biomass production for several consecutive years (three years in both studies) and the effects of the application of weed residues on SOC content. Nevertheless, these studies did not take into account different mineralogical and geochemical properties, since Mediterranean soils include soils with very different mineralogical properties (e.g. acid and basic soils). Therefore, it is necessary to focus on the relationship between biomass production and total SOC content in agroecosystems in Mediterranean conditions also considering different mineralogical soil properties.

2. Objectives

This chapter has three main objectives.

1. Quantify and provide a range of variability of the annual aboveground **biomass production, and organic carbon,** of spontaneous resident vegetation cover of **olive orchards**.
2. Assess the **effects of** spontaneous resident vegetation cover **on total SOC content** and also considering different depths.
3. Evaluate the influence of some **mineralogical properties on total SOC content**.

Two approaches were undertaken to fulfill these objectives:

1. Mensurative experiments (studies 1 and 2; objectives 1, 2 and 3). In these experiments the medium-term (> 8 years) implementation of a resident vegetation cover and the influence of different soil mineralogical properties, on aboveground annual biomass production were evaluated (figure 1).
2. A manipulative experiment. In this experiment the change in management (from bared to vegetation covered soil) is changed to evaluate the changes (at the short term) of some soil properties (Study 3; objective 1) (figure 1).

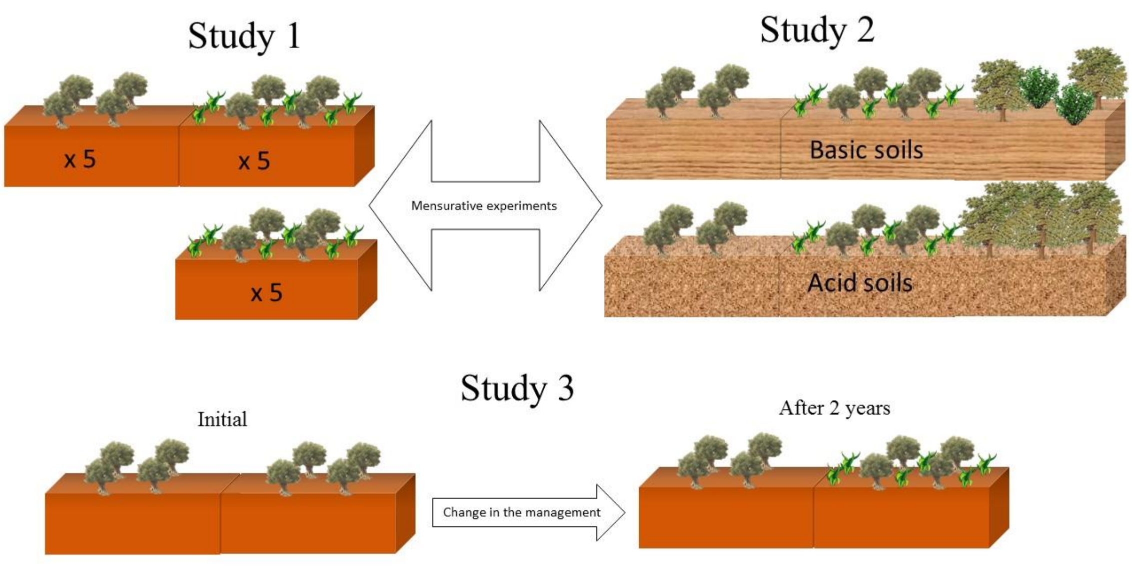


Figure 1. Schematic representation of the mensurative (1 and 2) and manipulative (study 3) experiments.

3. Materials and methods

3.1 Experimental design

Study 1

Quantification of the variability of the annual aboveground **biomass production of** spontaneous resident vegetation cover of **olive orchards**.

Assess the **effects of** spontaneous resident vegetation cover **on total SOC content** and considering different depths.

<u>Site, managements and biomass production</u>

Ten commercial olive oil farming in which spontaneous vegetation cover were implemented each year during at least the last eight years were selected in different areas of southern west Andalusia. Spontaneous vegetation in all farms were mechanically cleared each year during March and spontaneous vegetation residues left on the soil surface. Typically, spontaneous vegetation covered between 75 to 90 % of the olive oil farming area. Soils in these farming differed in a range of characteristics some of which are showed in Table 1. Five of these ten spontaneous vegetation covered olive farms were paired with a nearby (within a distance of tens of meters) and comparable olive oil farms (in terms of climatology, orientation, slope, soil properties and farming characteristics such as tree density and age), except for no spontaneous vegetation was allowed to grow for the last 8 years. In the olive oil farms lacking of spontaneous vegetation, these were controlled with various passes of the chain mower and by applying pre-emergence herbicides in autumn. Thus, differences between these five pairs of olive oil farming were attributed primarily to the presence of spontaneous vegetation cover and to the management related to the control of weeds. All olive oil farming consist of a tree density of 90 – 120 trees per hectare, aged 35 to 45 years and trees were distributed in a regular fruity arrangement with a canopy cover typically of about 30 % of the farming area. Fertilizer was not considered as a source of variation as these were only applied locally under the tree canopy.

Study 2

Assess the **effects of** spontaneous resident vegetation cover **on total SOC content**.

Evaluate the influence of some **mineralogical properties on total SOC content**.

Sites

Two different sites in Andalusia were selected for this study. One is situated in Valle de los Pedroches, in Sierra Morena, in the province of Córdoba (acid soils) (figure 2) and the other one is located in Sierra Mágina (basic soils), in the province of Jaén (figure 3). Therefore, two contrasted-mineralogy sites (siliceous and carbonated) were selected. The acid soils were under granitic parent material, whereas the basic soils were under marls. Table 2 show differences in general soil properties between the two locations, whereas Table 3 shows differences in mineralogical soil properties.

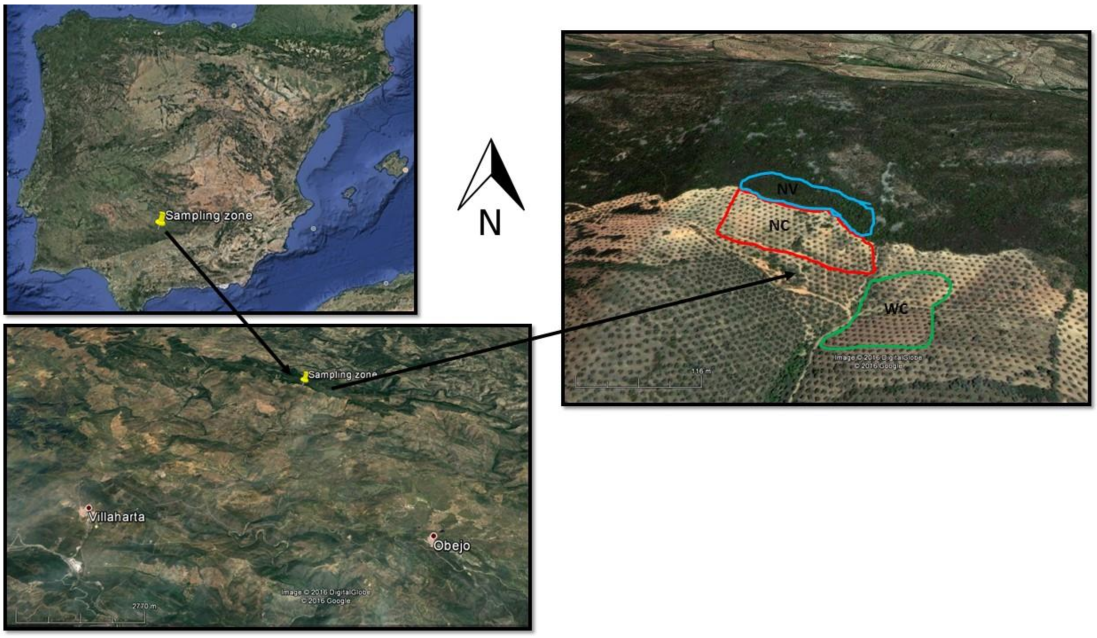


Figure 2. Images of the three managements Sierra Morena (Córdoba): olive orchards with bare soil (NC), with spontaneous natural vegetation (WC) and forest (NV).

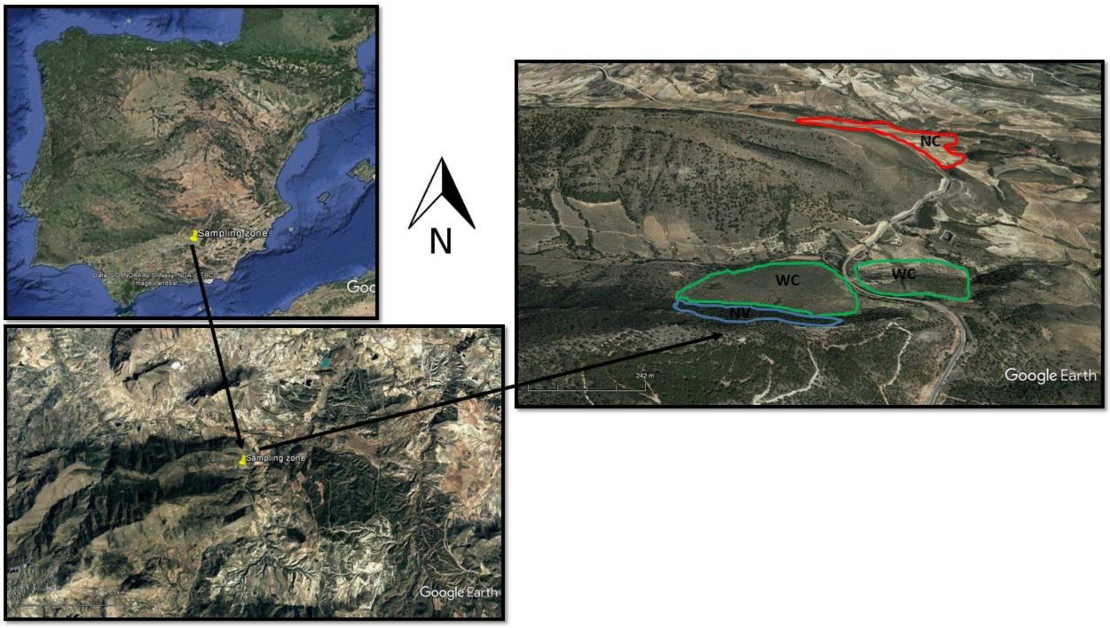


Figure 3. Images of the three managements Sierra Mágina (Jaén): olive orchards with bare soil (NC), with spontaneous natural vegetation (WC) and forest (NV).

Managements

Three different managements were considered for each site of contrasting mineralogy. Figures 4 and 5 shows the results of the difractograms of each type of soil for the acid and basic parent materials, respectively. i) Natural vegetation (NV), or no management, mainly consisting of a Mediterranean forest dominated by *Quercus ilex* in the acid soils and of a typical Mediterranean scrublands in the basic site (figures 6a and 7a), ii) soils under an olive orchard covered by spontaneous vegetation (WC), in the intercanopy area of the orchard. The vegetation was typically cleared early in the spring by mowing and leaving the aerial biomass on the soil surface (figures 6b and 7b), and iii) non-covered soils (NC), consisting of a bare soil due to a tillage combined with the use of pre-emergence herbicides. The soil was only occupied by olive trees (figures 6c and 7c). The three plots corresponding to the three managements in each site were close to each other (tens of meters). It is hypothesised that three managements in each of the two sites may provide a SOC gradient.

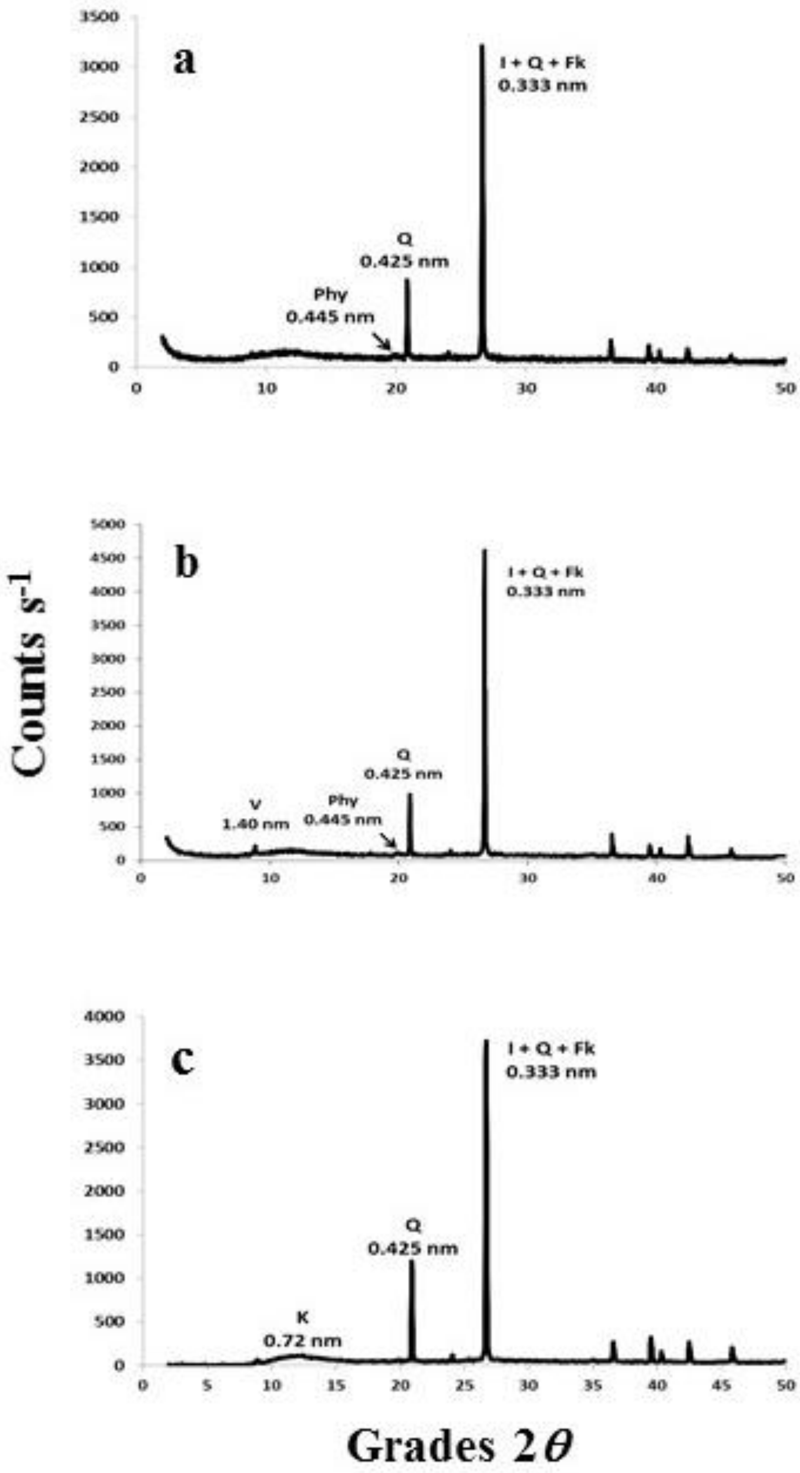


Figure 4. Results of the diffractograms of the acid soils (Sierra Morena) in the natural vegetation (a), weed-cover (b) and non-cover (c) managements. Peaks represent the content on illite (I), quartz (Q), Potassium Feldspar (Fk), Potassium (K), vermiculite (V) and laminar phyllosilicates (Phy). Differences on the height of the peaks represent different amounts of each mineral. The three graphics are very similar, indicating similar mineralogical composition. Quartz is the most characteristic mineral in these soils.

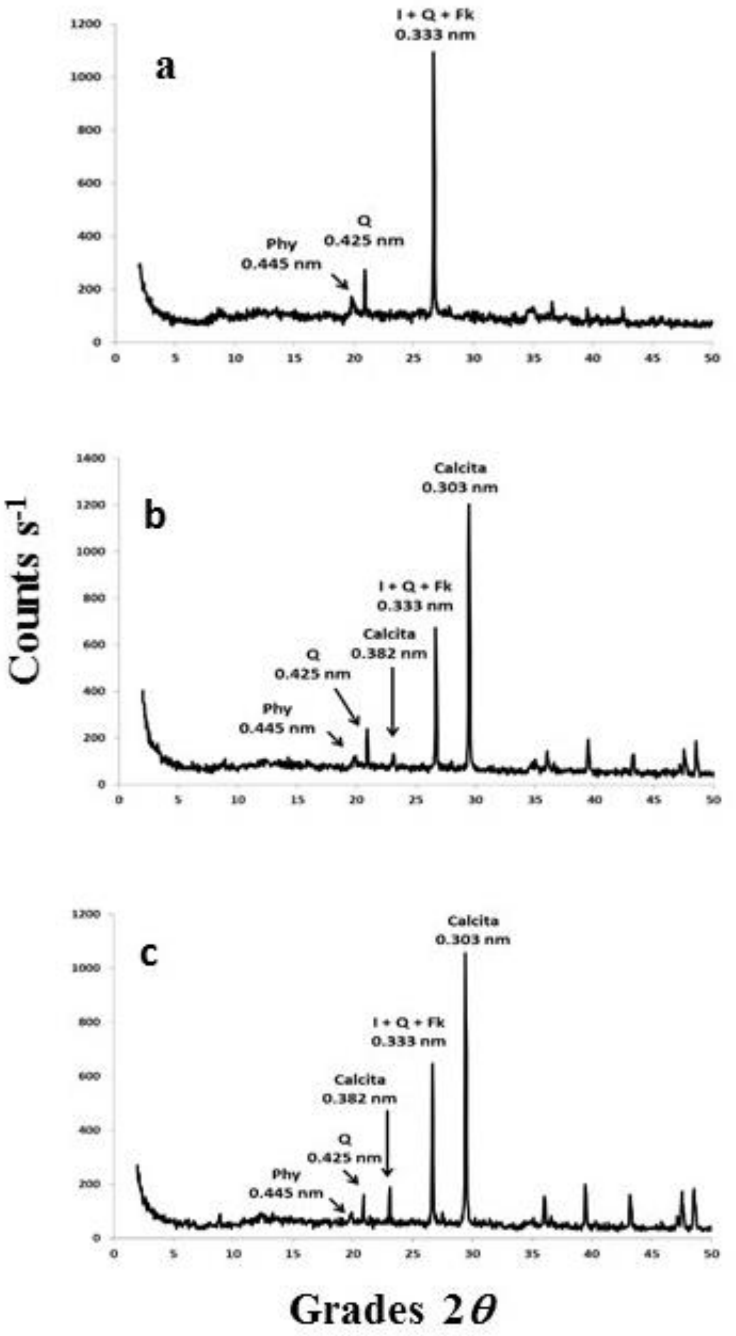


Figure 5. Results of the diffractograms of the basic soils (Sierra Mágina) in the natural vegetation (a), weed-cover (b) and non-cover (c) managements. Peaks represent the content on calcite (calcite, i.e. carbonates), illite (I), quartz (Q), Potassium Feldspar (Fk), Potassium (K) and laminar phyllosilicates (Phy). Differences on the height of the peaks represent different amounts of each mineral. The difractograms of the weed-cover and non-cover managements are very similar, indicating similar mineralogical composition. However, carbonates do not appear in the soil under natural vegetation, which is decarbonated due to its edaphic evolution. The results of the diffractograms are more complex than those of the acid soils, appearing the calcite with the quartz (except in the natural vegetation management).

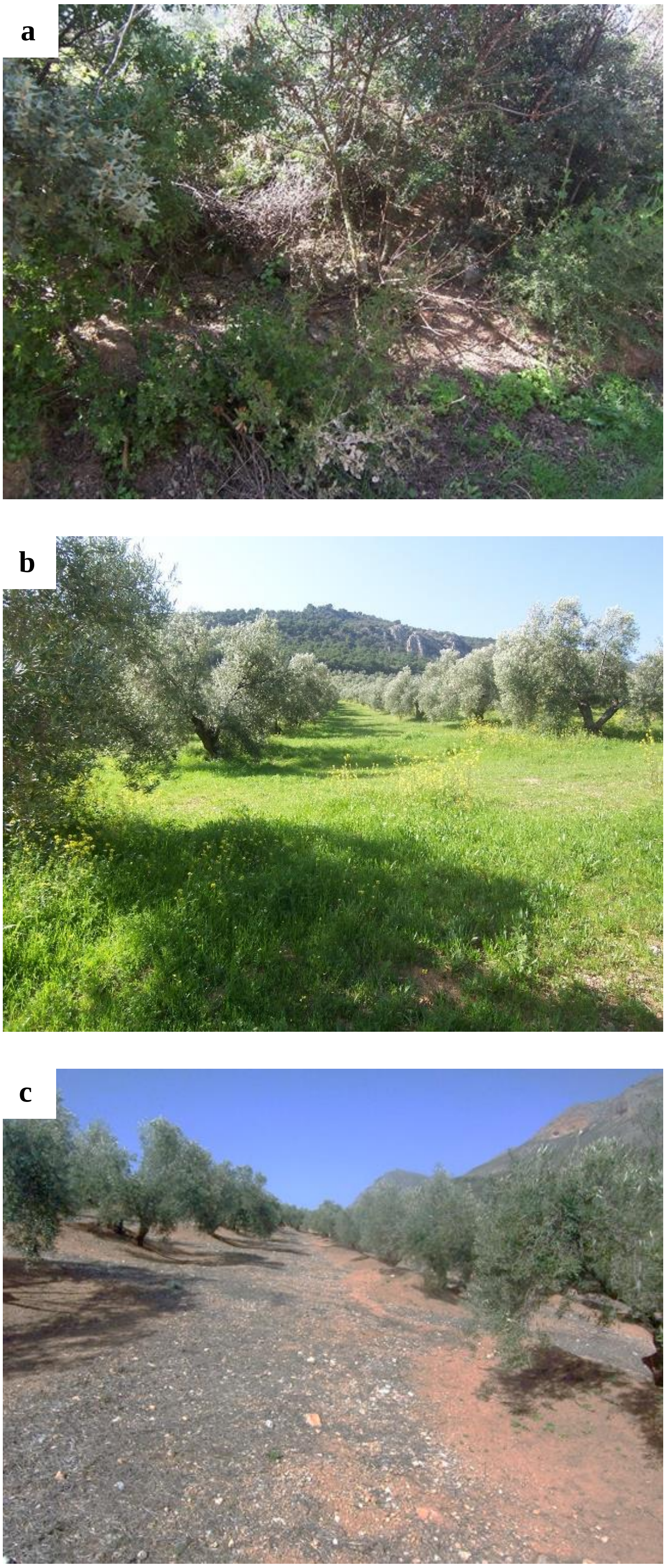


Figure 6. Images of the three managements in Sierra Mágina (Jaén): natural vegetation (a), weed-cover (b) and non-cover managements (c).

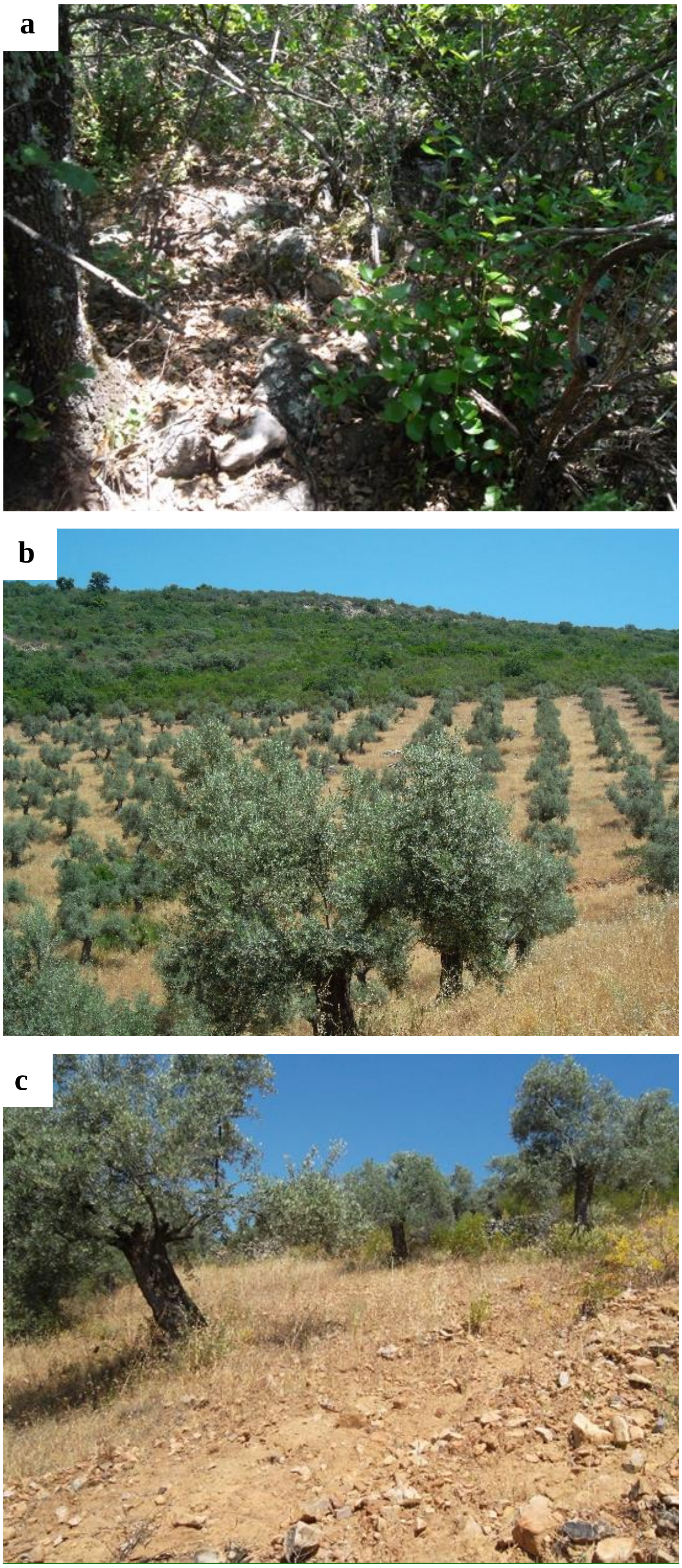


Figure 7. Images of the three managements in Sierra Morena (Córdoba): natural vegetation (a), weed-cover (b) and non-cover managements (c).

Study 3

Quantification of the variability of the annual aboveground **biomass production of** spontaneous resident vegetation cover of **olive orchards**.

Site and managements

Two nearby plots were selected within the same olive orchard belonging to the Castillo de Canena Property, in Úbeda (Guadalquivir River Valley, Jaén). Until 2014 both plots had the same management consisting on a spontaneous plant cover in the inter-row area but the biomass was usually controlled by sheep grazing. In 2014 in one of the plots (about 1 ha) a spontaneous vegetation cover in the inter-row area was implemented as before but was controlled by mowing during early spring (WC). Plant residues were left on the soil surface (figure 8). On the other hand, the other plot pre and post-emergence herbicides were applied to avoid the development of a spontaneous vegetation cover (NC), and therefore soil was bared all year round. Fertilisation was not considered as a source of variation because it was done under the tree canopy and not in the inter-row area.

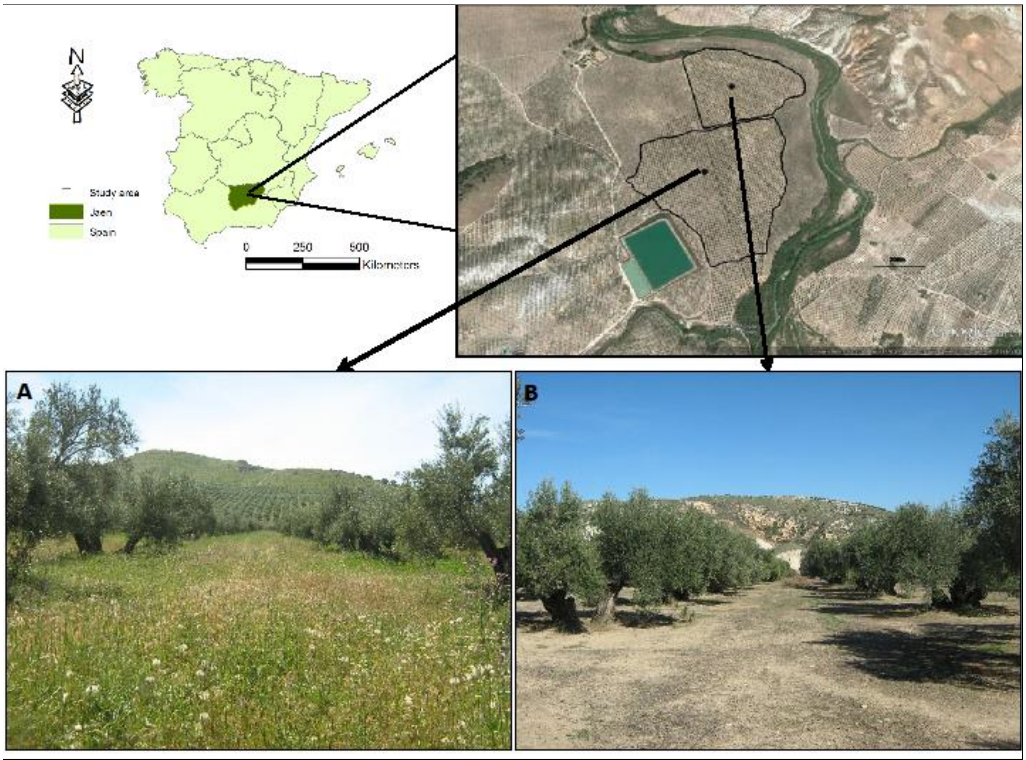


Figure 8. Images of the location of the Castillo de Canena property (Jaén) and the two managements: weed-covered (a) and non-covered (b). Image taken from Chamizo *et al*. (2017).

3.2 Sources of variation

Considering the three studies described previously were: management (covered vs non-covered soils), sampling depth (0 – 5 and 5 – 15 cm) and parent material (acid vs basic soils).

3.3Dependent variables

Biomass production

Biomass production was measured in the ten olive orchards covered by spontaneous vegetation cover in 2010. Sampling and aboveground biomass, carbon and nitrogen were measured as described in Chapter II (1.2, 2.2 and 2.3 sections). Sampling was carried out at the end of March or beginning of April and a couple of days before clearing the spontaneous vegetation cover.

Aboveground biomass in the plots selected in the Study 3, in Gualdalquivir valley, was quantified during early April in two consecutive years (2015 and 2016), one year after the change of management.

Total SOC content

In the Study 1, soil below the frames used to collect spontaneous aboveground biomass was collected during the end of March early April 2010 following the methodology described in Chapter II (1.1 section) at depths of 0 – 5 and 5 – 15 cm. In the spontaneous vegetation free olive oil farms, soils of the intercanopy area were collected in the same day and in the same way than that of olive orchards covered by spontaneous vegetation.

A similar methodology was followed in the case of the Study 2 (the effect of other mineralogical characteristics on total SOC is analyzed in Chapter VI), but in this case only one depth was considered (0 – 15 cm). The number of soil replicates per plot depended on soil variability and ranged from 5 to 10 replicates.

In the case of the Study 3, experiment is still running and “final” soil samples have not been collected yet and thus it is not possible to assess the influence of the biomass production on the total SOC content. Nevertheless, soil samples were collected at the beginning of the experiment (October 2014). For each treatment samples were collected from two depths (0 – 5 and 5 – 15 cm).

Other soil properties related to soil fertility.

< 2 mm meshed soil was analyzed for size particle distribution, cation exchange capacity (CEC), N, pH, Phosphorous, total carbonates, Potassium according to the corresponding methods described in Chapter II (2.3 section).

Mineralogical soil properties.

To obtain the diffractograms has been used the fine fraction (< 2 mm) throughout the use of the disorientated technique (diffractometer Siemens D5000, using Cu Kα in an angular range of 3 – 50º 2θ). The following phases have been identified: chlorite (0.700 nm reflexion), laminars (0.445 nm reflexion, including illite, paragonite, smectite, kaolinite and interstr 2:1 phases), quartz (0.425 nm reflexion), goethite (0.418 nm reflexion), Potassium Feldspar (0.325 nm reflexion), Sodium Feldspar (0.318 nm reflexion), calcite (0.303 nm reflexion), dolomite (0.289 nm reflexion) and hematite (0.269 nm reflexion). The percentages of these phases has been established depending on the relative surface of the peaks (specifics for each mineral) by using the following factors (only factors different from 1 are cited): laminars, 0.1; quartz, 0.5, goethite and hematite, 0.25.

For the analysis of the phyllosilicate mineralogy a combination of samples has been used for each plot (previously the clay fraction, < 2 µ, was isolated by sedimentation). The samples were treated with H_2O_2 and dithionite-citrate-bicarbonate to eliminate the organic matter and Fe, respectively, and to achieve a good orientation of the sample.

Plates with oriented aggregated were prepared from Mg- and K-saturated samples (Magnesium and Potassium chloride, respectively) according to the protocol of Whitting and Allardice (1986), which includes conditions of : i) air dry, ii) saturation in ethyleneglycol vapor (only for Magnesium samples), and iii) glycerol solvation (only for Magnesium samples).

The analysis of the orientated aggregates have been carried out in the diffractometer (Siemens D5000 in an angular range of 2 – 30º 2θ). In the diffractograms of the orientated aggregate (ethyleneglycol saturated) the following mineral have been identified: smectite (> 1500 nm reflexion), vermiculite (1400 – 1500 nm reflexion), illite (1000 nm reflexion), chlorite (0.705 nm reflexion), Kaolinite (0.720 nm reflexion); quartz (0.425 nm reflexion), calcite (0.303 nm reflexion), and the different interstratified phases (i.e. illitic interstr, 1000 – 1400 nm reflexion). The percentages of these phases have been established according to the relative surface of the peaks, specific of each mineral, by using the following factors (only cited those different than 1): smectites, 4.0; vermiculites, 2.0; Kaolinite, 2.0; quartz, 2.0. To the interstratified phases medium factors were applied (i.e. 2.0 to illite-smectite).

For the exactly identification of the interstrstratified phases a decomposition of the 3 – 10º 2θ zone of the diffraction patrons of the Mg-ethileneglycol-saturated samples by using the program DecompRX (Lanson, 1997), following the recommendations of Barré *et al*. (2008). Once done the decomposition e identified the phyllosilicates, the percentage of each phase was estimated according to the relative surface of each specific peak (Velde and Barré, 2010).

The results of the diffractograms are those shown previously in the figures 4 and 5.

3.4 Statistical analysis

In the Study 1 the effects on the total SOC of the two factors (depth, management) and their interactions were assessed using two-way ANOVA procedure, software STATISTICA (StatSoft, 2001). Pearson correlation coefficients were used to assess the significance of the correlation between total SOM and biomass production. Values of the variables for each depth were averaged in order to better focus on the difference between depths and managements.

In the case of the Study 2 the effects on total SOC of the factor (management) and their interactions were assessed using one-way ANOVA procedure (IBM SPSS Statistics 22.0).

4. Results and discussion

4.1 Aerial biomass production

Annual weed biomass production in the weed-covered olive orchards varied greatly from an average of 0.65 t ha^{-1} found at CT to 2.53 t ha^{-1} of JA site, with and overall mean of 1.48 t ha^{-1}. Organic C content of the biomass of the full set of farming averaged 37.4 %. Annual input of C due to aboveground weed biomass averaged 0.56 t ha^{-1} with a minimum and a maximum of 0.24 and 1.0 t ha^{-1}, respectively (Table 1). A summary of these results is shown in the figure 9.

For another hydrological year and in the site of Castillo de Canena (Úbeda is very close to the Guadalquivir River), annual biomass of the spontaneous vegetation cover amounted 2.2 t ha^{-1} and 2.44 t ha^{-1} after one and two year of cover implementation, respectively. These values were in the upper range found for the Study 1. Organic C content of the biomass averaged 36 and 30% in 2015 and 2016, respectively. This resulted in an annual organic C input of 0.79 and 0.74 t ha^{-1} for the two years, an averaged 0.77 t ha^{-1}) (Table 5), which was 38% higher than the average value obtained in the Study 1.

Data of annual production of aboveground weed biomass were in the lower end of the 2.6 to 4.0 t ha^{-1} yr^{-1} on annual average (data from 4 years) reported by Castro *et al*. (2008) for an olive oil farm which allowed weed to grow during 28 years, or that of 5 – 10 t ha^{-1} yr^{-1} reported by Bugg *et al*. (1996) in Californian vineyards with weeds, or lower than the average values of 1.5 and 11 t h^{-1} found by SAN (1998) with different types of cover crops. However, data are in the range of 1 and 4 t ha^{-1} obtained by Repullo *et al*. (2012b) in an olive weed-covered farm in Córdoba (Spain) during a period of three agricultural years. Annual rate of biomass entering to the soils throughout crops residues is usually much higher. For instance, Allmaras *et al*. (1998) provided the most probable crops residues yield values for the most abundant crops of United States, which ranged from 2.9 of the wheat to 7.3 t ha^{-1} yr^{-1} of the Corn. However, our values were similar to that of Álvaro-Fuentes (2009), who found for different tillage treatments and cropping system of barley (continuous barely versus barley-fallow rotation) in a semiarid site of north Spain (average annual precipitation of 340 mm) a 4-year annual average of above-ground crop residue C inputs of between 0.32 – 1.3 t C ha^{-1}. Relatively low annual values of aboveground weed biomass production in olive oil groves is not surprising since the alley area of the olive oil farming is neither fertilized nor irrigated.

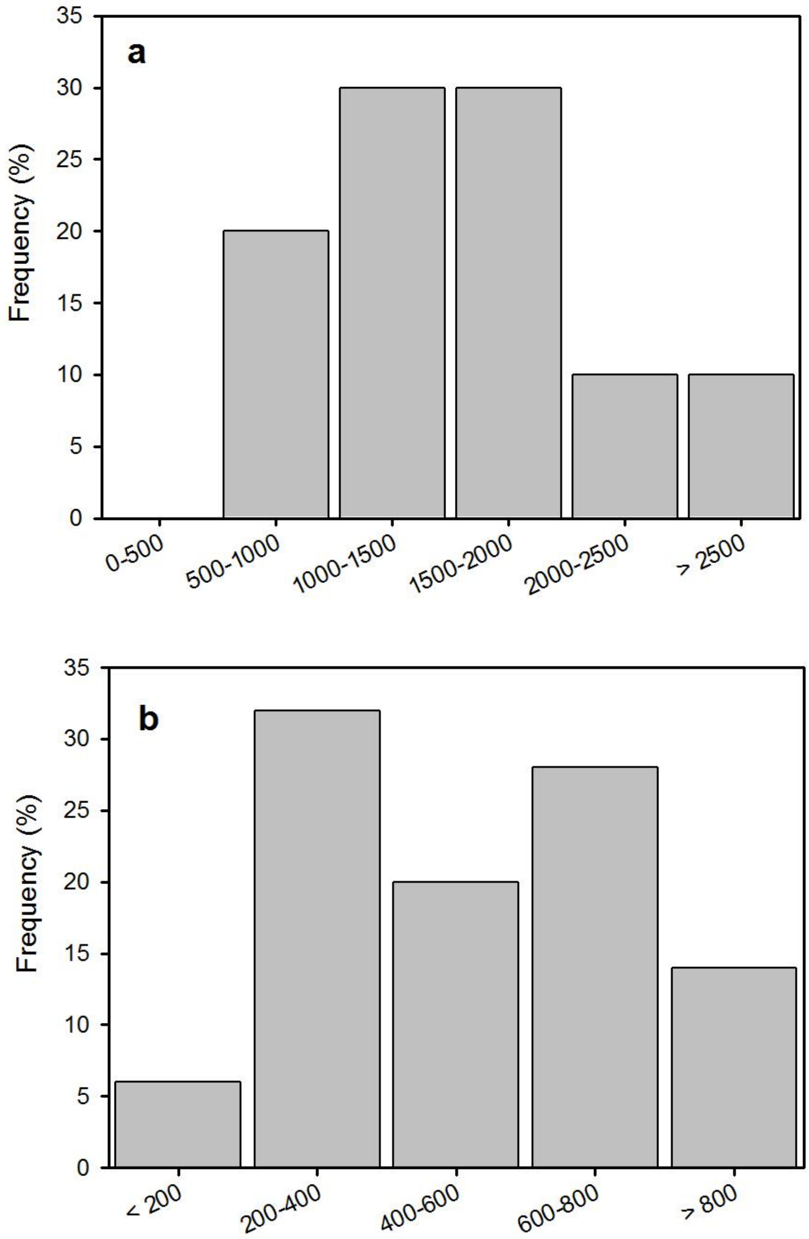


Figure 9. Histogram showing the range of aboveground biomass production (a) (kg of dry matter ha^{-1} yr^{-1}) and range of aboveground plant carbon production (b) kg of dry matter ha^{-1} yr^{-1}). The histogram shows that a great part of the biomass production is in the range of between 1 and 2 t ha^{-1} yr^{-1}. It corresponds to a range of organic carbon input of 0.2 – 0.8 t ha^{-1} yr^{-1}.

On the other hand, annual inputs of organic C throughout weed biomass are close to the C sequestration potential outlined by several researchers under scenarios of application of crops residues. Thus, Freibauer *et al.* (2004), based on Smith (2000), estimated, although with great uncertainty, that between 0.2 to 0.7 t C ha^{-1} yr^{-1} is the potential soil carbon sequestration rate after applying annually residues of major crops in Europe, and Hutchinson *et al.* (2007) found average rates of potential carbon gain from 0.1 to 0.5 t C ha^{-1} yr^{-1}. However, the extent which the input of weed-derived C increase the soil C stock will ultimately depend on decomposition rate of weed-C which, in turn, depends on many factors including weed quality (e.g. C-to-N ratio and lignin and polyphenol contents), edaphic and environmental conditions and weed residues management (e.g. residue location) (Kumar and Goh, 2000). Weeds C-to-N ratio grand mean of this study was 17.1 (min 14.4; max 24.0) (Table 1) and thus relatively high decomposition rate is expected from these relatively low C-to-N ratio, especially taking into account that in Mediterranean type climate highest precipitation overlaps with relatively high air temperature. On the other hand, weed residue-C decomposition is expected to slow down as typically they are left on soil surface. Cooper *et al.* (2006) found that Oilseed rape residue decomposition was lower, and thus more organic carbon was retained, at the surface as mulch compared to when residues were mixed within the soil matrix. Unfortunately, we have not found data on weed residue decomposition under Mediterranean climate. However, our own 343 days experiment of weed-residue litter-bag decomposition on the soil surface in the DEI farming of this study showed that only 20 % of the added weed residue-C remained in the litter bag, indicating that the other 80 % was respired or had entered into the soil as < 1mm (mesh size) particle fragments (Gómez-Muñoz *et al*., 2014). This value was lower than the 43 % of Vazquez *et al*. (2003) for litter-bag decomposition of mixed weeds laid on the ground surface from December to July.

The high variability of the biomass production observed in the Study 1 would be caused by the different soil and weather conditions of the 10 plots sampled. In other words, it is very difficult to choose a single variable as the main variable causing differences on spontaneous vegetation biomass production.

Table 1. Main values and standard deviation (in brackets) of some characteristics of the soils of the Study 1 from Cambil (CA1 and CA2), Cortijo Tobazo (CT), Moraleda (MO), Loja (LO), Deifontes (DEI1 and DEI2), Pegalajar (PE), Jaén (JA), Jaén (AL): depth (cm), weed production (t ha^{-1}), organic carbon production through the biomass (t ha^{-1}), nitrogen content in the biomass (Kg ha^{-1}), C/N of the biomass, necromass (t ha^{-1}), soil organic matter (%), cation exchange capacity (meq $100g^{-1}$), total Nitrogen (%), Olsen Phosphorus (g Kg^{-1}), Potassium (g Kg^{-1}), sand (%), silt + clay (%) and texture. WC = Weed-cover, NC = Non-cover managments.

Sites	Management	Depth	Weed production	C in the biomass	N in the biomass	C/N of biomass	Necromass	SOM	CEC	TN	Olsen P	K	Sand	Silt+clay	Texture
CA1	WC	0 -5	1.10 (0.37)	0.40 (0.12)	22.3 (2.2)	17.6 (2.2)	1.69 (1.3)	4.30 (0.5)	19.0 (0.5)	0.23 (0.02)	42.3 (9.5)	722 (67)	30.9 (1.3)	69.0 (0.9)	loam clay
CA1	WC	5-15						2.47 (0.4)	17.8 (1.5)	0.13 (0.01)	15.5 (3.2)	517 (3.5)	26.1 (1.7)	73.8 (1.2)	loam clay
CA1	NC	0 -5	-				-	1.10 (0.3)	10.4 (0.9)	0.06 (0.01)	7.1 (0.4)	220 (42)	32.5		clay
CA1	NC	5-15						1.08 (0.3)	10.7 (0.4)	0.06 (0.02)	6.0 (1.0)	254 (22)	-		clay
CA2	WC	0 -5	0.89 (0.44)	0.32 (0.15)	19.5 (5.7)	16.1 (2.9)	2.03 (1.5)	5.35 (1.98)	23.4 (4.9)	0.25 (0.07)	28.8 (11.0)	720 (339)	34.4 (8.1)	65.5 (8.13)	clay
CA2	WC	5-15						2.77 (1.35)	22.9 (9.07)	0.14 (0.05)	7.55 (2.33)	357.5 (244)	21.2 (0.0)	78.8 (0.0)	clay
CA2	NC	0 -5	-				-	2.17 (0.18)	20.8 (1.5)	0.13 (0.01)	17.3 (2.0)	455 (91.9)	21.5 (1.4)	78.5 (1.0)	clay
CA2	NC	5-15						1.9 (0.11)	20.0 (2.8)	0.11 (0.00)	9.9 (2.3)	322 (116.7)	21.8 (0.9)	78.1 (0.6)	clay
CT	WC	0 -5	0.65 (0.15)	0.24 (0.05)	16.9 (3.5)	14.4 (0.9)	1.38 (0.75)	6.42 (0.8)	18.7 (0.3)	0.30 (0.01)	11.7 (0.2)	580 (98.9)	40.7 (4.6)	59.2 (3.2)	loam clay
CT	WC	5-15						3.00 (0.2)	17.3 (0.3)	0.13 (0.01)	4.60 (0.0)	490 (20.5)	33.7 (1.7)	66.2 (1.2)	clay
CT	NC	0 -5	-				-	1.87 (0.5)	11.6 (0.5)	0.10 (0.01)	8.45 (0.9)	275 (13.4)	40.4 (0.0)	59.6 (0.0)	loam
CT	NC	5-15						1.33 (0.1)	11.4 (0.8)	0.07 (0.01)	4.45 (0.6)	270 (21.21)	40.2 (4.3)	59.7 (3.0)	loam clay
MO	WC	0 -5	1.50 (0.41)	0.58 (0.14)	39.1 (8.6)	14.7 (1.12)	2.63 (2.07)	3.78 (0.7)	14.1 (0.2)	0.21 (0.04)	30.60 (1.1)	371 (21.9)	43.9 (0.8)	56.1 (0.6)	loam
MO	WC	5-15						1.59 (0.1)	12.1 (0.2)	0.10 (0.01)	15.30 (2.4)	305 (56.5)	39.8 (0.7)	60.2 (0.5)	loam
MO	NC	0 -5	-				-	1.14 (0.18)	7.0 (0.9)	0.07 (0.01)	56.2 (0.4)	192 (47)	39.4 (1.7)	60.6 (1.2)	loam
MO	NC	5-15						1.05 (0.08)	7.0 (0.3)	0.06 (0.01)	40.2 (8.3)	204 (37)	42.8 (0.6)	57.2 (0.4)	loam
LO	WC	0 -5	1.95 (0.15)	0.70 (0.09)	29.3 (4.0)	24.0 (1.5)	0.34 (0.25)	1.72 (0.1)	15.4 (0.9)	0.10 (0.01)	30.30 (6.36)	355 (70.7)	28.1 (2.7)	71.9 (1.9)	loam clay
LO	WC	5-15						1.29 (0.07)	15.1 (0.5)	0.08 (0.00)	26.90 (5.1)	283.5 (30.4)	25.9 (0.4)	74.1 (0.3)	loam clay

DE1	WC	0 -5	1.07 (0.16)	0.39 (0.06)	26.7 (5.6)	14.9 (1.3)	0.54 (0.19)	7.62 (1.2)	17.3 (2.6)	0.33 (0.04)	26.50 (3.1)	364 (2.8)	57.2 (3.5)	42.8 (2.5)	loamy sand
DE1	WC	5-15						3.25 (0.1)	20.1 (0.1)	0.15 (0.01)	7.35 (0.1)	235 (57.2)	44.1 (1.7)	55.8 (1.2)	loam
DE1	NC	0 -5	-				-	6.2 (0.21)	19.8 (0.3)	0.31 (0.01)	30.45 (0.78)	531 (15.56)	43.75 (1.77)	56.25 (1.77)	loam
DE1	NC	5-15						4.79 (2.5)	18.6 (2.0)	0.24 (0.11)	21.75 (17.32)	408 (222.7)	46.25 (1.2)	53.75 (1.2)	loam
DE2	WC	0 -5	2.04 (0.49)	0.79 (0.17)	47.8 (5.6)	16.4 (2.5)	0.12 (0.09)	2.57 (0.2)	16.6 (0.5)	0.13 (0.01)	46.35 (10.5)	337 (54.0)	40.7 (2.8)	59.3 (2.8)	loam
DE2	WC	5-15						2.31 (0.17)	17.2 (2.8)	0.14 (0.03)	8.65 (0.92)	103 (3.53)	38.2 (2.8)	61.8 (2.8)	loam
PE	WC	0 -5	1.83 (0.49)	0.68 (0.19)	47.9 (12.4)	14.4 (2.2)	0.53 (0.31)	3.34 (0.3)	30.8 (2.5)	0.19 (0.02)	26.85 (1.3)	630 (127.2)	23.7 (5.3)	76.0 (3.7)	clay
PE	WC	5-15						2.56 (0.4)	31.3 (4.6)	0.14 (0.01)	15.15 (2.2)	398 (10.6)	19.6 (0.6)	80.0 (0.4)	clay
JA	WC	0 -5	2.53 (1.3)	1.02 (0.46)	47.5 (18.7)	21.2 (3.0)	1.12 (0.61)	2.62 (1.3)	26 (0.8)	0.14 (0.06)	16.75 (2.1)	415 (28.2)	17.5 (3.5)	82.2 (2.5)	clay
JA	WC	5-15						1.19 (0.1)	24 (0.2)	0.08 (0.00)	7.55 (0.2)	378 (38.8)	12.7 (0.3)	87.3 (0.2)	clay
AL	WC	0 -5	1.21 (0.57)	0.48 (0.20)	27.4 (13.1)	17.8 (2.2)	0.25 (0.16)	1.80 (0.09)	23.7 (0.6)	0.10 (0.01)	12.1 (0.8)	308 (3.5)	28.7 (7.0)	71.3 (5.0)	clay
AL	WC	5-15						1.31 (0.16)	25.7 (2.3)	0.08 (0.01)	8.0 (2.0)	283 (88)	17.9 (2.5)	82.0 (1.7)	clay

Table 2. Mean values and standard deviation (in brackets) of physical and mineralogy properties of soils of the Study 2. Sand (%), silt (%), clay (%), total soil organic carbon (%), total Nitrogen (%), C/N, Olsen Phosphorous (g Kg^{-1}), pH, total carbonates (%), cation exchange capacity (meq 100 g^{-1}) and Potassium (g Kg^{-1}). Bellow, the results of the ANOVA. Notes that natural vegetation (NV) is not included in the ANOVA.

Mineralogy	Management	Sand	Silt	Clay	SOC	N	C/N	Olsen P	pH	T. Carbonates	CEC	K
Siliceous	NV	58.8 (4.6)	30.6 (4.2)	10.2 (1.3)	10.5 (2.5)	0.40 (0.12)	26.7 (6.7)	10.4 (4.4)	6.2 (0.2)	1.8 (0.4)	17.4 (2.9)	210 (162)
	WC	58.3 (2.9)	27.7 (4.0)	13.9 (2.6)	1.5 (0.3)	0.09 (0.02)	17.5 (2.3)	4.8 (5.1)	6.4 (0.2)	1.5 (0.8)	10.9 (1.5)	110 (46)
	NC	48.8 (16.6)	24.4 (3.4)	26.8 (17.5)	0.8 (0.2)	0.04 (0.02)	23.0 (11.8)	3.0 (1.6)	5.9 (0.5)	1.8 (0.9)	12.8 (6.6)	39 (10)
Carbonated	NV	38.0 (6.8)	30.2 (4.4)	32.0 (6.9)	5.8 (0.4)	0.35 (0.08)	19.0 (3.1)	5.8 (1.6)	7.7 (0.1)	3.5 (2.1)	43.6 (7.6)	631 (134)
	WC	35.3 (4.1)	37.9 (4.7)	26.9 (5.6)	3.0 (1.4)	0.22 (0.14)	14.7 (3.4)	11.9 (13.9)	7.9 (0.1)	34.2 (13.7)	28.8 (7.3)	387 (316)
	NC	30.9 (8.4)	33.5 (2.9)	35.5 (8.5)	1.5 (0.6)	0.09 (0.04)	12.2 (1.5)	11.3 (4.8)	8.0 (0.1)	53.7 (12.1)	22.1 (4.0)	175 (86)
	Management	* WC>NC	ns	** NC > WC	** WC >NC	** WC >NC	ns	ns	ns	* NC>WC	ns	ns
	Mineralogy	*** Si>Ca	*** Ca>Si	*** Ca>Si	** Ca>Si	** Ca>Si	** Si>Ca	** Ca>Si	*** Ca>Si	*** Ca>Si	*** Ca>Si	** Ca>Si
	Man * Min	ns	ns	ns	ns	ns	*	ns	***	**	*	ns

WC = covered management; NC= non-covered management; Si = siliceous mineralogy; Ca = carbonated mineralogy. *, **, *** and ns = significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$ and no significant differences, respectively.

Table 3. Mean percentage values and standard deviation (in brackets) of soil minerals of the Study 2: laminar silicates, quartz, goethite, hematite, chlorite, potassium feldspar, sodium feldspar, calcite and dolomite. Bellow, the results of the ANOVA. Notes that natural vegetation (NV) is not included in the ANOVA.

Mineralogy	Management	Laminar S.	Quartz	Goethite	Hematite	Chlorite	K Feldspar	Na Feldspar	Calcite	Dolomite
Siliceous	NV	30.7 (7.2)	61.3 (9.1)	3.4 (1.8)	0.39 (0.53)	1.6 (1.3)	1.2 (0.9)	1.4 (0.6)	0.0 (0.1)	0.0 (0.0)
	WC	39.7 (18.4)	51.9 (20.1)	3.1 (1.5)	0.26 (0.43)	1.8 (1.1)	1.5 (0.8)	1.9 (1.6)	0.0 (0.0)	0.0 (0.0)
	NC	31.6 (7.2)	61.5 (7.1)	2.7 (0.7)	0.00 (0.00)	1.8 (0.6)	1.0 (0.3)	1.3 (0.5)	0.0 (0.1)	0.0 (0.0)
Carbonated	NV	60.9 (5.4)	23.8 (4.0)	3.3 (1.2)	1.43 (0.80)	2.4 (0.8)	2.0 (0.6)	3.0 (0.6)	2.3 (1.1)	0.9 (0.6)
	WC	46.7 (11.9)	14.8 (2.9)	2.2 (1.3)	0.08 (0.26)	3.2 (2.8)	0.9 (0.6)	1.0 (1.0)	30.4 (11.6)	0.7 (0.4)
	NC	34.5 (8.8)	9.7 (4.0)	1.4 (1.0)	0.00 (0.00)	2.0 (0.6)	0.7 (0.6)	0.7 (0.8)	50.2 (11.5)	0.9 (0.6)
	Management	* WC>NC	ns	* WC>NC	* WC>NC	ns	ns	ns	ns	ns
	Mineralogy	ns	*** Si>Ca	* Si>Ca	ns	ns	ns	* Si>Ca	*** Ca>Si	*** Ca>Si
	Man * Min	ns	ns	ns	ns	ns	ns	ns	**	ns

WC = covered management; NC= non-covered management; Si = siliceous mineralogy; Ca = carbonated mineralogy. *, **, *** and ns = significant differences at P < 0.05, P < 0.01 and P < 0.001 and no significant differences, respectively.

Table 4. Depth (cm), sand (%), silt (%), clay (%), soil organic carbon (%), N (%), C/N, Olsen Phosphorous (g Kg^{-1}), pH, CEC (meq $100g^{-1}$), Potassium (g Kg^{-1}) and texture of the soils under the weed-cover and non-cover managements of the Study 3. These results corresponds to soil properties at the beginning of the experiment (year 0, 2014). Values in brackets correspond to the standard deviation.

Management	Depth	Sand	Silt	Clay	SOC	N	C/N	Olsen P	pH	CEC	K	Texture
Weed-covered	0 - 5	15 (2)	30 (3)	55 (3)	2.06 (0.59)	0.32 (0.13)	4.63 (1.86)	23.3 (12.2)	8.25 (0.09)	33.7 (2.5)	779 (190)	Clayey
	5 - 15	13 (4)	32 (2)	55 (5)	1.69 (0.26)	0.30 (0.06)	4.97 (2.96)	20.0 (8.9)	8.26 (0.11)	34.0 (1.7)	671 (188)	Clayey
Non-covered	0 - 5	33 (5)	36 (2)	32 (3)	1.30 (0.64)	0.14 (0.06)	9.93 (5.10)	19.7 (7.0)	8.44 (0.21)	22.0 (1.7)	312 (137)	Clay loam
	5 - 15	33 (1)	32 (2)	34 (1)	1.1 (0.18)	0.13 (0.08)	8.00 (4.37)	14.0 (4.4)	8.55 (0.10)	21.7 (1.5)	209 (48)	Clay loam

Table 5. Weed production (t ha^{-1}), organic carbon content of aerial biomass (%) and annual inputs organic C to the soil through weeds (t ha^{-1}) of the Study 3. Values in brackets correspond to the standard deviation.

Year	Aerial biomass production	Organic C content (%)	organic C in weeds rsidues
2015	2.20 (0.58)	36.1 (1.30)	0.79 (0.21)
2016	2.44 (0.92)	30.3 (3.34)	0.74 (0.28)

4.2 Is total SOC content linked to biomass production?

In the Study 1, when assessed 10 weed-covered plots (5 of them contrasted with a nearby non-covered plot), it was not found a significant relationship between weed biomass production and SOC content at the two considered depths (data not shown) – although significant differences were found between managements –. Many long-term agroecosystem field experiments, in which treatments consisted of applying different levels of C inputs, show soil C stocks that appear related, linearly or following a saturation behaviour, to the average amount of C returned to the system (e.g. Kong *et al*., 2005; Paustian *et al*. 1997; Stewart *et al*., 2007). Our result was not unexpected since weed-organic carbon production was measured in a single year and the pool of soil organic carbon is the result of the accumulated balance between organic carbon inputs and decomposition during many years.

Moreover, the relationship between levels of C inputs and soil C stock observed by the above researchers is only clear for a wide range of C inputs (i.e. from 1 t C to more than 5 t C) and in our results the highest carbon production was about 1 t C. In addition, annual weed biomass production of olive oil farming shows a high inter annual variability, mainly driven by annual precipitation which is highly variable in Mediterranean regions, with a inter annual coefficient of variation reference reaching values of between 30 and 35% in the Andalusia Region (Martín-Vide, 1996). Thus, Castro *et al*. (2008) showed that annual aboveground weed biomass varied one order of magnitude in 3 years, whereas Guzman and Foraster (2011) recorded a 4-fold variation for the same management and olive oil farming. Finally, C decomposition rate might differ among sites, resulting in different soil organic carbon stocks for a similar level of weed biomass C input. For instance, SOC in CT soils was the highest but aboveground weed carbon production of this site was the lowest.

Thus, these results show that long-term studies are needed in order to assess the influence of the biomass production on total SOC content. Single values of aboveground biomass production in Mediterranean conditions are not enough to study this relationship.

4.3 Olive orchard soils under weed-cover management had higher SOC than non-covered soils

Despite we did not find a relationship between the biomass production and the total SOC content the results of the Study 1 showed that a weed-cover management increases significantly the total SOC content (2.75 times in the 0 – 5 cm layer and 1.96 times in the 5 – 15 cm layer). Therefore, the result is that the upper layer had 1.7 times higher SOC content than the 5 – 15 cm soil layer (Table 6). According to the results of the stock[4] of SOC in the first 15 cm of soil, with the weed-cover management was found a SOC sequestration of about 16 t C ha^{-1} in the first 15 cm of soil. Considering that these plots allowed the growth of a vegetation cover as a maximum of last ten years, the C sequestration rate would be about 1.6 t C ha^{-1} yr^{-1}. This value is higher than the average value found of 1 t C ha^{-1} yr^{-1} in olive grove soils under weed-covered management (Chapter III).

On the other hand, according to the results shown in Table 6 the influence of the biomass production among the depth is clear. In the weed-cover management there is a significant higher total SOC content in the first 5 cm than in the 5 – 15 cm soil layer, whereas in the non-cover management there were not significant differences in the total SOC content between depths.

Table 6. Total soil organic carbon content (mg C g^{-1} soil) and stock (t C ha^{-1}) of soils the study 1 in the non-covered (NC) and weed-covered (WC) managements (standard deviation in brackets) for the two sampled depths: 0 – 5 and 5 – 15 cm. The stocks are shown for the whole depth of 15 cm.

Depth	SOC (mg C g^{-1} soil)		Depth	SOC (t C ha^{-1})	
	NC	WC		NC	WC
0 - 5	12,3 (3,2) Ba	33,9 (8,9) Aa	0 – 15	13.7 (1.3) B	29.8 (6.3) A
5 - 15	10,1 (3,1) Ba	19,8 (5,7) Ab			

Different first letter means significant differences between managements and second letter means significant differences between depths for the same management ($p < 0.05$).

[4] SOC stocks have been calculated according to the equation 4 showed in Chapter III (3.3 section).

Thus, it is possible to calculate the stratification ratio of the SOC, which means the ratio between the SOC content in the upper layer to that of the deeper layer. In this case, for the non-covered soils the stratification ratio was 1.22, whereas for the weed-covered soil it was 1.71. These values were similar to those found by Franzluebbers (2002) in soils of Georgia, Texas and Alberta/British Columbia in crops for the conventional management (1.1, 1.2 and 1.9, respectively) and lower to those found for the non-tillage management (3.4, 2.0 and 2.1).

This results are also in line with Castro *et al*. (2008), who found that olive grove soils under weed-cover management showed much higher stratification ratios than the non-covered after 28 years of treatment. Similar results were found by other authors in rotations in Mediterranean conditions. Hernanz *et al*. (2002) found stratification ratios of about 1.5 for the zero tillage, 1.1 for the minimum tillage and 1.0 for the conventional tillage in depths of 0 – 5 and 10 – 20 cm, respectively. A similar trend was found by Álvaro-Fuentes *et al*., 2008 in Mediterranean semiarid conditions when comparing no-tillage, reduced tillage, subsoil tillage and conventional tillage.

The stratification ratio is very important, since it provides information about soil quality. Franzluebbers (2002) highlighted that total SOC content does not provide information about soil degradation, as two soils can have the same total SOC content but one of them could be much more degraded than the other one. For that reason, in that study it was hypothesized that the degree of stratification of SOC with soil depth could indicate soil quality or soil ecosystem functioning, since the OM content of the upper layer plays a key role in the erosion control, water infiltration and conservation of nutrients. Therefore, it is possible to conclude according to these data that the non-covered soils would be more degraded than the soils under the weed-cover management.

In the case of the Study 2, assessing the SOC also in contrasted parent material soils, figure 10 shows clearly significant differences between the total SOC content in the non-covered and weed-covered managements (average values of 11.7 and 23.4 mg C g^{-1} soil, respectively) (i.e. 24.0 and 43.3 t C ha^{-1} as SOC stocks[5], respectively), being two times higher in the weed-covered management. This difference is in line with those obtained in the Study 1 for the two depths. The SOC sequestration in weed-covered plots in the first 15 cm would be about 19 t C ha^{-1}, which is similar than the 16 t C ha^{-1} found in the Study 1.

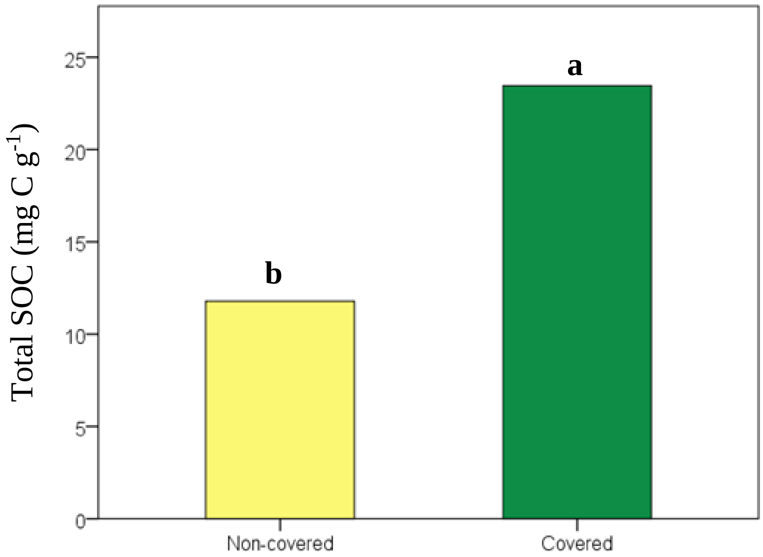


Figure 10. Total soil organic carbon content (mg C g^{-1} soil) in the Study 2 in the non-covered and weed-covered plots. Different letters means significant differences ($p < 0.05$).

Therefore, results of the studies 1 and 2 highlight the importance of allowing the growth of a vegetation cover in the inter-row area on the total SOC content. Thus, with a vegetation cover the total SOC content increases by at least 2 times in the first 15 cm of soil in comparison with the non-covered management, and this differences are higher in the upper layer. However, single values of biomass production should not be used as a predictor of the SOC level due to the high variability of the biomass production in Mediterranean climates

[5] SOC stocks have been calculated according to the equation 4 showed in Chapter III (3.3 section). In this case, the bulk density has been estimated according to the equation 5 showed in Chapter III (3.3 section).

Nevertheless, as it was mentioned before, other variables, such the edaphic features (mineralogical and geochemical properties) might also affect biomass production and, thus, total SOC content. So, it is necessary to focus on the relationship between the total SOC content and some edaphic features.

The Study 2 assessed the influence of two contrasted parent material sites (acid vs basic) on total SOC. As it was shown above, in these plots the total SOC content was approximately two times in the covered than in the non-covered plots. However, regarding the figure 11 significant differences were found also for the comparison between siliceous and carbonated soils (12.4 and 23.1 mg C g^{-1} soil, respectively) (i.e. 25.2 and 42.8 t C ha^{-1} of SOC stocks[6], respectively). These results show that olive groves under carbonated soils accumulated almost two times more total SOC content in the first 15 cm of soil, representing about 18 t C ha^{-1} higher, than the siliceous soils. This might be due to a higher biomass production in the carbonated soils due to its better physicochemical features in comparison to the siliceous soils. Nevertheless, this behavior is intimately related to the SOC fractions dynamics, so the discussion of these results will take place in Chapter VI.

[6] SOC stocks have been calculated according to the equation 4 showed in Chapter III (3.3 section). In this case, the bulk density has been estimated according to the equation 5 showed in Chapter III (3.3 section).

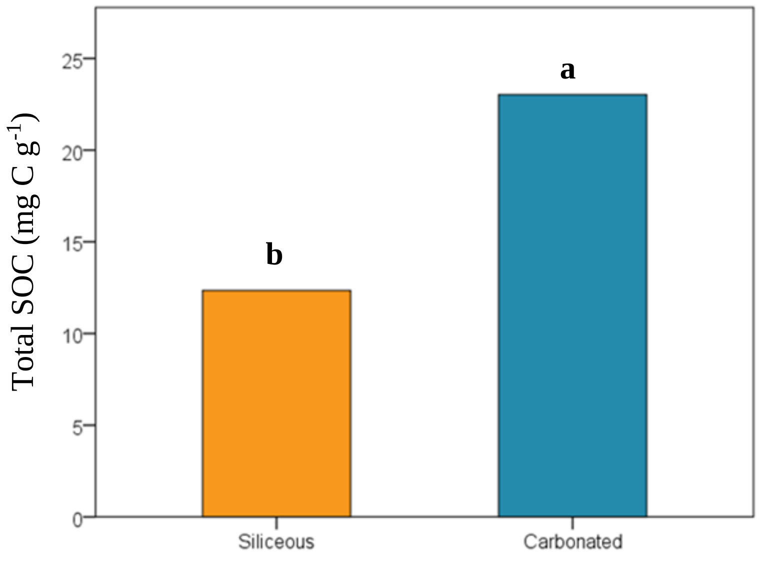


Figure 11. Total soil organic carbon content (mg C g^{-1} soil) in the study 2 in the siliceous and carbonated plots. Different letters means significant differences ($p < 0.05$).

5. Conclusions

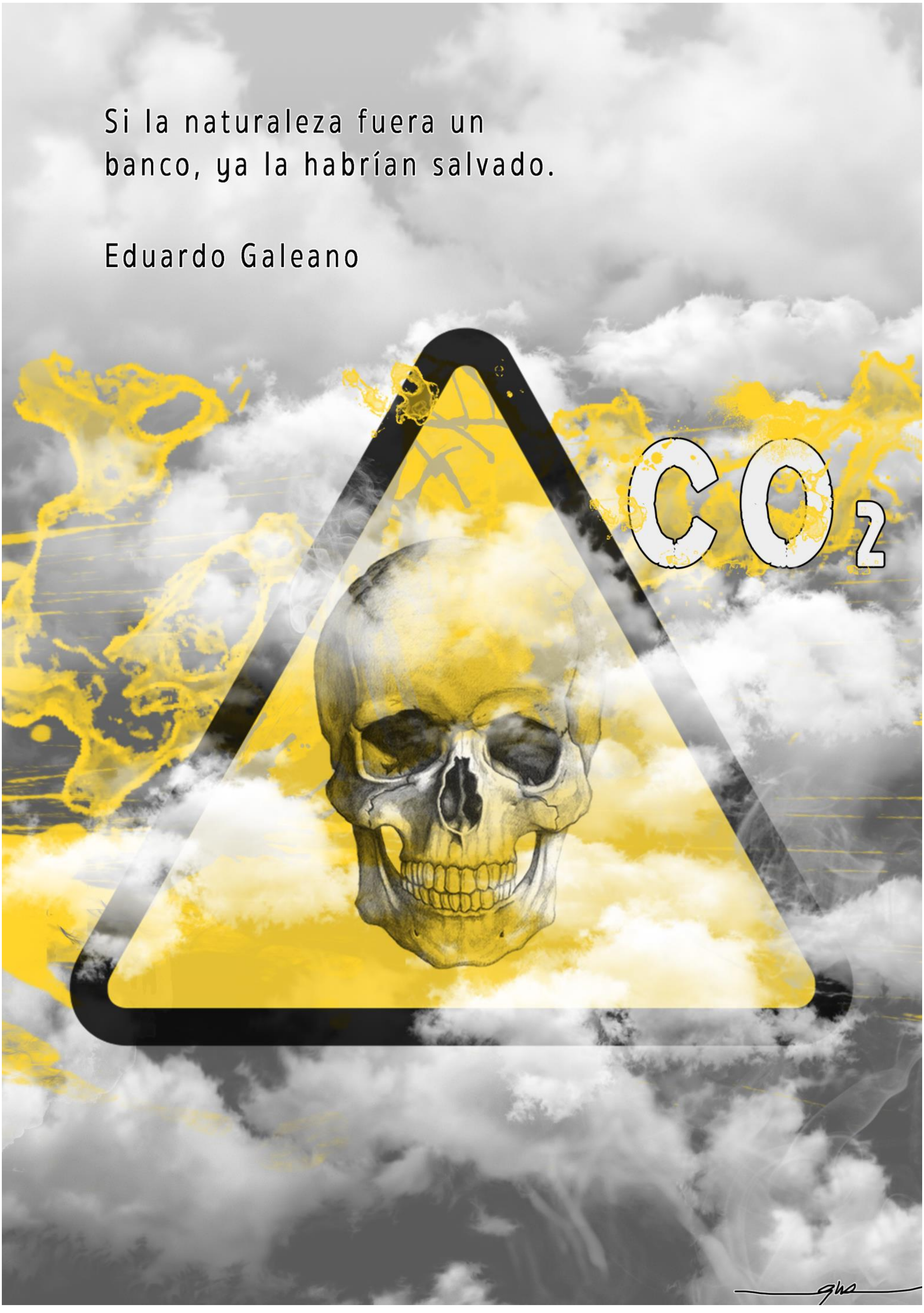
Considering the results showed in this chapter is possible to conclude the following:

- **Annual organic carbon input due to residues of the vegetation cover** ranged 0.24 and 1 t C ha^{-1} yr^{-1}. These are in the lower range of annual organic carbon input due to the application of crop residues

- Soils of olive orchard with a **weed-cover had higher SOC** at the **top 5 cm** and at the **5 – 15 cm** soil layer than comparable olive orchard with bare soils.

- **Soil organic carbon stratification ratio was higher under a weed-cover management**. These higher stratification ratios indicate better soil quality/fertility being more resistant to soil erosion and other degradation processes.

- **Soil parent material affected total SOC content**, as under the same management, SOC in siliceous soils was half of that of the carbonated soils.


Si la naturaleza fuera un
banco, ya la habrían salvado.
Eduardo Galeano
CO_2

CHAPTER V

CO_2 emissions and organic C inputs in olive grove soils in laboratory and field conditions

1. Introduction

As it has been shown before, the presence of a spontaneous resident vegetation cover is a management practice which provides several environmental benefits, such as the reduction of soil erosion and soil disturbance, and increase the amount of C added to soil thus increasing SOC as well as soil fertility. As interest increases in both promoting organic C storage and alternative uses of cover crops or spontaneous vegetation cover residues, it is crucial to understand the relative stabilization efficiency of added residue C as well as its stability in the soil.

In some long-term agroecosystems having treatments with varying C addition levels do not show an increase of the equilibrium SOC stocks at higher C addition levels (Paustian *et al*., 1997; Huggins *et al*., 1998; Reicosky *et al*., 2002), suggesting that soil C content becomes saturated with respect to C inputs at equilibrium (Six *et al*., 2002; Stewart *et al*., 2007). However, multiple C input level treatments at some long-term agroecosystem experiments produced no increase in SOC stocks at equilibrium for straw, residue or stover retention (Soon, 1998; Huggins *et al*., 1998; Reicosky *et al*., 2002, respectively) suggesting soil C content becomes saturated respect to C inputs. Limits to SOC storage have been proposed for specific C pools including the silt+clay-protected C pool (Hassink, 1996, 1997; Six *et al*., 2000), the soil aggregate-protected C pool (Jastrow, 1996; Paustian *et al*., 2000; or Six *et al*., 1998, 1999, 2000), the biochemically protected C pool (Cadish and Giller, 1997; Baldock and Skjemstad, 2000) and non-protected C (Six *et al*., 2000). Therefore, soil properties such as texture, mineralogy and cation availability determine the C saturation level of the silt+clay and physically protected C pools, while the biochemically protected C pool is constrained by the type of C and the non-protected C pool by the dynamic balance between the decomposition rate and C input levels. According to these premises the maximum C protective capacity in soil is determined using the relationship between soil texture and mineral C content develop by Hassink *et al*. (1997) and Six *et al*. (2002). The difference between a soil's theoretical saturation level and the current C content of the soil is defined as saturation deficit (Stewart *et al*., 2007). Therefore, after organic matter application soils with larger C saturation deficit (e.g. agriculture soil) are expected to be able to stabilize more C than soils with smaller C saturation deficit (e.g. forest soil) and, thus, losing lower amounts of C-CO_2. Data from long-term agroecosystem experiments suggested a decrease of soil fraction stabilization efficiency at high C contents (as a proxy for C input levels), even in soils that do not show decreased stabilization in the whole soil (Stewart *et al.*, 2007). However, in these studies, C saturation has been evaluated using experiments investigating effects of management (i.e., tillage, fertilization, and crop rotation) on yield or soil C across sites differing in climate, soil texture, mineralogy and decomposition kinetics (Stewart *et al.*, 2007, 2008b).

The hypothesis is that the proportion of stabilized residue-derived C would be greater in soils with a larger saturation deficit compared to those with smaller C saturation deficit and the relative stabilization efficiency of added C would be higher for the low C input level compared to the high C input level. In addition, lower stabilized residue-derived C would be expected when residue is of low quality (e.g. high recalcitrance level) compared to high quality organic carbon.

On the other hand, CO_2 emissions from soil are only a small part of the CO_2 balance of an agroecosystem. Calculating the CO_2 emissions balance of an olive grove with a conventional and a weed-cover management would be a suitable tool in terms of SOC sequestration assessment. Therefore, in order to figure out this balance it is necessary to measure soil CO_2 respiration on site, in field conditions, considering also climate and daily conditions (rain, temperature, daylight duration…). Thus, these CO_2 soil emissions measures could be used in the C-balance of the whole agroecosystem.

2. Objectives

Objectives are as follow (figure 1 shows a scheme):

- Assess the influence of the presence of a **resident vegetation cover and environmental conditions (temperature and rainfall) on CO_2 emissions in field conditions**.
- Evaluate the role of **organic carbon quality** on CO_2 emissions and organic carbon retention in laboratory conditions.

- Assess, in laboratory conditions, the influence of the application of **different doses of the two types of organic inputs** (biomass residues and olive mill pomace) of **contrasting quality** on CO_2 emissions and organic carbon retention.
- Evaluate, in laboratory conditions, the influence of **SOC saturation deficit** on CO_2 emissions and organic carbon retention.

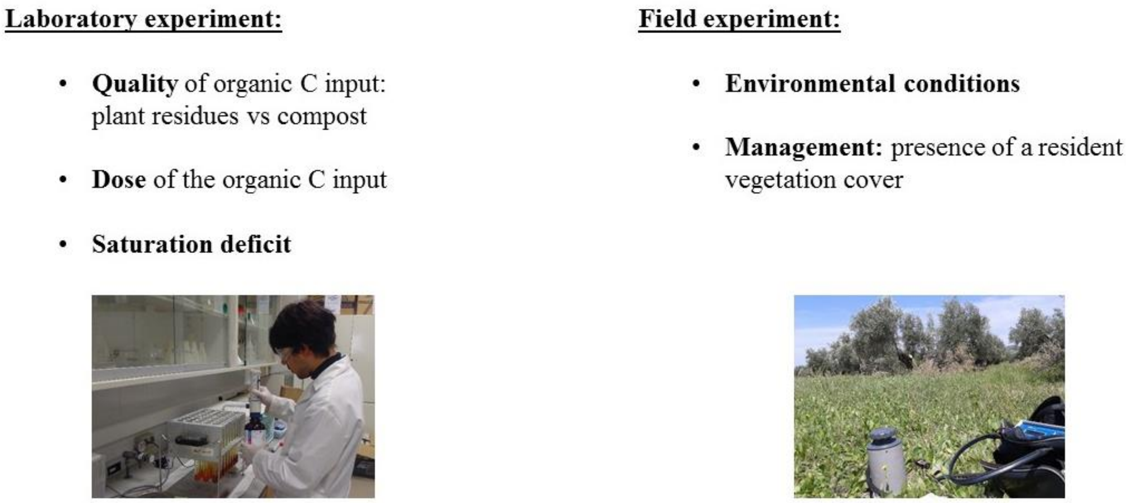


Figure 1. Schematic representation of the objectives of Chapter V. Words in bold correspond to the variables affecting CO_2 emissions studied in the two types of experiments carried out (laboratory and field).

3. Material and Methods

3.1 Experimental design

To development the following objectives;

- Evaluate the role of organic C quality on CO_2 emissions and organic C retention in laboratory conditions.
- Assess, in laboratory conditions, the influence of the application of **different doses of the two types of organic input**s of contrasting quality on CO_2 emissions and organic carbon retention.

- Evaluate, in laboratory conditions, the influence of **SOC saturation deficit** on CO_2 emissions and organic carbon retention.

a lab experiment with the following rationale was performed.

Rationality of the lab experiment

Soils with differing C saturation deficits are required to examine the effect of soil C saturation deficit on the stabilization of added C residue. Field-level experiments are not appropriate to directly test C saturation deficit effect due to confounding variation between SOC content and climatic factors that could alter C input as well as decomposition. Therefore, laboratory incubations were used to directly test the effect of saturation deficit, C input level and quality of the organic carbon source on C stabilization where residue addition, soil characteristics and decomposition factors were controlled.

On the other hand, the field experiment was carried out in order to develop the following objective:

Assessment of the influence of the presence of a **resident vegetation cover and environmental conditions (temperature and rainfall) on CO_2 emissions in field conditions**.

The experimental design is that described in Chapter IV (Study 3). Briefly, in 2014, one plot of about 1 ha, initially with a weed-cover management, was changed to a non-cover management. Another nearby plot mantaining the weed cover was selected for the study. From March 2015 to August 2016 monthly CO_2 respiration measures were done in each plot. For that purpose, 10 different cores were settled in the inter row area of each of the plots differing in the presence of vegetation cover. Soil CO_2 emissions were measured with PPsystem without disturbance (figure 2).

Figure 2. Images of the soil cores in the two managements: bare (left) and weed-covered (right) in Castillo de Canena property (Jaén).

3.2 Main sources of variation

Laboratory experiment

Soils of contrasted physicochemical properties, total SOC content and saturation deficit from a forest ecosystem and from four olive orchards were selected and collected (see sampling method in 1.2 section in Chapter II). These soils consisted of a soil with high SOC (forest soil; Mag, hereafter), three soils of olive groves (CT, Dei and Peg) with medium level of SOC and one soil with low SOC collected from an olive grove (Mor, hereafter). To obtain soils with similar maximum capacity for C sequestration but with different saturation deficit, soil samples from two different depths (0 – 5 and 5 – 15 cm) were collected at three of the selected soils (CT, Dei and Peg).

Therefore the levels of this source of variation were 8 (e.g. 8 soil types). Table 1 shows the main soil physico-chemical and biological properties of the soils.

The highest Silt+Clay contents for the 0 – 5 cm soils were found from Peg and Mar with 76.3 and 73.0%, respectively, being lower in the soil from CT, Dei and Mor ranging 56.3 to 60.6 %. The Silt+Clay content increased significantly with the depth in the soil from CT and Peg, whereas this was not observed in that from Dei. As expected, soil organic nitrogen (SON) content on 0 – 5 cm soil was significantly higher in Mag (forest soil) with

0.54%, whereas olive grove soils at CT and Dei showed similar SON content with 0.31 and 0.30%, respectively, being these values higher than those found at Peg. Finally, the lowest SON content was found in Mor (conventional olive groves) with 0.07 %. In all cases the SON content decreased in soil with the depth except in Peg soil. Similar results were found for OM content, where the highest values from 0 – 5 cm were obtained in Mag (data not shown), followed by CT and Dei (6.42 and 6.21 %, respectively), the OM content of Peg soil was significantly lower with 3.34% and the lowest values were observed in Mor soil (1.14%). As for the SON, the OM content decreased among depth, being lower in the 5 – 15 cm range in CT and Dei, whereas at Peg both values were similar. SOC content at 0 – 5 cm was significantly higher for Mag (74.0 mg g^{-1}), followed by CT with 44.8 mg g^{-1}, Dei with 34.5 mg g^{-1}, whereas the lowest content in SOC were found to Peg and Mor soils with 21.8 and 13.3 mg g^{-1}, respectively. For the three soil tested the SOC was significantly higher in the 0 – 5 cm compared to 5 – 15 cm. In general, the SOC/SON ratio in soil from 0 – 5 cm ranged between 11.3 to 20.5, being the lowest values those of Dei and the highest of Mor and increasing among depth in all cases. Due to the high Silt+Clay content in Peg and Mag for soils from 0 – 5 cm, these sites showed the high protective capacity to store C with 174 and 168 mg g^{-1}, respectively. Mor showed intermediate levels with 142.0 mg g^{-1} and the lowest protective capacity to store C was obtained to CT and Dei with 139.2 and 132.9 mg g^{-1}, respectively. In CT and Peg the protective capacity was higher in deeper soils, but these differences were not found at Dei. The saturation deficit for soils from 0 – 5 cm resulted in the following classification: Mor > Peg > Dei > CT > Mag, in all cases tested the saturation deficit increased in soil from 5 – 15 cm, except in Dei soil.

Table 1. Main properties of the soils of CT, Dei, Peg, Mor and Mag sites. Note that at CT, Dei and Peg soils were also sampled at 5 – 15 cm, whereas at Mor and Mag plots were only sampled at 0 – 5 cm. Data are the mean and standard deviations are shown within brackets. Different letters shows significant differences between sites, managements and depths for the same variable ($p < 0.05$).

	CT		**Dei**		**Peg**		**Mor**	**Mag**
	0 - 5	**5 - 15**	**0 - 5**	**5 - 15**	**0 - 5**	**5 - 15**	**0 - 5**	**0 - 5**
Sand (%)	40.8 (4.6)[af]	33.8 (1.8)[b]	43.8 (1.8)[ac]	46.3 (1.2)[c]	23.8 (5.3)[d]	19.6 (0.6)[e]	39.4 (1.7)[f]	27.0 (2.2)[d]
Silt + Clay (%)	59.3 (4.6)[af]	66.3 (1.8)[b]	56.3 (1.8)[ac]	53.8 (1.2)[c]	76.3 (5.3)[d]	80.4 (0.6)[e]	60.6 (1.7)[f]	73.0 (2.2)[d]
Total N (%)	0.30 (0.01)[a]	0.13 (0.01)[bd]	0.31 (0.01)[a]	0.24 (0.11)[ac]	0.19 (0.02)[bc]	0.14 (0.01)[bd]	0.07 (0.01)[d]	0.54 (0.12)[e]
OM (%)	6.42 (0.83)[a]	3.00 (0.25)[b]	6.21 (0.21)[a]	4.79 (2.51)[c]	3.34 (0.35)[b]	2.56 (0.44)[b]	1.14 (0.18)[d]	-
SOC (%)	44.8 (2.5)[a]	27.1 (2.8)[bc]	34.5 (2.6)[ab]	34.3 (3.7)[ab]	21.8 (0.7)[bc]	17.3 (0.7)[c]	13.3 (1.5)[c]	74.0 (25.3)[d]
SOC/SON ratio	15.2 (0.88)[ac]	21.7 (2.18)[b]	11.3 (0.91)[a]	15.5 (4.72)[ac]	11.8 (1.07)[a]	12.4 (0.37)[a]	20.5 (2.25)[bc]	14.9 (6.52)[a]
WHC (%)	1.24 (0.09)[a]	0.67 (0.05)[bc]	1.16 (0.34)[a]	0.86 (0.26)[b]	0.89 (0.09)[b]	0.69 (0.06)[b]	0.44 (0.02)[c]	-
Maximum protective capacity (mg C g^{-1})	139.2 (6.8)[af]	153.9 (2.6)[b]	132.9 (2.6)[ac]	127.6 (1.8)[c]	174.9 (7.9)[d]	183.6 (0.8)[e]	142.0 (2.5)[f]	168.1 (3.2)[d]
Saturation Deficit (mg C g^{-1})	0.68 (0.02)[a]	0.82 (0.02)[bc]	0.74 (0.02)[ab]	0.73 (0.03)[ab]	0.88 (0.01)[c]	0.91 (0.00)[c]	0.91 (0.01)[c]	0.56 (0.15)[d]

C saturation deficit calculations of the soils were based on SOC content of the soil and SOC content of these soils at maximum organic carbon protective capacity (g C Kg^{-1} soil) which was calculated according to the empiric model of Six *et al*. (2002):

$$\text{Maximum organic carbon protective capacity} = 0.21 \times (\text{silt} + \text{clay}\ \%) + 14.76$$

Organic C saturation deficit (Stewart *et al*., 2007) was calculated as:

$$\text{C saturation deficit} = 1 - \left(\text{SOC content} \middle/ \begin{matrix}\text{Maximum organic carbon} \\ \text{protective capacity}\end{matrix} \right)$$

<u>Organic carbon quality and doses</u>

Two sources of organic carbon of contrasting quality were assayed to evaluate the effects of the quality and doses of organic C in soils with different properties and saturation deficit: weed-cover residues (PR) and composted olive mill pomace (COMP). Table 2 shows carbon, nitrogen and C-to-N ratio of the plant residues and composted olive mill pomace.

Table 2. N (%) and C (%) contents, and C/N ratio of plant residues (PR) and composted olive mill pomace (COMP). Standard deviation is shown in brackets. Different letters mean significant differences (P < 0.05) after one-way ANOVA.

	N	**C**	**C/N**
PR	1.86[a] (0.05)	40.5[a] (0.12)	21.8[a] (0.58)
COMP	2.72[b] (0.04)	32.5[b] (0.73)	11.9[b] (0.23)

On the other hand, the effects of the magnitude of the C input on CO_2 emissions and organic carbon retention were assayed at four PR and COMP C doses (2, 4, 8 and 12 mg C g^{-1}) (i.e. 3.4, 6.3, 11.5 and 16.4 t C ha^{-1}) in Mag and Mor soils, whereas for CT, Dei and Peg only two doses were assayed (4 and 12 mg C g^{-1}) (i.e. 6.3 and 16.4 t C ha^{-1}).

Field experiment

As it was previously shown in the figure 1, the sources of variation of the field experiment are two: the management (covered vs non-covered soils) and the environmental conditions, which vary over time in temperature and precipitations.

3.3 Dependent variables

CO_2 emissions, cumulative CO_2 emissions, and decomposition rate and the pools of labile and recalcitrant organic C were measured. In the case of the field study, only direct CO_2 emissions from soil were measured.

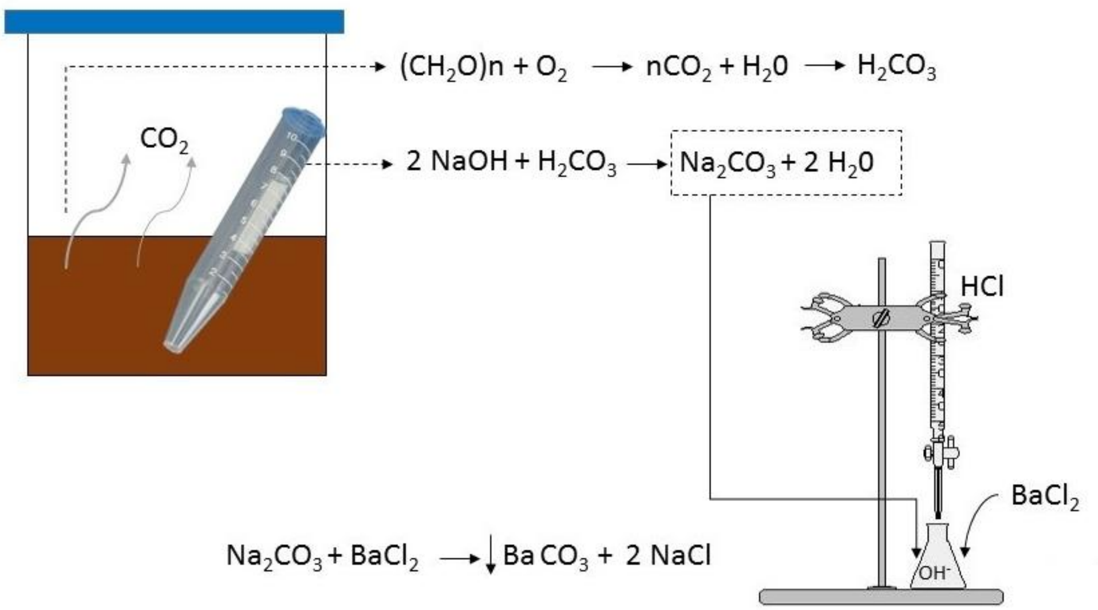


Figure 3. Schematic respresentation of the alkali traps method (Anderson, 1982).

CO_2 emissions in lab were determined using the alkali traps method (Anderson, 1982) (figure 3). A small vial containing 10 ml of NaOH was introduced into each recipient containing the soil samples with or without (control) amendments and were hermetically closed. The miliequivalent of OH^- in the vials were those according to the expected respiration rate (which was dependent on the incubation period, soil and source and doses of organic carbon) and typically ranged from 5 to 20. At different intervals, depending on the expected respiration rate, vials with the NaOH were taken and Cl_2Ba was added to precipitate the fixed C as $BaCO_3$. The unused OH^- miliequivalents were quantified by titration with a known normality of HCl. A scheme of the process is shown in the figure 3.

Procedure of soil incubation in laboratory conditions

Pre-incubated (at 60% of WHC during a few days 3 – 7 day) triplicate 100 g dry soil equivalent of the different soils were incubated in a 500 ml flask with an equivalent amount of different doses of organic carbon: 2, 4, 8 or 12 mg C g^{-1} of PR or COMP. Un-amended soil of each type of soil was incubated as control. Distilled water was added to reach 60 % of the WHC for each soils and mix thoroughly. Incubation was performed at 25 ºC and darkness. After 4, 7, 13, 20, 29, 40, 49, 69, 110, 140, 187, 253 and 313 days, CO_2 emissions were evaluated. Figure 4 shows a schematic representation of the experiment.

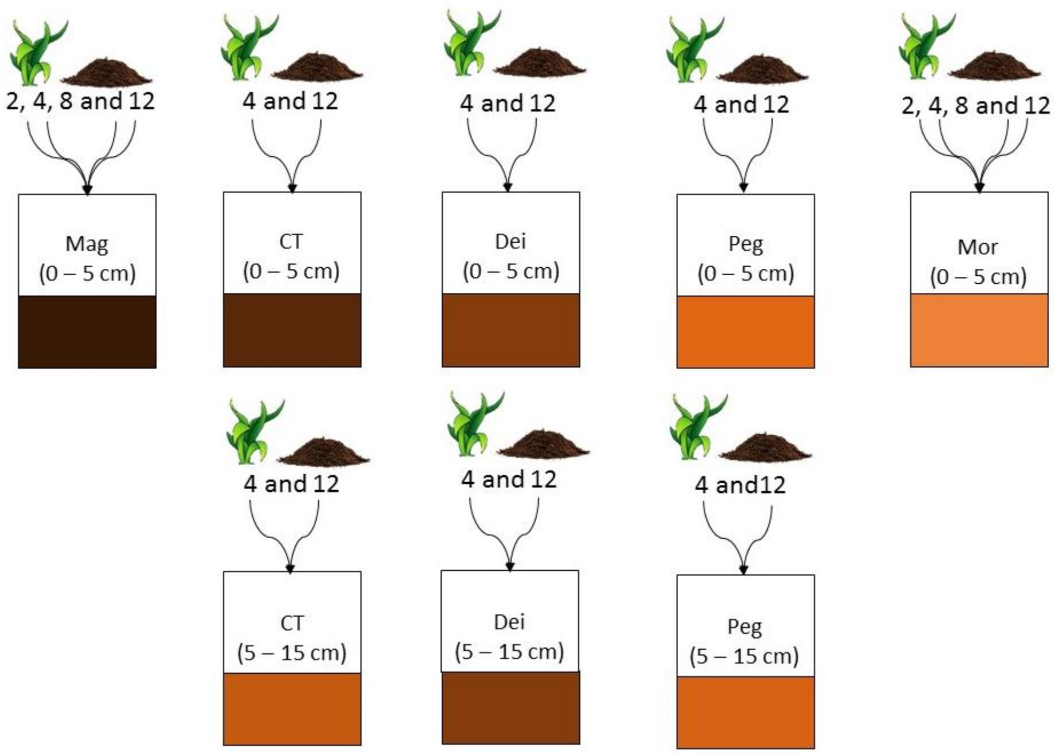


Figure 4. Scheme of the incubation experiment with different soils (different colours show different SOC levels), depths and doses (mg C g^{-1}) of the plant residues and compost.

Methodology of the soil respiration measurement (field conditions)

For the respiration measurement a PPsystem (EGM-3 Environmental Gas Monitor) was used. The measurement consisted on inserting the instrument on the core previously (months) buried into the soil (figure 5). Although there were 10 soil cores in each of the treatment, soil respiration was usually measured only in 5 randomly selected cores. Three measurements for each soil core were done and the time of each measurement was 120 seconds as a maximum (in high soil respiration periods of time the measurement usually took less than 120 seconds). Therefore, 15 measurements were done for each treatment. CO_2 measurements were performed between 11:00 am to 14:00 pm. At the end of the day, data were transferred from the instrument to the PC. After that, it was necessary to carry out some transformations of the results to correct the measurements and then, calculate the respiration rate.

Figure 5. Images of the soil respiration measurements with PPSystem in Castillo de Canena property (Jaén).

Corrections of the soil respiration rates (field conditions)

The instrument measures the amount of the CO_2 per volume unit (density of CO_2) of the soil respiration chamber, whereas the mix of CO_2 (mass of CO_2 per mass of dry air) must be used for the calculation of the respiration rate, since it is the only variable that does not change among changes in the air density of the chamber (due to changes in the temperature, pressure or water vapour content), and it only varies because of variations of the CO_2 emissions from the soil.

The instrument gives the values in terms of mole fraction of CO_2 (moles of CO_2 to moles of humid air; ppm) at reference temperature and pressure (273.15K and 100000Pa, respectively). Thus, for the estimation of the mole fraction of CO_2 at real temperature and pressure (correction by temperature and pressure), it is necessary to recalculate the density of CO_2 using the Ideal Gas law:

$$\rho_c = \frac{n_c \omega_c P_{ref}}{T_{ref} R} \quad (1)$$

Where n_c is the molecular weight of CO_2 (0.044 Kg mol^{-1}), ω_c is the mole fraction of CO_2 at reference temperature and pressure (T_{ref} = 273.15 K and P_{ref} = 100000 Pa) and R is the ideal gas constant (8.314 m^3 Pa K^{-1} mol^{-1}).

To calculate the mole fraction of CO_2 at real pressure (P) and temperature (T) of the measurement the following equation was applied:

$$\omega_{c(T.P)} = \frac{\rho_c RT}{n_c P} \quad (2)$$

These two steps can be simplify by substituting ρ_c of the equation 2 by that of the equation 1.

$$\omega_{c(T.P)} = \omega_c \frac{P_{ref} T}{P T_{ref}} \quad (3)$$

By definition, the mole fraction of CO_2 is referred to the humid air and. Thus, this variable is highly dependent on air humidity. Therefore, this mole fraction must be referred to dry air (mixing ratio) following equation 4.

$$C = \frac{\omega_{c(T.P)}}{1-\omega_v} \quad (4)$$

Where ω_v is the mole fraction of the water vapour (mol mol^{-1}) which is not directly measured by the instrument but it can be calculated from the Dalton's law of partial pressures, knowing the partial pressure of the water vapour (e) and the total pressure (P), which were measured.

$$\omega_v = \frac{e}{P} \quad (5)$$

Soil respiration calculation

The soil respiration flux per area unit was calculated from the increase of mixing ratio of CO_2 (C) in the chamber during the time of measurement (respiration rate). During a short period of time at a low density of CO_2 it is possible to assume that the respiration rate is constant among time. Nevertheless, if the time of the measurement is longer or the CO_2 emissions from the soil are very high, some problems of air leaks or saturation of CO_2 into the chamber may appear. These situations may lead to an apparent decrease in the respiration rate along the measurement period.

To avoid this problem, the increase of the mixing ratio of CO_2 in the chamber (C = y) thought time (T = x) is adjusted to a quadratic relationship ($C = a + bT + cT^2$) and, thus, the soil respiration rate is calculated as $\left(\frac{dC}{dT}\right)_{T=0} = b$

To calculate the CO_2 respiration flux to area unit (Fc_{soil}; µmol (CO_2) m^{-2} s^{-1}), the Ideal Gas law must be applied referring the result to the area and volume of the chamber:

$$Fc_{soil} = b * \frac{V}{S} * \frac{P_0(1 - \omega_{v0})}{RT_0}$$

where b is the slope of the quadratic relationship ($\left(\frac{dC}{dT}\right)_{T=0} = b$) in ppm s^{-1}, V is the volume of the system (m^3), S is the area (m^2), P_0 is the initial atmospheric pressure (Pa), ω_{v0} is the initial mole fraction of the water vapour (mol mol^{-1}), R is the ideal gas constant (8.314 m3 Pa K^{-1} mol^{-1}) and T_0 is the initial air temperature (K).

3.4 Statistical analysis

In the laboratory study, differences in some physicochemical properties of soil were tested using a one–way ANOVA. Differences between CO_2 respiration measured in different sampling were tested using repeated measures ANOVA. Correlation among measured variables and the main processes related with C mineralization were tested with the Pearson–moment correlation coefficient. Significance was accepted at $P < 0.05$ in all cases.

In the case of the field study a repeated measures ANOVA was carried out with the data from 17 dates. In each management from 3 to 7 soil cores were measured. This resulted in 76 cores in the non-cover management and in 80 for the weed-cover management.

4. Results

4.1. Effects of quality and quantity of the organic carbon sources on CO_2 production

In all cases highest rates of C mineralization were found during the first month of incubation. After 313 days, the cumulative amount of organic C mineralized in the amended soils was significantly different than respective controls. Highest cumulative C mineralization in unamended soils was found in Mag soils (2578 µg C g^{-1}) followed by CT with (1296 µg C g^{-1}), Dei (1200 µg C g^{-1}) and Peg (917 µg C g^{-1}). The lowest was obtained in Mor soils with 442 µg C g^{-1} (graphics not shown).

Cumulative C-CO_2 production in soil amended with PR or COMP at doses of 4 and 12 µg C g^{-1} is shown in figure 6 and Table 3. Independently of the type of material (PR and COMP) and the rate of application (4 and 12 mg C g^{-1}) the highest rate of C mineralization occurred within the first two weeks after adding the sources of organic carbon, and typically decreased exponentially along the incubation period for all types of soils. Cumulative C mineralization at the end of experiment ranged between 1337 to 2315 µg C g^{-1} for soil amended with 4 mg C g^{-1} of PR (figure 6a and Table 3) and between 3138 to 3782 mg C g^{-1} for soil amended with 12 mg C g^{-1} of PR (figure 6b and Table 3). In both cases, the highest C mineralization was obtained in Mag and the lowest in CT soils, whereas similar and intermediated values were observed for the other soils.

Cumulative CO_2 production of soils amended with 4 mg C g^{-1} of COMP ranged 161 to 348 µg C g^{-1} (figure 6c and Table 3); whereas when 12 mg C g^{-1} of COMP was added, values were between 468 to 849 µg C g^{-1}(figure 6d and Table 3). Respired organic C under COMP for both doses was similar in soils of CT, Dei and Peg, but significantly lower than those found in Mor and Mag (Table 3). On average, for the whole treatments, cumulative respired C followed the following sequence Mag > Mor = Dei > Peg = CT (Table 3).

As expected, the amount of mineralized C when dose was of 12 mg C g^{-1} was significantly higher than that of 4 mg C g^{-1}, and this was independently of the SOC content of the soils and the two types of sources of organic carbon. Quality of the organic C source had a significant effect on cumulative CO_2 emissions. Thus, cumulative CO_2 production in soils amended with 4 mg C of PR g^{-1} was between 4.7 to 10.8 times higher than that of 4 mg C of COMP g^{-1}. This also was true when 12 mg C g^{-1} was applied, although differences were typically lower (between 4.1 to 6.8 times higher under PR) (Table 3).

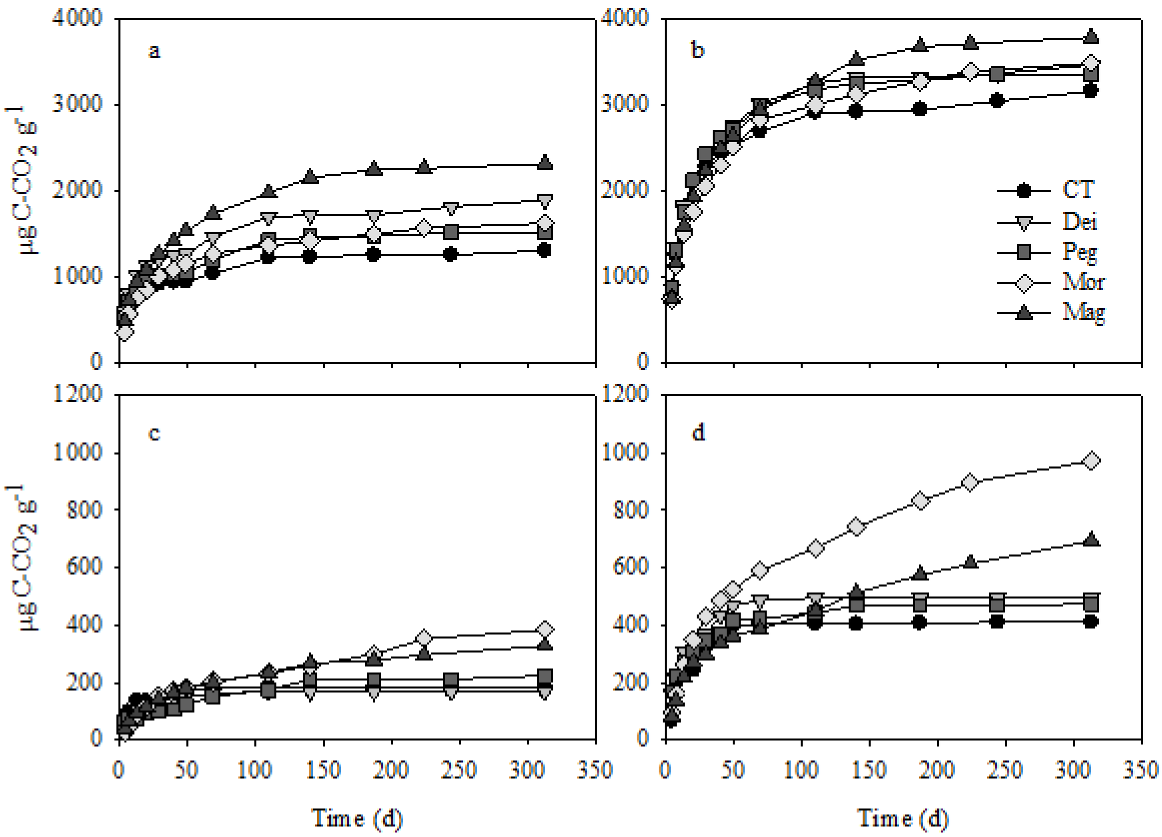


Figure 6. Mean cumulative amount of CO_2 respired during the 313 days of incubation in soil of contrasted SOC of the five locations amended with PR (a and b) or COMP (c and d) at doses of 4 (a and c) or 12 (b and d) mg C g^{-1}. Values of controls (unamended soils) have been discounted.

Table 3. Mean cumulative amount of CO_2 respired after the 313 days of incubation in soil of contrasted SOC of the five locations amended with PR or COMP at doses of 4 or 12 mg C g^{-1}. Data are the mean of three replicates. Different first letters (lowercase) shows significant differences between different sites in the same treatment ($p < 0.05$). Different second letters (capital) shows significant differences between treatments in the same site ($p < 0.05$). “All inputs” column corresponds to the mean of all treatments for each site.

Site	4 PR	12 PR	4 COMP	12 COMP	All inputs
CT	1337.3 (70.0)^{dB}	3199.9 (73.6)^{cA}	195.1 (17.8)^{bD}	467.8 (81.2)^{cC}	1300.0 (42.6)^{c}
Dei	1739.1 (167.7)^{bB}	3461.9 (45.9)^{bA}	161.3 (12.8)^{cD}	571.8 (86.2)^{cC}	1483.5 (30.4)^{b}
Peg	1496.4 (29.1)^{cB}	3138.2 (245.8)^{cA}	209.2 (16.5)^{bD}	537.6 (69.9)^{cC}	1345.3 (55.0)^{c}
Mor	1646.8 (17.6)^{bB}	3508.2 (9.2)^{bA}	348.2 (2.4)^{aD}	848.8 (6.1)^{aC}	1588.0 (6.8)^{b}
Mag	2314.7 (28.19^{aB}	3781.7 (10.4)^{aA}	327.9 (9.9) ^{aD}	693.9 (5.4)^{bC}	1779.6 (6.1)^{a}

Effects of dose of organic carbon input on C-CO_2 emission and SOC retention

The percentage of the cumulative respired C respect to the total added C (after substracting controls) was significantly higher for the doses of 2 and 8 mg C g^{-1} (8.01 and 7.58 %, respectively) when COMP was added (Table 4). Lowest values were achieved for doses of 4 and 12 mg C g^{-1} (5.65 and 4.89 %, respectively) (figure 7a and Table 4). When soils were amended with PR, and removing the values of the controls, the percentage of the cumulative respired C respect to the total added C was indirectly proportional to the doses ($R^2 = 0.81$; $P < 0.05$) (figure not shown): highest value (61.2%) was observed for the lowest dose and lowest value (28.0 %) at the highest dose. On average, there was a decrease of 2.8 units in the percentage for each mg C g^{-1} added. On contrary, regression of the COMP treatment was not significant.

The percentage of the cumulative respired C respect to the total added C was significantly higher under PR, although was dependent on the dose; highest differences (about 7 times higher under PR) were achieved at the lowest dose and lowest at the highest dose (about 5 times higher under PR).

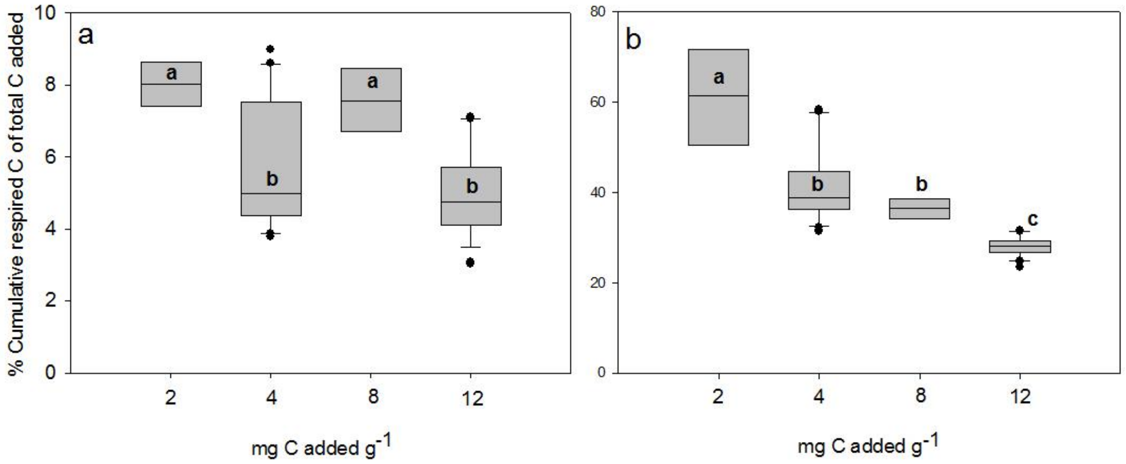


Figure 7. Box plot showing the percentage of cumulative respired C of total C added at different rates of C application in form of composted olive mill pomace (COMP) (a) and plant residues (PR) (b) for all sites. Boundaries of the boxes closest to, and furthest from zero indicate the 25th and 75th percentiles, respectively. Bars above and below the box indicate the 90th and 10th percentiles, respectively. Outliers are represented as black dots. Average values with the same letter indicate no significantly differences between different C doses applied ($p < 0.05$).

Table 4. Mean values of the percentage of the respired C respect to the added for the composted olive mill pomace (COMP) and plant residues (PR) treatments and different C doses. Different letters shows significant differences ($p < 0.05$). Soil emissions from controls were removed from the final values.

Dose (mg C g^{-1})	COMP (% respired C of total added)	PR (% respired C of total added)
2	8.0 (0.7)[a]	61.2 (11.7)[a]
4	5.7 (1.7)[b]	41.0 (7.9)[b]
8	7.6 (0.9)[a]	36.4 (2.3)[b]
12	4.9 (1.1)[b]	28.01 (2.1)[c]

Effect of the saturation deficit on the cumulative respired C

In the unamended soils, cumulative CO_2 production was inversely proportional to the saturation deficit of the soil (figure 8). The highest the saturation deficit, the lowest was the cumulative amount of CO_2 production. Soils with a saturation deficit lower than about 0.60 (e.g. SOC content of the soil was 60 % of that at maximum protective capacity) respired about four times more C than the soil with the highest saturation deficit (i.e. lowest total SOC content).

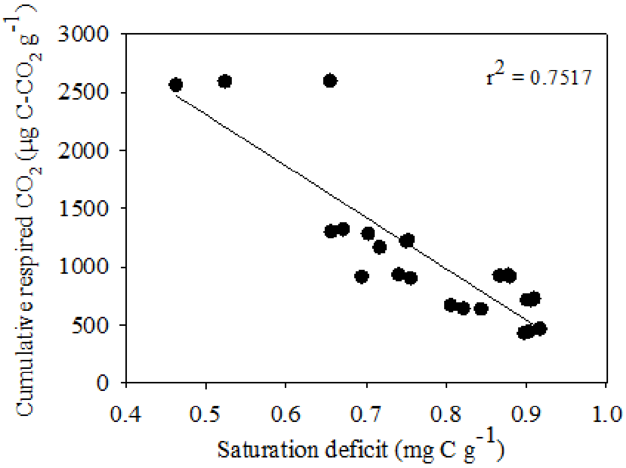


Figure 8. Linear relationship between the saturation deficit of the soils and cumulative amount of C respired (mg g^{-1} soil) for non-amended samples (control) of 5 types of soil.

This pattern was similar for the soils amended with 4 and 12 mg C g^{-1} of PR or COMP (figure 9).

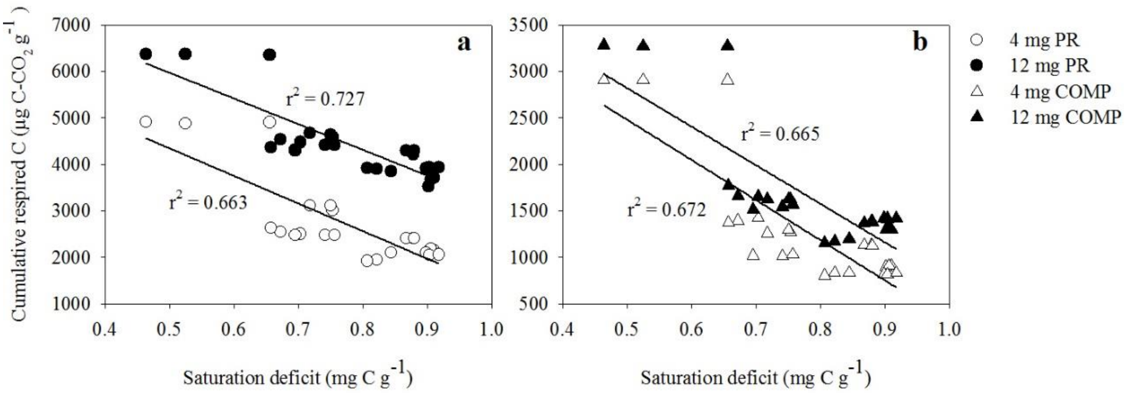


Figure 9. Linear relationship between the saturation deficit of the soils and cumulative amount of C respired (mg g^{-1} soil) for the 5 soils amended with 4 and 12 mg C g^{-1} of PR (a) and COMP (b). Note that in this case emissions from controls have not been eliminated.

For COMP or PR at 4 or 12 mg C g^{-1}, there was also a significant inverse relationship between the cumulative CO_2 production and saturation deficit. However, at a given soil saturation deficit, cumulative CO_2 production was much lower when soils were amended with COMP. In addition, cumulative CO_2 production was also lower for a given saturation deficit at 4 mg C g^{-1} dose than at 12 mg C g^{-1}, although differences between 4 and 12 mg g^{-1} doses were not high when COMP was added.

4.2 Effects of the presence of spontaneous vegetation cover and environmental conditions (temperature and rainfall) on CO_2 soil emissions in field conditions

Figures 10 and 11 show the intra-annual variability of the air temperature and rainfall during the studied period. The highest temperatures were reached in July and August (about 40 ºC), whereas lowest were found in January and February (between 0 to -5 ºC). In the studied period, the highest precipitations were reached in spring. Autumn had also relatively high precipitations and, finally, winter also accumulated some precipitations. Summer is a period of important water scarcity, but sometimes some storms can appear as it was the case for the summer of 2016. During spring and autumn were the periods with “a priori” significant respiration activity, since relatively high soil moisture and warm temperatures overlap.

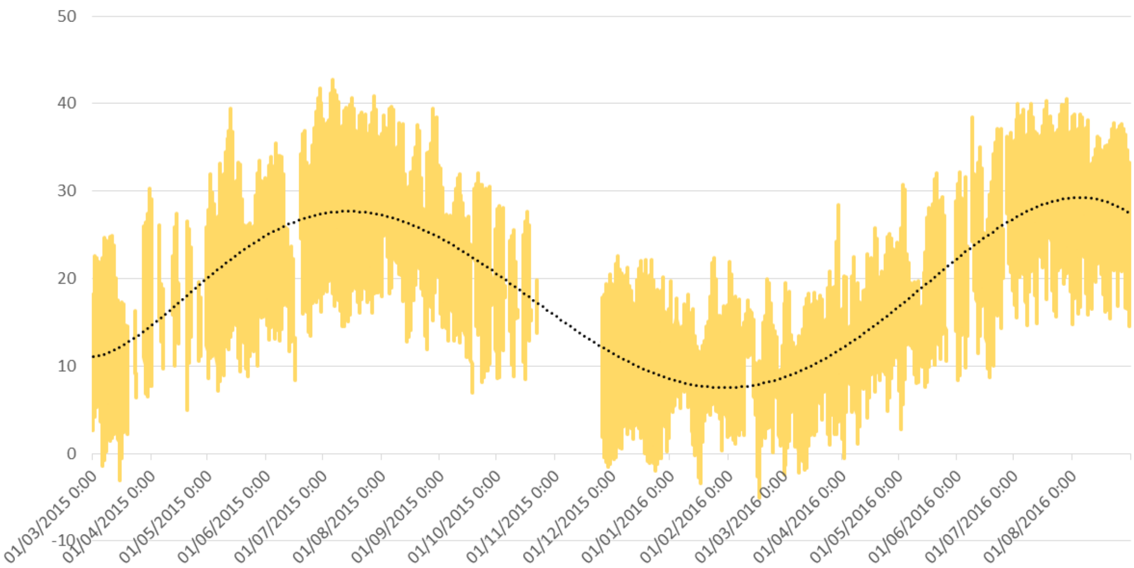


Figure 10. Average 30-minutes temperature (º C) (yellow line) and daily average temperature (º C) (black dotted line) from 01/03/2015 to 01/08/2016 in Úbeda (Jaén) (Consejería de Agricultura, Pesca y Desarrollo Rural, 2016). Note that Úbeda is only 7 Km far from the plots.

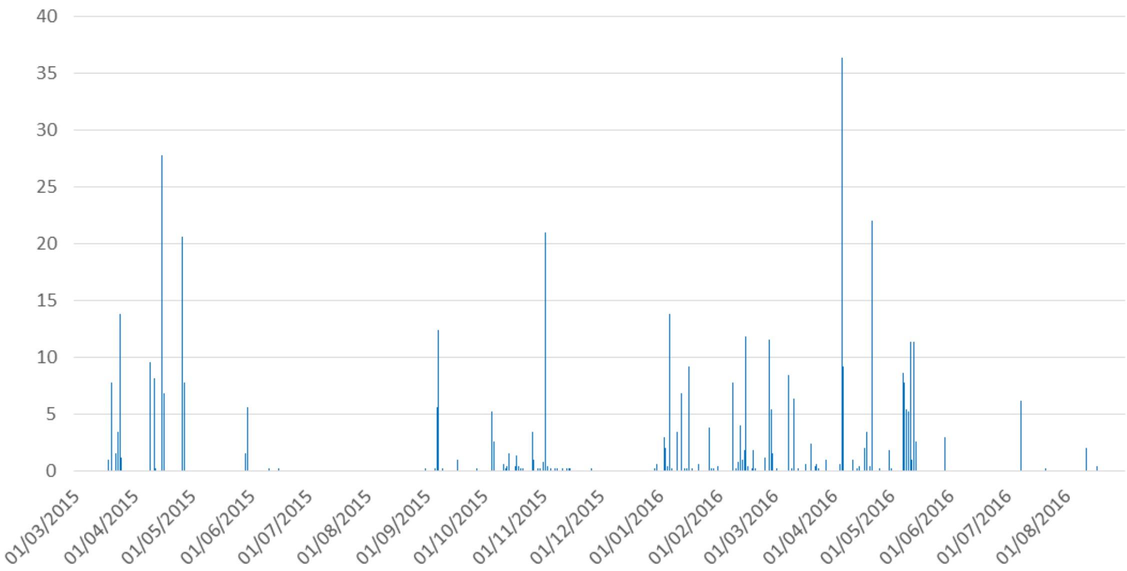


Figure 11. Daily rainfall (mm) from 01/03/2015 to 01/08/2016 in Úbeda (Jaén) (Consejería de Agricultura, Pesca y Desarrollo Rural, 2016). Note that Úbeda is only 7 Km far from the plots.

Figure 12 and Table 5 show the intra-annual soil CO_2 emission in the bare and weed-covered plots, and the significance of the effects of the two factors (management and season) and the interaction on the CO_2 emissions, respectively.

At the bare soils, CO_2 emissions was rather constant along the studied period and values ranged 0.5 to 1.6 μmol m^{-2} s^{-1}. However, at the soils covered by weed, values varied greatly from 0.6 to 9.3 μmol m^{-2} s^{-1}. During summer and winter months, soil CO_2 emissions were similar for both managements.

However, during the spring of the two years, the rate of CO_2 emissions was significantly higher in the weed-covered plots than in the bare soils. In both springs the peak of the CO_2 emissions in the weed-covered plots was about 9 μmol m^{-2} s^{-1}, whereas in the same periods, the emissions of the non-covered plots were about 1 μmol m^{-2} s^{-1}.

The sharply decrease in CO_2 emission in the weed-covered soils after the early spring peak in 2015 did not occur in 2016. In 2015, weeds were cleared at the end of April, whereas clearing was done at the end of June in 2016, and therefore the decrease after the CO_2 emission of peak of 2016 was more leisurely. During autumn soil CO_2 emissions in weed-covered soils increased significantly up to 3 μmol m^{-2} s^{-1}, whereas in the bare soils there was also an increase but significantly lower than that of weed-covered soils.

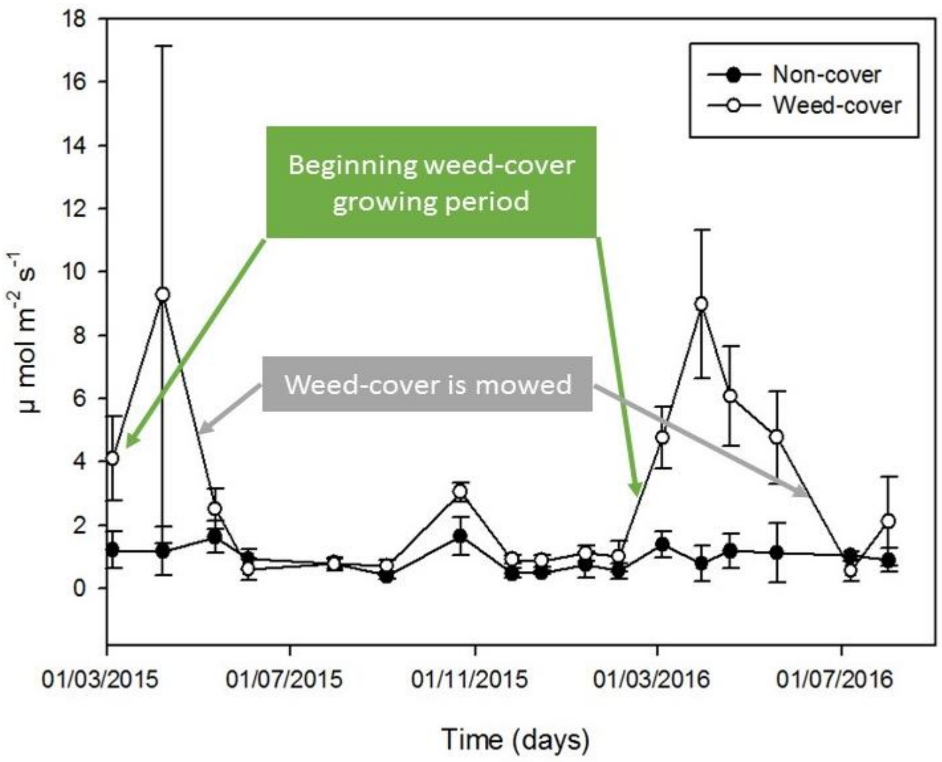


Figure 12. Evolution of the soil respiration (micro mole of CO_2 m^{-2} s^{-1}) from 05/03/2015 to 01/08/2016 in the non-cover and weed-cover managements.

Table 5. The effects of the management, time and the interaction between the two factors on soil CO_2 emissions (repeated measures ANOVA).

Effect	P	Interpretation
Management (M)	<0.001	Weed-covered > bare
Time (T)	<0.001	Early spring > late autumn> other periods
T x M	<0.001	Weed-covered > bare specially in Early spring > late autumn

5. Discussion

5.1. Influence of the saturation deficit on soil respiration

After one year of incubation, it was found that C saturation deficit, the amount of C added and the quality of the organic carbon source influenced the cumulative CO_2 emissions (used here as a proxy of residue-derived C stabilization). As expected, in soils with no addition of organic C (controls), CO_2 emission was related to initial SOC. This result is in line with those found by Kimetu *et al.* (2009), who obtained higher respiration rates per gram of soil in rich-SOC (forest and low disrupted agricultural soils) than in poor-SOC soils (most degraded soils).

There was negative linear relationship between CO_2 emissions and saturation deficit. Therefore, the higher are the differences between current SOC levels of a soil respect to the maximum capacity to protect SOC, the lower is the cumulative soil CO_2 emission by soil respiration. This finding agrees well with the SOC saturation hypothesis. According to this hypothesis when SOC pools that are stabilized (e.g. protected) cannot longer increase in size, soils are considered “C saturated”. At this point, an increase in steady-state C input rate does not increase steady-state stabilized SOC. Alternatively, when an increase in steady-state C input rate does increase steady-state protected SOC, soils have an unsatisfied capacity to store C that is referred to as the “saturation deficit” (Hassink, 1997). A soil with no protected SOC has a saturation deficit of 100 %, while a soil that is C-saturated has a saturation deficit of zero. New C inputs can be stored as protected pools only when there is a saturation deficit. In our case, forest soils (Mag) and olive grove soils rich in organic matter (CT and Dei) which showed saturation deficit lower than 0.7, had the highest cumulative CO_2 emission. The observed increase in cumulative CO_2 emissions of soils with a smaller C saturation deficit supported other work that has observed a limit to C stabilization in organic carbon protected pools, such as silt+clay pool, especially when C saturation deficit is relatively low (Hassink *et al*., 1997; Roscoe *et al*., 2001; Jolivet *et al*., 2003; Dieckow *et al*., 2006; Stewart *et al*., 2008).

Similar pattern was found when a source of C was added. According to the SOC saturation hypothesis the addition of an organic source to a soil results in: i) a greater proportion of the added C is stabilized/protected when C saturation deficit of the soil is large, at least compared with soils with a smaller C saturation deficit, and ii) a lower relative stabilization efficiency of added C (e.g. amount of organic carbon which is stabilised compared with that added) at higher compared with that of lower organic carbon inputs. C stabilisation was not measured in this study. However, cumulative CO_2 emission was assumed to be an inverse proxy of the stabilised organic carbon. This assumption was based in the fact that daily CO_2 production rate in amended soils was similar to that of the control soils at the end of the experiment. Therefore, the higher the cumulative CO_2 production was in amended soils, the lower was the stabilized organic carbon. The fact that higher cumulative CO_2 emissions (e.g. lower C stabilization) was achieved in soils with lower C saturation deficit suggests that sites which are near to their C saturation level sequester a small part of the added organic carbon. This finding agrees well with that of Stewart *et al.* (2009) who found that C stabilization efficiency of the chemically and biochemically protected organic carbon pools was higher in soils with lower saturation deficits.

Figure 9 showed that increasing three times the dose of the organic C input, the cumulative respired C increased between 2 to 3 times. Thus, at higher doses the proportion of the stabilized C was higher, and this was so for the relative stabilization efficiency, than at lower dose. This was true independently of the quality of the organic carbon source. This finding does not fit with the saturation hypothesis. However, Stewart *et al*. (2008b), who did a similar experiment but measuring stabilized C after adding a source of organic carbon, found similar results; most of the protected organic carbon pool showed no general trend in C accumulation with organic carbon addition level and only in four out of six soils there was a higher stabilization efficiency in the chemically protected organic carbon at lower doses.

The fact that at higher doses of organic carbon, stabilization efficiency was higher could be due to different “a priori” uncontrolled sources of variations. At low SOC and high saturation deficit sites, such as Mor site, soil microorganism growth might be limited by organic carbon availability. Thus, under a low level of addition of organic carbon, a great part of organic carbon of the unprotected forms might fueled soil respiration needed to build microorganism biomass, resulting in relatively low organic C stabilization efficiency as lower amount of the added organic carbon is transformed in other protected organic carbon pools. In addition to this, soil respiration at higher doses of organic C input in soils with lower saturation deficit, might be limited by available nutrient, and thus a proportion of the unprotected organic carbon pool which should be respired are not because of the scarcity of available nutrient. Indeed, it has been described that the addition of available N in rich organic matter soil supplemented with organic C, stimulated further CO_2 production (Kirkby *et al*., 2013). Overall, the possibility of a limitation of the CO_2 emissions exerted by a scarcity in the available nutrients when high levels of organic carbon is added, might explain the higher C stabilization efficiency at higher doses of organic carbon.

5.2 Influence of the quality of the source of organic carbon on C stabilization efficiency

Plant litter is the primary source of all organic carbon in the soil. However, the processes of litter decomposition and organic C stabilization are often considered separate (Sollins *et al*., 2007). Litter decomposition research has focused mainly on the effects of litter quality on short-term mineralization and nutrient release (Parton *et al*., 2007), whereas SOM stabilization research has focused on organo-mineral interactions that slow SOM turnover relative to total SOM due to physicochemical protection by mineral association and microaggregate occlusion (Six *et al*., 2002; Stewart *et al*., 2008a). To understand, model, and manage the response of SOM to global environmental change, litter decomposition must be linked to SOM stabilization (Prescott, 2010; Dungait *et al*., 2012).

In this preliminary work it was intended to assess for the effect soil organic matter quality in CO_2 production and SOC retention. Cumulative C-CO_2 production was much higher (up to an order of magnitude) after the addition of PR than that of COMP. Therefore the organic C retained in the soil and thus the C stabilization efficiency was significantly lower when a source of high quality organic C was added, and this was true for the two doses applied. Indeed, only less than 10 % of the added COMP-carbon was respired whereas values were between 30 – 60 % for PR.

Values for the COMP were very similar to that reported by Gómez-Muñoz *et al.* (2012), who evaluated the cumulative respired C after 240 days of incubation of 8 types of composted olive mill pomace. The very high C stabilization efficiency of the composted olive mill pomace is likely due to the refractory nature of this source of organic carbon owing the condensation and complexation processes during the composting period. Plaza *et al.* (2007) observed during the composting period that C, H, S content, the C:N ratio and aliphatic compounds (easily decomposable) of the composting olive mill pomace decreased. They also observed an increase in the N, O, COOH and OH associated to phenols, C:H and O:C ratios and aromatic compounds, all of these properties provide stability properties. This enrichment in recalcitrant compounds of the composted olive mill pomace might be the responsible of the high C stabilization rate of the added C. On the other hand, fresh plant residues are made of a mix of organic components, such polysaccharides (starch, cellulose, hemicellulose and pectin 50 – 60%) and lignin (15 – 20%), and other components (proteins, polyphenols, chlorophyll, cutin and suberin, lipids and waxes, 10 – 20%), a relatively high percentage of which are considered labile at the short-medium term. Therefore, it was not surprising that C stabilization efficiency of this source of organic carbon was relatively low.

5.3 Comparing evolution of CO_2 emissions in field conditions between conventional and spontaneous resident vegetation cover managements

CO_2 fluxes out of the soils were greatly affected by the presence of a weed cover in olive orchards (figure 12). This finding was similar to that of Bertolla *et al.* (2014) who found that soil respiration was higher in a weed-covered olive orchard compared to a non-covered one. This was not unexpected as plant residues are an important source of organic C to the soil. At this site, about 750 Kg C ha^{-1} (see Study 3 in Chapter IV) on average for two consecutive years, mostly as unprotected organic C, were deposited on the soil surface, promoting soil microbial growth and, thus, respiration. In addition to this, the contribution of the roots of the species composition of the weed-covered community to the CO_2 emissions not only cannot be dismissed but it is suspected that it may be very important.

On the other hand, the relatively very low CO_2 emissions found in the non-covered soils in summer and winter (between 0 – 1 µmol CO_2 m^{-2} s^{-1}) were in line with those found by Testi *et al.* (2008), who obtained daily values ranging from 0 – 1 µmol CO_2 m^{-2} s^{-1} in an olive grove of Córdoba (Andalusia, Spain). However these authors only measured from June to September, so results of spring and autumn of this study, when the CO_2 respiration seems to be higher, cannot be fully compared to those of Testi *et al.* (2008).

Spring and autumn were the periods with higher CO_2 fluxes out of the soil and when differences between managements we more marked. This was due to the fact that in these periods the temperature and soil water content were most favorable for weed growth and soil microbial activity. Nevertheless, there are some differences between spring and autumn. In spring, CO_2 fluxes out of the soil were mainly due to SOM and also from the decomposition of the fresh organic matter and roots respiration. The effects of other variables directly or indirectly affected by the presence of plants, such as soil water content or soil temperature, on CO_2 fluxes were not accounted for. However, during autumn CO_2 efflux magnitude and the differences between managements were much lower. With many cautions, these facts could be explained by the lack of root respiration in the weed-covered site, and thus the contribution of roots to the overall fluxes of CO_2 might be important. In addition to this, the higher CO_2 fluxes in the weed-covered site in periods when plants were lacking could be due to differences in SOC content. Initial SOC content was higher in the weed-covered plots (2.06 and 1.69% in the 0 – 5 and 5 – 15 cm layer, respectively) than in the non-covered soils (1.30 and 1.1% in the 0 – 5 and 5 – 15 cm layer respectively) (data shown in Table 4 in Chapter IV).

On the other hand, the effect of the weed-cover mowing is clear. Usually, at the end of February is when the spontaneous resident vegetation cover starts to grow. The growth is relatively fast and at the beginning of March the effect of the presence of the vegetation cover is measurable. The effect of the vegetation cover finishes after it is mowed. This effect is clear. For example, in the first year (2015) the vegetation was mowed at the end of April. Nevertheless, the effect of the mowed vegetation over the soil surface also affected the CO_2 respiration in May, but in this case the emissions were only about 2.5 $\mu mol\ m^{-2}\ s^{-1}$. This is 3.6 times lower than the peak achieved. In June, values of the two managements were similar. In the spring of 2016 the respiration started to increase, again, in March. In this case the vegetation was mowed in early June, which means much later than in 2015. The peak was again achieved at later March or earlier April but the decrease in the soil respiration was progressive. For example, at the middle of May the respiration in 2016 was about two times than that found in 2015 when the vegetation was already mowed. In July of 2016 the effect of the vegetation in the weed-cover management disappeared.

Nevertheless, despite annual CO_2 fluxes under weed-covered management were much higher than under non-covered, it is assumable that gross C assimilation was also much higher due to the photosynthetic activity of the spontaneous vegetation cover during the growing period. Although, we did not measure gross or net C assimilation. Thus, in this line, and in our same experiment Chamizo *et al*. (2017) found that at the annual balance, non-cover management degreased C-CO_2 from the atmosphere uptake by 50% compared to the weed-cover treatment. They found that the annual C balance at the olive grove scale (e.g. no only soil with or without spontaneous weed vegetation but also taking into account olive trees) under weed-covered was -1.4 t C ha^{-1} , whereas figures for the site lacking of vegetation cover was of -0.71 t C ha^{-1}. Assuming that tree growth rates and olive fruit production were similar under both managements, net C accumulation due to the presence of the weed-cover amounted for 0.69 Kg C ha^{-1}, which is a value similar to the mean (two consecutive years) annual organic carbon production of the weed biomass in the weed-covered site.

6. Conclusions:

- Soils with **higher SOC content** (i.e. lower saturation deficit) accumulated **higher respired C-CO_2.** Nevertheless, it was true **only in soils with no addition of an organic input.**
- **Higher amounts of the PR and COMP inputs led to lower cumulative respiration rates.** This might be related to the nutrient availability (e.g. N content) for soil microorganisms.
- **Soils amended with PR respired much more CO_2 than those amended with COMP, reaching in some cases a difference of one order of magnitude.** This was probably due to the much faster incorporation to the soil of the PR than the COMP, since the COMP is formed by a high proportion of recalcitrant substances and, on contrary, PR is formed mainly by labile substances.
- Therefore, a **management combining PR and COMP could be a suitable solution** in order to, on the one hand, increase relatively fast the SOC content and,

on the other hand, to add an organic C input with a slow incorporation rate to the soil.

- Lastly, the field study highlighted that **the respiration of soils with a weed-cover management was higher than the respiration of the non-covered soils.** This fact was due to the higher SOC content and the presence of the spontaneous resident vegetation cover. However, this fact takes place only when warm temperatures and relatively high precipitations overlap (spring and autumn). Nevertheless, the total annual balance suggests that the C-CO_2 captured by the whole agroecosystem is higher (about a double) in the weed-cover management than in the non-cover due to the higher uptake of CO_2 by weeds of the vegetation cover.

No son las malas hierbas las que ahogan la buena semilla, sino la negligencia del campesino.

Confucio

CHAPTER VI

SOC fractions dynamics: influence of management, mineralogy and soil depth. Mechanisms of SOC sequestration in Andalusian olive grove soils

1. Introduction

As it has been shown earlier (Chapter I), that SOC is not a homogeneous pool, but it is composed by different fractions differing, among other aspects, in their dynamic in the soil. As already mentioned in the 2.3 section of the Chapter I, Six *et al.* (2002) recognised four soil organic fractions which can be relatively easy to identify and quantify with recognised functions: i) Unprotected, which is considered as fresh SOM relatively accessible to microorganisms; ii) Physically protected, which is that fraction of SOC protected within microaggregates (53 – 250 µm), iii) Chemically protected, which consists of SOC associated to silt and clay particles (< 53 µm), and iv) biochemically protected, which corresponds to the most recalcitrant SOC.

These fractions are affected by management practices, and thus the potential for SOC sequestration. Plaza-Bonilla *et al.* (2014) showed in soils of NE Spain that the most affected pool to a change in management practices was the unprotected SOC, the most labile fraction. Six *et al.* (2000) suggest that the conventional tillage decreases micro-aggregate formation and stability of aggregates by breaking macro-aggregates. Consequently, it is expected that the proportion of the SOC which is physically protected is lowered under conventional tillage. Tillage also might affect the concentration of soil organic carbon linked to silt+clay particles as made this fraction more accessible to soil microorganisms. Management practices might not only affect the SOC content but also the proportion of the different SOC pools.

Nevertheless, Six *et al.* (2004) proposed that aggregate formation and stabilization is influenced by five major factors: soil fauna, roots, microorganisms, environmental variables and inorganic binding agents. Conservative managements (e.g. weed-cover) in woody crops can affect soil fauna, roots and microorganisms, but it has no influence on the two latter factors. Thus, it is possible that other variables, such texture or geochemical properties may affect importantly the SOC distribution among different pools. Furthermore, climate conditions affect strongly mineralization process. In soils affected by Mediterranean climate the lack of moisture, especially during the summer, reduces the mineralization rate and, then, may affect the distribution of the SOC among the different pools in comparison to rainier temperate soils. In summary, the proportion of SOC among the four pools can be affected by management practices, edaphic properties and climate conditions. However, the majority of the studies have been focused on management practices or soil interactions, but they have not studied the interaction between management factors and soil properties.

On the other hand, the dynamics of the four SOC pools do not follow similar patterns. Stewart *et al.* (2008) studied the dynamics of the different SOC pools in eight long-term agroecosystem experiments across the United States and Canada and they found evidences of C saturation for some protected fractions. These results suggest that the amount of SOC which can be potentially sequestered – accumulated in the protected pools – is finite. Nevertheless, these results cannot be extrapolated since the dynamic of the SOC pools is affected by soil properties (e.g. phyllosilicate mineralogy, Barré *et al*., 2014) and type of crop (arable crops vs woody crops), thus, it is important to study the dynamic of the different SOC pools considering specific edaphic and climatic conditions for a specific crop. Furthermore, the influence of some soil properties on soil fertility is clear. For example, Oyonarte *et al*. (2008) in a study of Andalusian forest from Sierra Nevada (Granada) and Sierra de Cazorla (Jaén) found that CEC, Calcium and pH were significantly higher in carbonated than in siliceous soils. They also found higher total SOC and total N values in carbonated soils (1.3 times for both variables), but these differences were not significant. Nevertheless, Calero *et al*. (2013) found significant positive correlation between SOC and total N, and negative between SOC and pH. Therefore, currently, we clearly know that both management and parent material affect soil properties. However, we do not know the interaction between the managements and soil properties and how those factors affect the dynamics of the different SOC pools.

2. Objectives

Main objective of this chapter is to assess the **influence of** i) the presence of a **spontaneous resident vegetation cover** in the inter-row of olive groves, and ii) the **mineralogical and chemical properties** of the soil, **on SOC fractions** of different level of protection. **Different depths** were considered in some cases.

In addition, we tested for the **hypothesis of SOC saturation** in olive orchards.

For that purpose, two fractionation methods were applied. Thus, another objective of this chapter is to **look for the relationship between the fractions resulting from these two fractionation methods.**

3. Material and methods

Two mensurative experiments were performed to cope with the objective.

3.1 Experimental design

The experimental design is that described in the studies 1 and 2 of the 3.1 section in Chapter IV. Soil samples were taken according to 1.1 section of Chapter II.

Briefly, in the Study 1 samples were taken in 5 sites consisting of a weed-covered plot (WC) with a nearby non-covered (NC). Two depths were considered: 0 – 5 and 5 – 15 cm.

In the case of the Study 2, to assess for the effect of spontaneous vegetation cover on SOC fractions of different level of protection, soils under conventional and weed-cover management were selected. In addition, to assess the SOC saturation hypothesis and to obtain a gradient of SOC as wide as possible, soil samples under natural vegetation cover were also taken. Plots with different managements were nearby in order to sample soils with similar physicochemical properties. Furthermore, to evaluate the effect of the interaction between soil mineralogy properties and the presence of spontaneous vegetation cover on SOC fractions, soil samples were taken in two sites of contrasted parent material (acid vs basic soils).

3.2. Sources of variations

Briefly, to assess for the effects of spontaneous vegetation cover on SOC fractions, two sources of variations were selected: i) Presence of a spontaneous vegetation, with two levels; presence and absence, and ii) soil depth with two levels; top 5 cm and 5 – 15 cm depth. Main properties of the soils are showed in Table 1 in Chapter IV.

To assess for the effects of the interaction of spontaneous vegetation cover x soil mineralogical properties on SOC fractions, two were the main sources of variation; i) soil management, with three levels; olive orchard with bare soils, and comparable olive orchard with a spontaneous vegetation cover and a Mediterranean forests (this latter was used only for testing the saturation hypothesis), and ii) parent material, with two levels; soils under acid soils and under basic soils. Main properties of the soils are showed in Tables 2 and 3 in Chapter IV.

3.3 Dependent variables

Soils were analized for SOC fractions according to the methodology of Six *et al.* (2002) and Stewart *et al.* (2008). See 2.1 section (Chapter II) for the fractionation and 2.2 section (Chapter II) for the SOC analysis. In addition to this fractionation method, the humic substances fractionation method was also applied.

Humic substances characterization (traditional SOC fractionation method)

This fractionation method was carried out only for soil samples taken in the sites of contrasted parent material in plots differing in the management. Fractionation method for the analysis of humic substances was that of IHSS (International Humic Substances Society) (Swift, 1996). Total extractable organic carbon (TEC) for humic and non-humic substances was analized from extracts of soil by mechanically shaking the samples in a 0.1 M NaOH and $Na_4P_2O_7$ solution (1:10 w/v) at pH 14 for 24 h at 60 °C. The extracts were centrifuged and filtered (Millipore 0.45 μm). Separation of the TEC fraction into humic acids (HA) and fulvic acids (FA) was done by precipitation with H_2SO_4 (for HA) and purification with polyvinylpolypyrrolidone (for FA) to eliminate non-humic carbon, respectively. The FA fraction was considered the soluble carbon remaining from the humic extract, and the corresponding HA/FA ratio was accounted for. Finally, any remaining carbon was considered the alkali-insoluble fraction of SOM (humin). A scheme of the methodology is shown in the figure 1. Moreover, the humin was hydrolysed with HCl in the same conditions according to the methodology of Six *et al.* (2002) in order to study more deeply the relationship between the two fractionation methods.

Different informative ratios based on humic substances were also calculated:

Humification Index (HI). Quantitative (Sugahara and Inoko, 1981) relationship between the non-humic and humic organic C.

$$HI = \frac{NH}{(HA + FA)}$$

Where, NH is calculated as,

$$NH = TEC - (HA + FA)$$

where, NH corresponds to the non-humic substances, TEC to the total extractable carbon, HA to the humic acids and FA to the fulvic acids

Degree of Humification (DH). Relationship between the humic and total extracted organic C. It is both a qualitative and a quantitative index, calculated as.

$$DH\ (\%) = \frac{HA + FA}{TEC} \times 100$$

where, HA corresponds to the humic acids, FA to the fulvic acids and TEC to the total extractable carbon

Humification Ratio (HR). Relationship between the humic and total organic C. This is a quantitative index.

$$HR\ (\%) = \frac{HA + FA}{TOC} \times 100$$

where, HA corresponds to the humic acids, FA to the fulvic acids and TOC to the total organic carbon.

This fractionation method would be complementary to the other fractionation method, and provides information on chemical properties of the different substances forming the SOM. Therefore, with both fractionations it is possible to obtain an outlook about the origin, features and dynamic of the SOM.

Measurement of the E_4/E_6 ratio of the HA

This ratio is calculated from the HA fraction, and was only done in the soils samples taken in plots differing in the management of two sites of contrasted parent material. The E_4/E_6 ratio was calculated as the ratio of absorbances at 465 and 665 nm measured by a spectrophotometer UV – VIS on solutions of 3.0 mg of each HA dissolved in 10 ml of 0.05 M $NaHCO_3$ with the pH adjusted to 8.3 with NaOH (Chen *et al*. 1977; Senesi *et al*., 2003) (figure 1).

The E_4/E_6 ratio is considered to be inversely related to the degree of condensation and aromaticity of the humic substances and to the degree of humification (Stevenson *et al*., 1994; Senesi *et al*., 2003). In this study the E_4/E_6 ratio was used to characterize the organic compounds in the HA fraction of the SOM in olive groves differing in the management (presence of natural plant cover in the inter-canopy area) and the mineralogical properties (acid/basic soils).

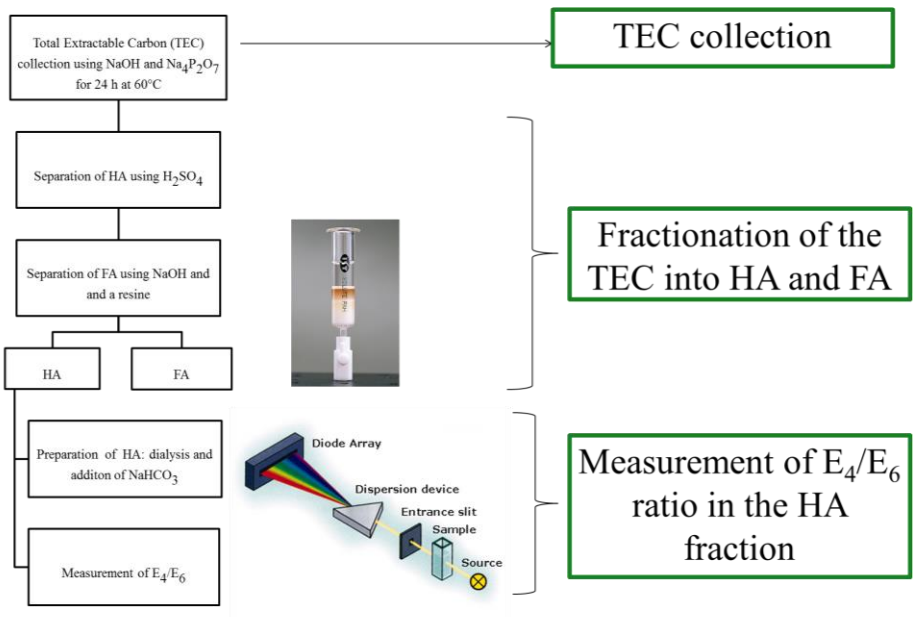


Figure 1. Scheme of the humic substances fractionation including the E_4/E_6 ratio measurement.

3.4 Statistical analysis

The effects on the total SOC and SOC fractions of the two factors (depth, management) and their interactions were assessed using two-way ANOVA procedure. Pearson correlation coefficients were used to assess the significance of the interrelations between total SOM and biomass production. The values of the variables for each depth were averaged in order to better focus on the difference between depths and managements.

For the study aimed to evaluate the effect of the interaction between parent material and weed-cover management, the effects on soil properties (texture, fertility and mineralogy variables) and on total SOC and SOC fractions of the two factors (management and mineralogy) and their interactions were assessed using two-way ANOVA procedure. R^2 was used to test saturation hypothesis of the SOC of the fractions, the correlations between TOC and fractions resulting from the humic substances fractionation, and between SOC fractions and mineralogical and geochemical properties. Principal component analysis (PCA) was used to assess the influence of the soil properties and management on the SOC content of the different fractions. In order to best adjust the axis of the PCA, a Varimax rotation was applied.

4. Results

4.1 Effects of the presence of spontaneous vegetation cover on SOC fractions

Soil organic carbon fractions of olive orchards under a spontaneous vegetation cover and different depths

The amount of SOC in the top 5 cm of the inter-row soils for weed-covered (WC) orchards ranged from 11.5 to 44.8 mg C g^{-1} and, as expected, these values were higher, about a 50 % on average, than those found in the 5 – 15 cm depth soil (Table 1 and figure 2). Unprotected and physically protected SOC of the top 5 cm of soils (10.0 and 5.2 mg C g^{-1}, respectively) were significantly higher than values obtained for the 5 – 15 cm (5.3 and 3.6 mg C g^{-1}, respectively) (Table 1 and figure 2). These differences between depth were not observed for the chemically and biochemically protected SOC. However, SOC density (i.e. mg C g^{-1} fraction) of unprotected, physically and chemically protected organic C was significantly higher in the top 5 cm (figure 3). However, this was not true for the biochemically protected organic carbon.

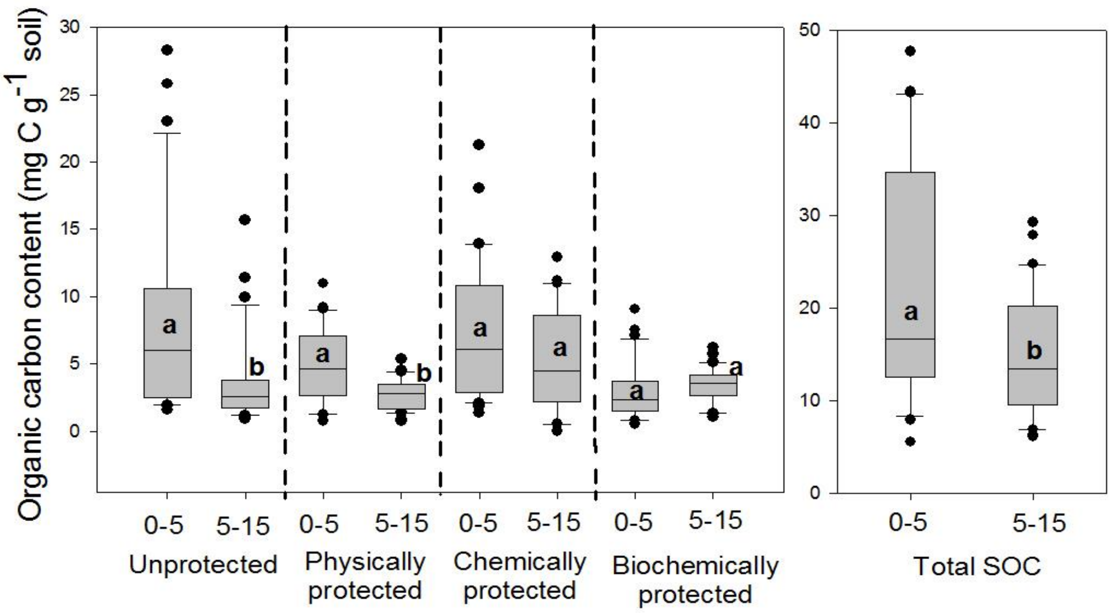


Figure 2. Box-plot representation of whole SOC and unprotected, and physically, chemically and biochemically protected organic carbon of soils (0-5 and 5 -15 cm) of WC olive oil farms. Boundaries of the boxes closest to, and furthest from zero indicate the 25th and 75th percentiles, respectively. Bars above and below the box indicate the 90th and 10th percentiles, respectively. Outliers are represented as black dots. Average values with the same letter indicate no significant differences between management types ($P < 0.05$).

Unprotected organic C compromised a relatively high proportion of the SOC with values ranging from 16.6 to 57.3 % and from 6.8 to 56.3 % for 0 – 5 and 5 – 15 cm soil depths, respectively; averaging 33.0 and 24.4 % for the whole set of soil samples (Table 1 and figure 4). The differences between depths were significant for the percentage of the unprotected fraction. However, the contribution of the physically, chemically and biochemically protected SOC to the total SOC did not differ significantly with depth (figure 4).

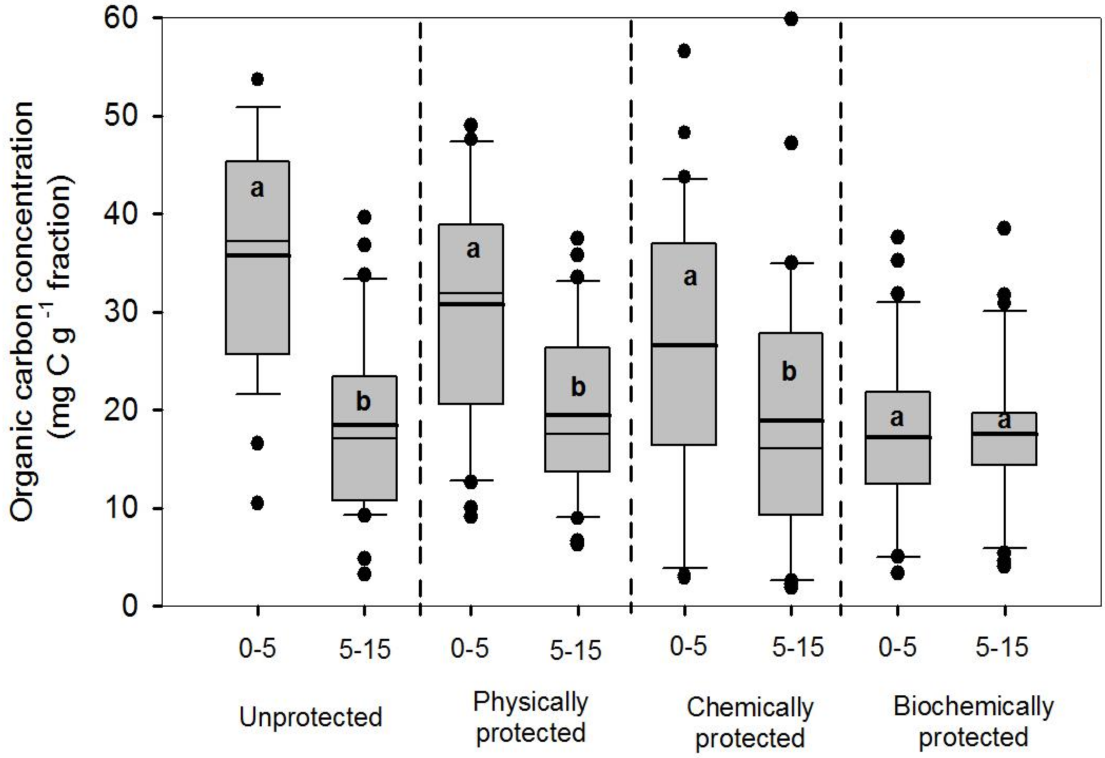


Figure 3. Box-plot representation of soil organic carbon concentration in the unprotected, and physically, chemically and biochemically protected organic carbon fractions of soils (0-5 and 5 -15 cm) of WC olive oil farms. Boundaries of the boxes closest to, and furthest from zero indicate the 25th and 75th percentiles, respectively. Bars above and below the box indicate the 90th and 10th percentiles, respectively. Outliers are represented as black dots. Average values with the same letter indicate no significant differences between management types ($P < 0.05$).

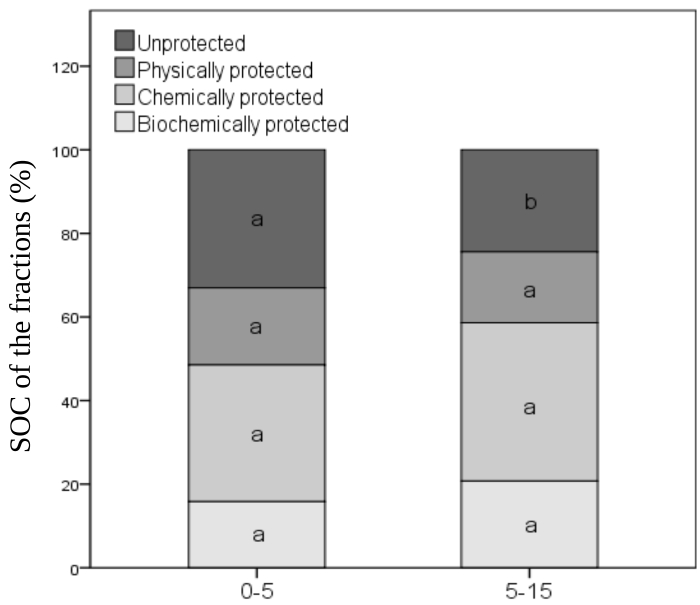


Figure 4. Percentage contribution (on average) of soil organic carbon fractions to the whole SOC (0 – 5 and 5 – 15 cm) of weed-covered olive oil farms. Average values with the same letter indicate no significant differences between the two soil layers ($P < 0.05$).

Effects of the C input due to aboveground vegetation cover biomass on SOC fractions

SOC content of the fractions in the WC orchards was significantly higher than in the paired non-covered (NC) orchards. This was true for both, 0-5 cm and 5 – 15 cm soil (figures 5a and 5b). SOC content of the unprotected, physically, chemically and biochemically protected was on average 4.5, 2.7, 3.2 and 1.9 times higher, respectively, in the top 5 cm and 2.7, 2.0, 3.0 and 1.8 times higher, respectively, in the 5 – 15 cm layer in the weed-covered than in the non-covered olive orchards (figures 5a and 5b and Table 1).

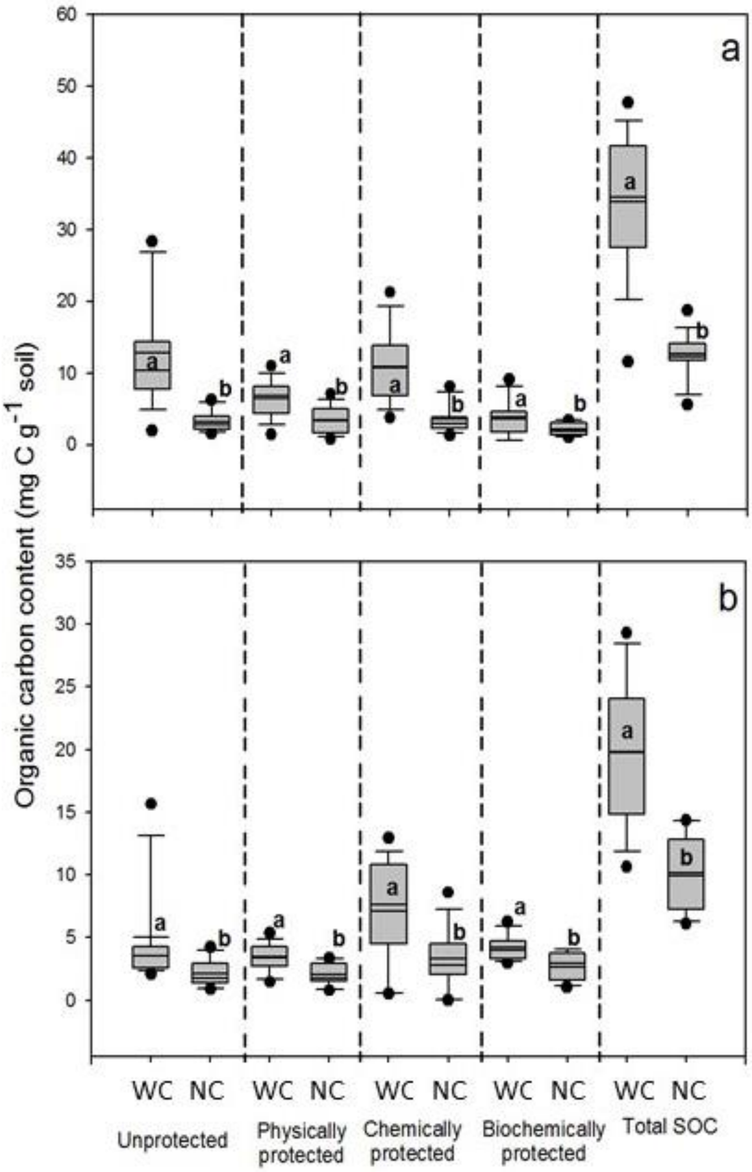


Figure 5. Box-plot representation of whole SOC and in the unprotected, and physically, chemically and biochemically protected organic carbon fractions of soils (0 – 5 (a) and 5 – 15 cm (b)) of weed-covered (WC) and comparable non-covered (NC) olive oil farms. Boundaries of the boxes closest to, and furthest from zero indicate the 25th and 75th percentiles, respectively. Bars above and below the box indicate the 90th and 10th percentiles, respectively. Outliers are represented as black dots. Average values with the same letter indicate no significant differences between WC and NC olive orchards ($P < 0.05$).

The higher SOC content in olive orchards under a spontaneous vegetation cover treatment was mainly due to the higher organic C concentration of the fractions. This was so for unprotected, physically and chemically protected fractions in both depths (figures 6a and 6b).

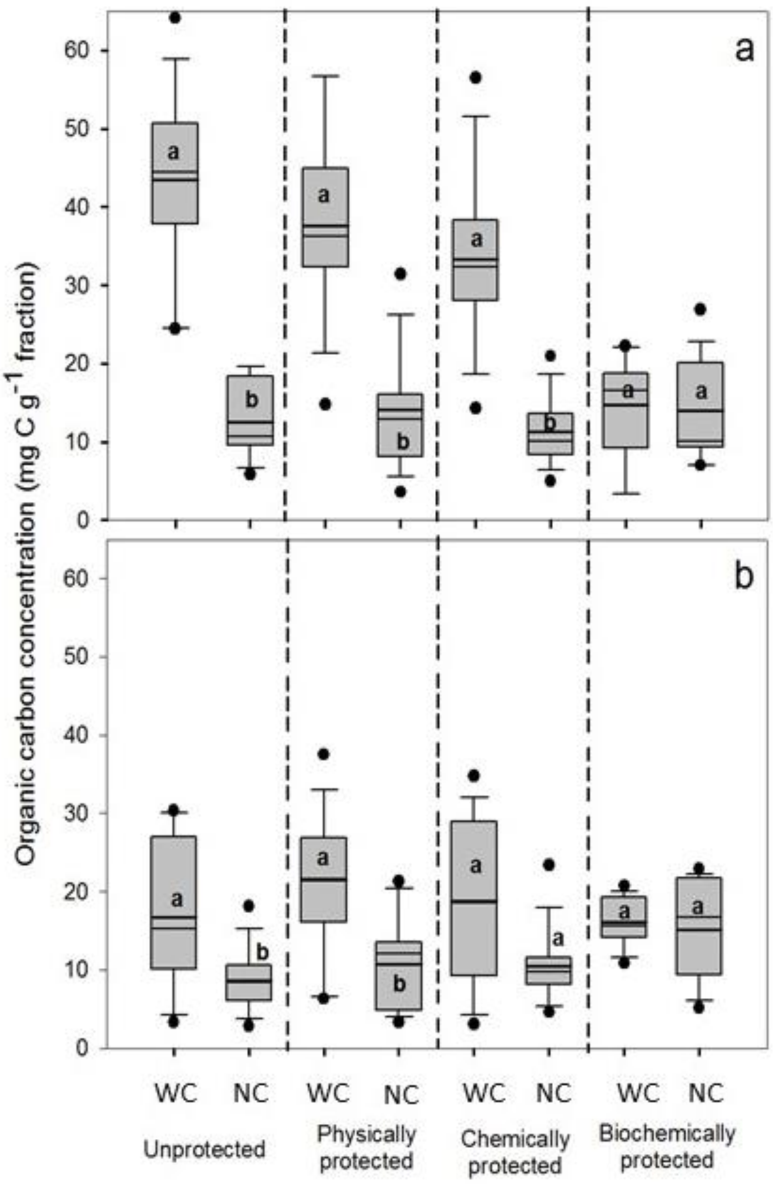


Figure 6. Box-plot representation of soil organic carbon concentration in the unprotected, and physically, chemically and biochemically protected organic carbon fractions of soils (0 – 5 (a) and 5 – 15 cm (b)) weed-covered (WC) and comparable non-covered (NC) olive oil farms. Boundaries of the boxes closest to, and furthest from zero indicate the 25th and 75th percentiles, respectively. The thin lines within the box mark the average. Bars above and below the box indicate the 90th and 10th percentiles, respectively. Outliers are represented as black dots. Average values with the same letter indicate no significantly differences between WC and NC olive orchards ($P < 0.05$).

Table 1. Values of soil organic carbon (SOC) present in soils. The amount of organic carbon present in the unprotected (U) and physically (PP), chemically (CP) and biochemically protected (BP) fractions (mg C g^{-1}) and their contribution (%) to the whole SOC in soils with (WC) and without (NC) spontaneous vegetation cover. Values show the mean and the standard deviation in brackets.

Site	Management	Depth (cm)	SOC (mg C g^{-1})	U (mg C g^{-1})	U (%)	PP (mg C g^{-1})	PP (%)	CP (mg C g^{-1})	CP (%)	BP (mg C g^{-1})	BP (%)
CA1	WC	0-5	33.3 (1.1)	8.4 (0.6)	25.31 (2.43)	7.2 (0.9)	21.65 (1.99)	13.2 (0.6)	39.60 (0.70)	4.5 (0.3)	13.44 (1.08)
CA1	WC	5-15	21.3 (1.4)	2.5 (0.04)	11.93 (0.89)	4.7 (0.6)	22.23 (2.17)	10.4 (0.9)	48.73 (1.47)	3.7 (0.4)	17.12 (1.15)
CA1C	NC	0-5	12.9 (0.5)	4.4 (1.9)	34.24 (13.64)	1.8 (0.6)	13.69 (4.27)	4.7 (1.8)	37.19 (15.36)	1.9 (0.05)	14.84 (0.90)
CA1C	NC	5-15	10.5 (3.6)	2.3 (1.5)	20.84 (7.63)	1.3 (0.5)	14.06 (7.43)	5.3 (2.9)	48.98 (9.60)	1.5 (0.4)	16.19 (8.43)
CA2	WC	0-5	35.2 (8.1)	8.6 (2.5)	24.49 (4.56)	3.5 (2.0)	10.48 (6.15)	16.8 (5.2)	47.12 (4.25)	6.4 (1.7)	17.91 (1.04)
CA2	WC	5-15	21.2 (5.6)	3.5 (0.9)	16.58 (1.34)	2.2 (1.0)	10.51 (3.46)	10.6 (2.7)	50.18 (3.12)	4.9 (1.5)	22.75 (2.16)
CA2C	NC	0-5	14.8 (3.5)	2.1 (0.1)	14.81 (2.85)	3.8 (1.1)	25.11 (1.41)	5.7 (2.2)	37.62 (5.28)	3.2 (0.2)	22.47 (3.81)
CA2C	NC	5-15	13.3 (1.3)	1.3 (0.4)	9.79 (2.30)	3.0 (0.5)	23.05 (5.45)	5.6 (1.0)	41.55 (4.69)	3.4 (0.4)	25.63 (1.37)
CT	WC	0-5	44.8 (2.5)	25.7 (2.6)	57.26 (3.73)	7.3 (2.1)	16.43 (5.44)	11.2 (2.5)	24.87 (4.25)	0.6 (0.2)	1.44 (0.40)
CT	WC	5-15	27.0 (2.8)	12.3 (3.0)	45.56 (9.09)	3.5 (0.6)	13.18 (2.64)	8.0 (2.6)	29.31 (8.12)	3.2 (0.2)	11.94 (1.75)
CTC	NC	0-5	7.2 (1.4)	2.3 (0.7)	32.56 (4.94)	1.3 (0.5)	17.29 (3.86)	2.0 (0.6)	27.45 (3.87)	1.5 (0.3)	22.70 (9.54)
CTC	NC	5-15	7.0 (1.3)	1.8 (0.5)	25.68 (2.76)	1.5 (0.6)	21.63 (6.90)	0.6 (1.0)	9.85 (16.76)	3.0 (1.5)	42.80 (20.02)
MO	WC	0-5	34.6 (1.8)	14.0 (0.4)	40.78 (2.84)	9.0 (2.1)	26.23 (6.30)	7.01 (1.5)	20.33 (4.35)	4.5 (4.0)	12.66 (10.92)
MO	WC	5-15	12.1 (1.3)	2.9 (0.8)	23.50 (4.80)	3.6 (0.4)	29.66 (0.93)	0.6 (0.07)	5.13 (0.92)	5.0 (0.6)	41.75 (5.15)
MOC	NC	0-5	13.1 (0.8)	3.8 (0.2)	29.36 (3.32)	4.0 (1.1)	30.68 (9.17)	2.5 (0.5)	19.00 (4.99)	2.8 (0.5)	21.46 (2.42)
MOC	NC	5-15	12.6 (0.4)	3.4 (0.7)	27.01 (6.71)	2.7 (0.4)	21.73 (3.50)	2.6 (0.4)	20.43 (2.79)	3.9 (0.3)	30.93 (1.46)
LO	WC	0-5	11.5 (1.8)	2.0 (1.1)	17.39 (7.07)	1.8 (1.1)	15.46 (6.94)	1.6 (0.3)	14.39 (4.22)	6.0 (0.7)	52.76 (9.99)

LO	WC	5-15	8.7 (1.4)	0.8 (0.4)	8.75 (3.89)	1.2 (0.3)	13.86 (2.36)	4.2 (1.3)	47.07 (8.35)	2.6 (0.5)	30.32 (9.81)
DE1	WC	0-5	21.7 (8.8)	7.3 (3.8)	30.50 (12.06)	5.2 (1.7)	25.97 (9.29)	6.2 (2.3)	29.49 (4.76)	3.0 (1.0)	14.04 (1.55)
DE1	WC	5-15	17.2 (1.7)	3.8 (0.4)	22.28 (4.68)	3.1 (1.2)	18.26 (7.54)	6.1 (2.3)	34.86 (9.74)	4.2 (0.6)	24.63 (1.78)
DE1C	NC	0-5	13.3 (1.5)	3.5 (2.0)	25.79 (12.05)	5.7 (1.5)	43.28 (13.74)	2.9 (0.7)	21.91 (4.65)	1.2 (0.2)	9.05 (0.77)
DE1C	NC	5-15	7.3 (0.4)	1.6 (0.2)	22.65 (2.30)	1.6 (0.1)	22.14 (0.76)	2.7 (0.2)	33.52 (0.67)	1.6 (0.3)	21.79 (3.51)
DE2	WC	0-5	34.5 (2.6)	16.1 (2.5)	46.44 (3.63)	8.5 (1.8)	24.75 (5.07)	6.5 (1.3)	19.06 (4.34)	3.3 (0.3)	9.76 (1.18)
DE2	WC	5-15	34.3 (3.7)	15.8 (3.7)	45.93 (5.85)	8.6 (1.9)	25.33 (6.29)	7.3 (2.7)	21.20 (8.22)	2.6 (0.5)	7.55 (1.81)
PE	WC	0-5	21.8 (0.7)	11.5 (0.8)	52.64 (4.86)	2.7 (1.9)	12.57 (8.74)	4.9 (0.4)	22.51 (2.19)	2.7 (0.4)	12.26 (1.95)
PE	WC	5-15	17.3 (0.7)	9.8 (2.4)	56.26 (11.52)	2.0 (0.08)	11.59 (0.81)	3.3 (1.3)	19.45 (7.97)	2.2 (0.6)	12.72 (4.74)
JA	WC	0-5	19.5 (0.5)	3.2 (0.2)	16.59 (0.56)	1.7 (0.7)	8.66 (3.54)	12.6 (0.9)	64.60 (3.37)	1.9 (0.4)	10.14 (2.41)
JA	WC	5-15	14.1 (0.7)	1.0 (0.2)	6.78 (1.28)	1.4 (0.3)	9.97 (2.34)	10.2 (0.9)	71.95 (4.51)	1.6 (0.1)	11.32 (0.89)
AL	WC	0-5	21.6 (3.1)	3.9 (1.4)	18.77 (9.04	4.8 (0.8)	22.41 (1.49)	9.8 (3.1)	44.88 (7.81)	3.0 (0.7)	13.95 (1.42)
AL	WC	5-15	13.8 (2.4)	0.9 (0.3)	6.80 (2.31)	2.1 (0.5)	15.10 (0.80)	7.0 (1.8)	50.55 (3.82)	3.8 (0.3)	27.58 (3.72)

4.2 Influence of the soil mineralogical and geochemical properties, and management on SOC fractions

Effects of parent material and management on SOC fractions

Figure 7 shows an overview of the SOC content in the different fractions in soils under different parent material and soil managements. Highest values of SOC were found in the calcareous soils. This was due to higher values of SOC measured in the unprotected, chemically protected and physically protected fractions. The content of unprotected SOC in the siliceous soils averaged 2.9 mg C g^{-1} whereas carbonated figures averaged 11.5 mg C g^{-1}. Values of the physically protected were 4.3 and 6.8 mg C g^{-1} and chemically protected averaged 2.0 and 3.2 mg C g^{-1}, in siliceous and in carbonated plots, respectively. Interestingly, SOC in the biochemically protected fraction was significantly higher in the siliceous soils (2.1 and 1.6 mg C g^{-1}) (Table 2 and figures 7 and 8b).

The percentage contribution of the fractions to the total SOC differs in both soil types. In the calcareous soils about 42 % of the SOC was considered unprotected, whereas the contribution of the physically protected fraction accounted for about 33 % (Table 3, and figures 7 and 8d). However, in the siliceous soils, the fraction which contributed the most was the physically protected (39%). The organic carbon in the unprotected fraction contributed to the total SOC half of that of the calcareous soil. Finally, the biochemically protected fraction accounted for 19 % of the total pool of SOC in the siliceous soils but less than 8 % in the calcareous (Table 3 and figures 7 and 8d).

As it was shown in Chapter IV for these soils, SOC in soils under an herbaceous cover was significantly higher than in non-covered soils (2.3 and 1.2%, respectively) (figure 8a), and this was true independently of the parent material. Unprotected, physically and biochemically protected organic C were significantly higher in the weed-covered plots (3.2, 1.9 and 1.6 times), whereas no significant differences were observed between both managements in the chemically protected pool (Table 2 and figures 7 and 8a). For the unprotected pool, interaction between management and parent material was significant (Table 2 and figures 7 and 8a). Thus, weed-covered soils had higher content of unprotected SOC than non-covered soils but only under a calcareous parent material.

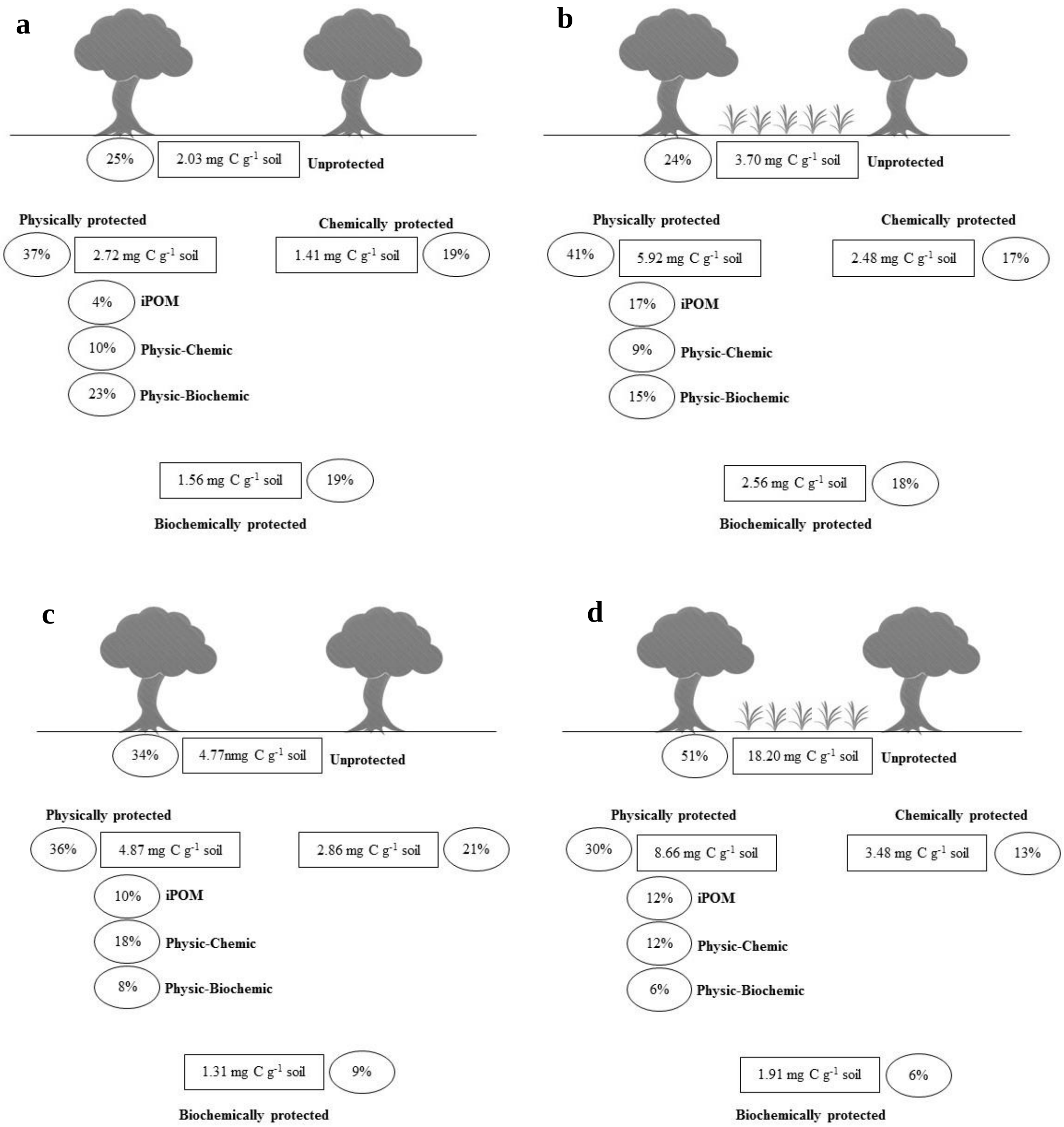


Figure 7. Mean values of soil organic carbon (mg C g^{-1} soil) and percentage of a) Non-covered siliceous plots, b) Weed-covered siliceous plots, c) Non-covered carbonated plots and d) Weed-covered carbonated plots. This figure is an overview of the results found in the experiment consisting on contrasting management and parent material. Thus, it can help to better understand figure 8 and tables 2 and 3.

Interestingly, the percentage contribution of the unprotected, physically protected and biochemically protected to the overall SOC did not differ between managements when data of different parent material were grouped (Table 3 and figures 7 and 8c). Only the percentage of organic C linked to the silt and clay was higher in the non-covered soils. However, the effects of management in the contribution of the different fractions were dependent on the parent material (Table 3 and figures 7 and 8d). Thus, contribution of the unprotected SOC to the total SOC were higher in the weed-covered plots only under carbonated parent material. However, contribution of the chemically and biochemically protected SOC was lower in the weed-covered plots but only in the carbonated soils. Typically, the biochemically protected C contributed the lowest to the total SOC in soils under contrasted parent material (Table 3 and figures 7 and 8d).

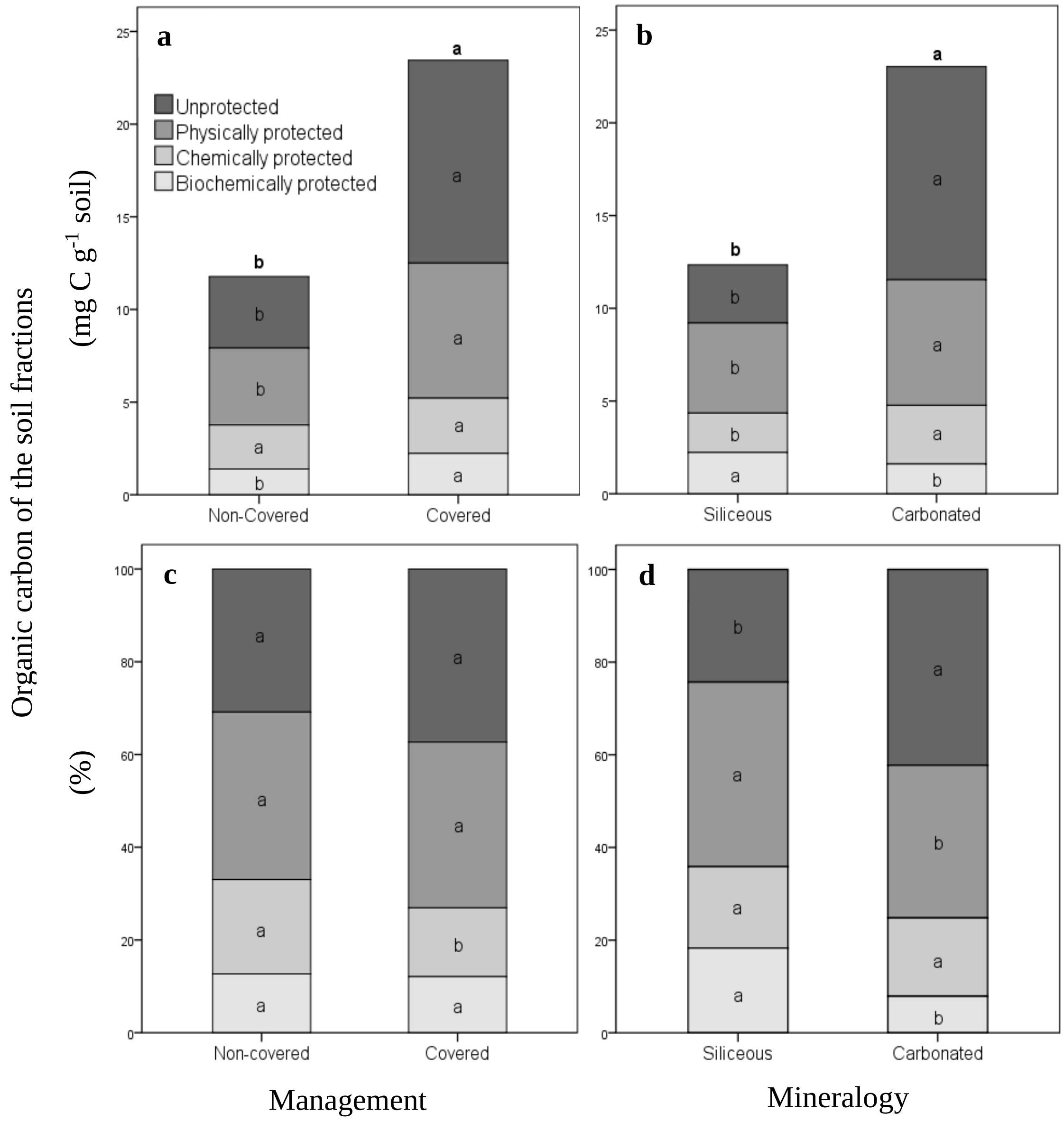


Figure 8. Mean values of soil organic carbon in the unprotected, physically, chemically and biochemically protected pools (in mg C g^{-1} soil and percentage of the total soil organic carbon). Different letters represent significant differences between managements (non-covered and weed-covered) and parent materials (siliceous and carbonated) at $P < 0.05$.

Table 2. Mean values and standard deviation (in brackets) of soil organic carbon content of the four fractions (unprotected, physically, chemically and biochemically protected) and the sub-fractions of the physically protected (mg C g^{-1} soil). Bellow, the results of the ANOVA.

Mineralogy	Management	Unprotected	Physically protected	Chemically protected	Biochemically protected	Phisico-biochemically protected	Physico-chemically protected	iPOM
Siliceous	NV	67.6 (15.9)	21.4 (2.9)	5.2 (0.8)	10.1 (1.4)	7.1(1.7)	3.1 (0.5)	11.1 (3.9)
	WC	3.7 (1.9)	5.9 (1.0)	2.5 (0.9)	2.6 (0.6)	2.2 (0.9)	1.3 (0.5)	2.5 (0.5)
	NC	2.0 (1.1)	2.7 (0.5)	1.4 (0.2)	1.6 (0.8)	1.6 (0.5)	0.7 (0.1)	0.3 (0.2)
Carbonated	NV	31.8 (14.7)	19.0 (3.8)	8.5 (0.7)	7.9 (1.6)	5.4 (1.7)	5.4 (1.8)	8.2 (3.8)
	WC	18.2 (15.1)	8.7 (3.2)	3.5 (1.0)	1.9 (0.8)	1.8 (0.9)	3.3 (0.7)	3.6 (1.8)
	NC	4.8 (2.4)	4.9 (2.0)	2.9 (1.1)	1.3 (0.7)	1.2 (0.7)	2.3 (0.9)	1.4 (0.7)
	Management	** WC>NC	** WC>NC	ns	** WC>NC	* WC>NC	ns	*** WC>NC
	Mineralogy	** Ca>Si	** Ca>Si	*** Ca>Si	** Si>Ca	** Si>Ca	*** Ca>Si	* Ca>Si
	Man * Min	***	*	ns	ns	ns	ns	***

WC = weed-covered management; NC= non-covered management; Si = siliceous mineralogy; Ca = carbonated mineralogy. *, **, *** and ns = significant differences at P < 0.05, P < 0.01 and P < 0.001 and no significant differences, respectively.

Table 3. Mean values and standard deviation (in brackets) of soil organic carbon content of the four fractions (unprotected, physically, chemically and biochemically protected) and the sub-fractions of the physically protected (% of the total soil organic carbon). Bellow, the results of the ANOVA.

Mineralogy	Management	Unprotected	Physically protected	Chemically protected	Biochemically protected	Phisico-biochemically protected	Physico-chemically protected	iPOM
Siliceous	NV	64.2 (5.8)	20.7 (3.0)	5.1 (1.1)	10.0 (2.8)	7.1 (2.7)	3.1 (0.8)	10.6 (2.6)
	WC	24.0 (8.0)	41.2 (4.8)	17.0 (3.9)	17.8 (2.9)	14.9 (5.5)	8.7 (3.2)	17.6 (4.9)
	NC	24.9 (9.3)	37.0 (11.9)	18.7 (2.3)	19.4 (7.1)	22.6 (10.5)	10.2 (3.1)	4.2 (1.3)
Carbonated	NV	45.5 (12.7)	29.6 (9.3)	12.9 (2.1)	11.9 (2.9)	8.4 (3.7)	8.6 (4.0)	12.6 (5.9)
	WC	50.7 (11.4)	30.3 (7.4)	12.6 (3.7)	6.4 (1.3)	6.0 (1.8)	12.4 (4.4)	11.8 (2.6)
	NC	33.8 (7.8)	35.6 (5.0)	21.2 (3.6)	9.3 (2.1)	8.2 (3.0)	17.5 (3.2)	10.0 (2.1)
	Management	ns	ns	*** NC>WC	ns	ns	** NC>WC	*** WC>NC
	Mineralogy	*** Ca>Si	** Ca>Si	ns	*** Si>Ca	*** Si>Ca	*** Ca>Si	ns
	Man * Min	***	***	***	***	***	Ns	***

WC = weed-covered management; NC= non-covered management; Si = siliceous mineralogy; Ca = carbonated mineralogy. *, **, *** and ns = significant differences at P < 0.05, P < 0.01 and P < 0.001 and no significant differences, respectively.

Principal Component Analysis (PCA)

Eigenvalues from the PCA indicate that the first two principal components (PC) accounted for 80 % of the variance of data (PC1: 43.73%, PC2: 36.34%). Soil pH, carbonate and clay and calcite contents were all significantly positively correlated ($P < 0.05$) with scores of PC1, whereas sand and quartz contents were negatively correlated ($P < 0.05$) (Table 4 and figure 9).

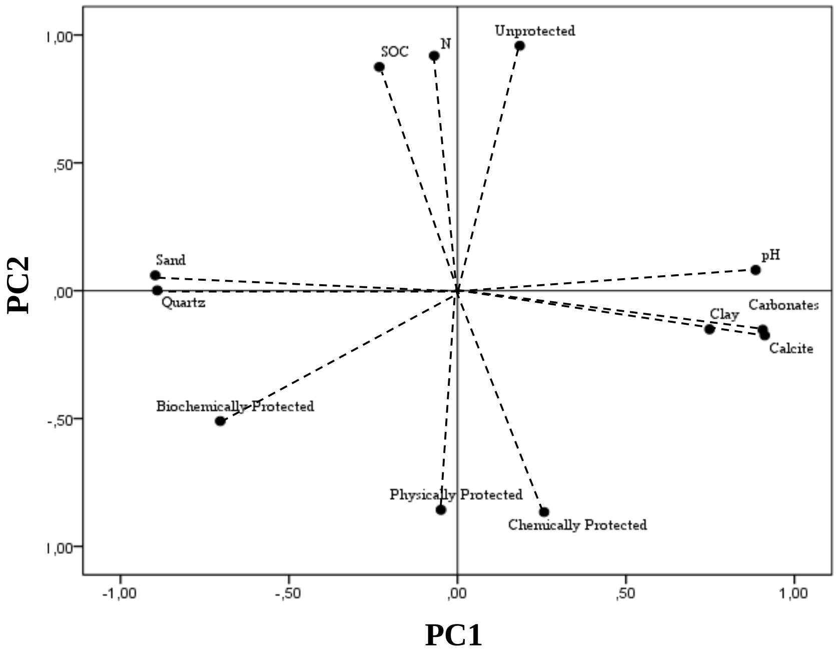


Figure 9. Ordination of the Pearson correlation coefficients of some of the physicochemical soil properties and percentage of the soil organic carbon fractions in the space defined by the PC1 and PC2 axis of the PCA analysis.

Table 4. Pearson correlation coefficients of the variables used in the PCA analysis for PC1 and PC2. SOC fractions were analized in terms of percentage of the total SOC.

Component	PC 1	PC 2
Sand	-0.897	0.060
Clay	0.748	-0.151
SOC	-0.232	0.875
N	-0.069	0.919
Quartz	-0.891	0.001
Ph	0.885	0.081
Carbonates	0.906	-0.153
Calcite	0.912	-0.175
Unprotected	0.185	0.958
Physically protected	-0.049	-0.857
Chemically protected	0.257	-0.866
Biochemically protected	-0.704	-0.510

Values with $r > 0.39$ are significant at $p < 0.01$ ($N = 45$).

Therefore, PC1 is clearly related to soil mineralogy. Total SOC and nitrogen (N), and the percentage of unprotected SOC were positively correlated with PC2, whereas physically and chemically protected SOC were negatively correlated with scores of this principal component (Table 4 and figure 9). PC2 seems to be related to presence of the spontaneous vegetation cover.

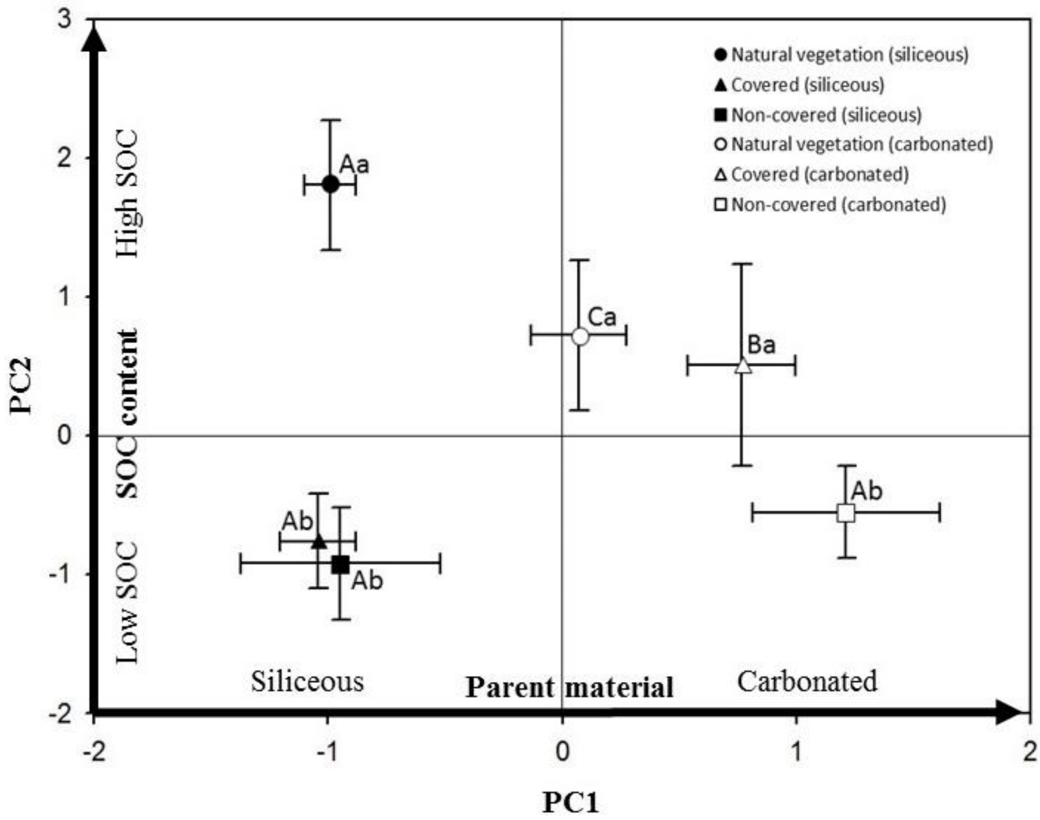


Figure 10. Ordination of the mean values of the siliceous and carbonated plots with the three different managements in the space defined by the PC1 and PC2 axis of the PCA analysis. Capital letters represent differences in the PC1 between plots with the same type of parent material at P < 0.05. Small letters represent differences in the PC2 between plots with the same type of parent material at P < 0.05. Differences between types of parent material are not shown in this figure.

Figure 10 shows the position of plots differing in vegetation management and soil mineralogy in the orthogonal space defined by the two PCs. Clearly, siliceous plots were allocated within the negative values of PC1 whereas the contrary was true for plots under calcareous parent material. On the other hand, soil samples collected in sites under Mediterranean forest had highest positive values of PC1 (e.g. higher content of total SOC, total N and percentage of unprotected SOC). The effect of the presence of the spontaneous vegetation cover differed according to the parent material. For siliceous soils non-covered and weed-covered plots distributed in a similar position, whereas for the calcareous soils, weed-covered plots showed higher values in PC2 than that of non-covered plots.

Correlations of some organic carbon sub-fractions with some soil properties

Table 5 shows that there is a significant positive correlation between the percentage of the chemically protected pools within soil microaggregates (53 – 250 µm) (H-µSilt + µClay) and the percentage of soil organic carbon linked to silt+clay in the fine fraction (< 53 µm) (H-dSilt + dClay), clay and total carbonates content, and soil pH.

The percentage of the pool of biochemically protected organic carbon within microaggregates (53 – 250 µm) (NH-µSilt +µClay) and in the fine fraction (< 53 µm) (NH-dSilt + dClay) showed an opposite behavior than the chemically protected pools. In this case these fractions showed significant positive correlations with sand and quartz content, and negative with pH and total carbonates. In the case of the biochemically protected pool in the fine fraction, it also showed a negative correlation with the clay content.

Table 5. Pearson-moment correlations between some soil physico-chemical properties and some SOC fractions (%). iPOM = internal particulate organic matter, H-µSil + µClay = organic carbon associated to silt and clay in the microaggregate, H-dSilt + dClay = organic carbon associated to silt and clay in the <53µm fraction, NH-µSil + µClay = biochemically protected organic carbon within microaggregates, NH-dSilt + dClay = biochemically protected organic carbon in the <53µm fraction. Data of the SOC fractions are shown in percentage (%) of total organic carbon. Significance: *< 0.05, **< 0.01 and ns = not significant. Higher intensity color indicates higher Pearson correlation (positive = green, negative = brown, no correlation = white).

Variables	Total Organic carbon	pH	Total carbonates	Sand	Clay	Quartz	NH-dSilt + dClay	H-dSilt + dClay	NH-µSilt + µClay	H-µSilt + µClay
Total organic carbon	1	ns	-.327*	ns	ns	ns	-.300*	-.767**	-.341*	-.587**
pH	ns	1	.771**	-.704**	.519**	-.861**	-.590**	ns	-.627**	.598**
Total carbonates	-.327*	.771**	1	-.729**	.559**	-.753**	-.554**	.366*	-.412**	.729**
Sand	ns	-.704**	-.729**	1	-.908**	.753**	.558**	ns	ns	-.602**
Clay	ns	.519**	.559**	-.908**	1	-.551**	-.401**	.309*	ns	.507**
Quartz	ns	-.861**	-.753**	.753**	-.551**	1	.601**	ns	.399**	-.579**
NH-dSilt + dClay (%)	-.300*	-.590**	-.554**	.558**	-.401**	.601**	1	ns	.520**	ns
H-dSilt + dClay (%)	-.767**	ns	.366*	ns	.309*	ns	ns	1	.312*	.678**
NH-µSilt + µClay (%)	-.341*	-.627**	-.412**	ns	ns	.399**	.520**	.312*	1	ns
H-µSilt + µClay (%)	-.587**	.598**	.729**	-.602**	.507**	-.579**	ns	.678**	ns	1

4.3 Humic substances fractionation

Results of the humic substances fractionation (TEC, HA and FA collection and the different index studied) and the analysis of the humin are shown in Table 6. Total extractable carbon (TEC) was 5.6 times higher in soils under natural vegetation cover than the soils under weed-cover management and 15.6 times higher than that of the non-covered soils in the siliceous soils. In the carbonated soils these values were lower, being 2.2 and 5.0 for the weed-cover and non-cover managements, respectively. These differences followed a similar behavior in the case of the humic acids (HA) and fulvic acids (FA). However the amount of the HA was higher, being the HA/FA ratio higher in the soils under natural vegetation cover followed by the weed-covered and the non-covered. However, values of HA/FA were closer between weed-covered and non-covered in the siliceous soils, whereas in carbonated soils HA/FA values were closer in the case of the soils under natural vegetation and weed-covered soils.

The humification index (HI), which measures the proportion of the non-humic to humic (HA + FA) substances in the siliceous than in the carbonated plots for WC and NC managements. However, the HI was higher in the NV carbonated soils. Thus, the proportion of the non-humic substances would be higher in the siliceous than in the carbonated plots for both NC and WC managements.

The degree of humification (DH), which measures the proportion of humic substances (HA + FA) to total extracted C (TEC). The DH was higher in carbonated than in siliceous plots, and in non-covered than in weed-covered soils, although these differences were much higher in the case of the siliceous soils. Therefore, these results suggest that soils under non-cover management would amount higher proportion – to the total extractable C – of humic substances than soils under a weed-cover management.

The humification ratio (HR) is very similar than the DH, but in this case measures the proportion of the humic substances to the total soil organic carbon. In this case results for the different managements were similar. Weed-covered and non-covered siliceous soils amounted about a 20 % of total humic substances of the total SOC. This value was higher in the carbonated soils, amounting about a 30 %. Thus, these results suggest that carbonated soils have higher proportion of humic substances than siliceous soils.

Table 6. Total soil organic carbon content (SOC; mg C g^{-1} soil), total extractable carbon (TEC; mg C Kg^{-1} soil), humic acids (HA; mg C Kg^{-1} soil), fulvic acids (FA; mg C Kg^{-1} soil), ratio humic/fulvic acids (HA/FA), Humification Index (HI), Degree of Humification (DH), Humification Ratio (HR), E_4/E_6 ratio, organic C in the humin (%), biochemically protected C in the humin (mg C g^{-1} humin).

Mineralogy	Management	SOC	TEC	HA	FA	HA/FA	HI	DH	HR	E_4/E_6	Organic C in the humin	Biochemically protected C in the humin
Siliceous	NV	105.1	47055	34215	4361	7.85	0.22	82.3	36.7	4.69	4.64	35.42
	WC	15.5	8460	2239	874	2.56	1.72	37.0	20.1	3.80	0.49	0.74
	NC	8.1	3018	1171	574	2.04	0.73	57.9	21.6	3.10	0.16	0.00
Carbonated	NV	57.8	37523	16424	4265	3.85	0.81	55.1	35.8	4.62	2.42	49.18
	WC	30.5	17210	7697	2179	3.44	0.68	60.6	33.8	4.62	1.68	25.97
	NC	15.3	7506	3014	1701	1.77	0.59	62.9	29.5	3.68	0.71	15.65

WC = weed-covered management; NC= non-covered management; NV = natural vegetation.

The E_4/E_6 ratio, which is inversely related to the content of recalcitrant substances of the humic acids, was higher in carbonated than in the siliceous soils. In both cases it was higher in weed-covered than in non-covered soils. Thus, these results suggest that the proportion of more labile organic carbon is higher in the carbonated and in the weed-coverd soils.

Unfortunately, the lack of replicates in the humic substances fractionation did not allow for the calculation of the significance of the differences due to the presence of spontaneous vegetation cover and parent material. Therefore, only some correlations were determined between total SOC content and humic substances.

There was a highly significant positive correlation between total SOC content and TEC, HA and FA contents (R^2 = 0.938; 0.997; 0.819, respectively, $P < 0.05$) (Table 7). The correlations of the total SOC content with the different humic index were significant although relatively low. Thus, there was a negative correlation with the HI (R^2 = -0.311), and positive correlations with the DH (R^2 = 0.504; $P < 0.05$) the HR (R^2 = 0.586; $P < 0.05$) (Table 7). A significant positive correlation was found for the E_4/E_6 ratio (R^2 = 0.577; $P < 0.05$) and also for the percentage of organic C in the humin (R^2 = 0.986; $P < 0.05$) and for the amount of the biochemically protected pool of organic carbon in the humine (R^2 = 0.555; $P < 0.05$).

Interestingly, the biochemically protected C in the humin in the non-covered siliceous soils was zero. That is surprising, since the organic C in the humine is supposed to be the most recalcitrant organic C and siliceous soils amounted higher biochemically protected C than the carbonated soils.

4.4 Linking SOC fractions from different fractionation methods

Table 8 shows the significances of the results of the correlations between SOC fractions measured with both fractionation techniques. Correlation between TEC and the unprotected SOC pool was highly significant (R^2 = 0.911; $P < 0.05$), and this was true for the relationship between the HA + FA and iPOM (R^2 = 0.945; $P < 0.05$), and HA + FA with the protected pool of organic carbon (R^2 = 0.791; $P < 0.05$).

Organic C content of humin was significantly related to the pool of organic carbon which is biochemically protected ($R^2 = 0.841$; $P < 0.05$), whereas the relation with the biochemically protected C in the humin was lower although significant ($R^2 = 0.566$; $P < 0.05$).

Table 7. Correlations resulting from the correlation between the total soil organic carbon content and: total extractable carbon, humic acids, fulvic acids, humic/fulvic acids ration, Humification Index, Degree of humification, Humification Ratio, E_4/E_6 ratio, content of the organic C in the humin, content of the biochemically protected C in the humin.

	TEC	HA	FA	HA/FA	HI	DH	HR	E_4/E_6	Organic C in the humin	Biochemically protected C in the humin
R^2	0.938	0.997	0.819	0.946	-0.311	0.504	0.586	0.577	0.986	0.555

All these correlations are significant at $p < 0.05$.

Table 8. Significance of the correlations between selected pairs of SOC fractions.

	TEC vs Unprotected	HA + FA vs iPOM	HA + FA vs Protected*	Organic C in the humin vs biochemically protected **	Biochemically protected C in the humin vs biochemically protected **
R^2	0.911	0.945	0.791	0.841	0.566

* Protected = Chemically + Biochemically + Phisico-chemically + Phisico-biochemically. It does not include the iPOM fraction.

** Biochemically protected = biochemically protected + physico-biochemically protected

All these correlations are significant at $p < 0.05$.

4.5 SOC fractions saturation

In this study it was assumed that SOC concentration is a proxy for soil C input. Stewart *et al*. (2008a) showed mathematically the relationship between the C concentrations of individual soil fractions and SOC concentration, allowing to express C saturation as a function of SOC concentration rather than soil C input. Nevertheless, we acknowledge the limitations to this analysis imposed by using soils from different environments, which will vary in their approximation of steady-state conditions.

Under this assumption, a linear relationship between whole SOC content and SOC content of the different fractions indicates the lack of C saturation dynamic, whereas fractions exhibiting either an asymptotic relationship are influenced by C saturation.

For the study of many weed-covered and non-covered olive orchards and the two depths (Study 1) (0 – 5 and 5 – 15 cm), data of SOC and SOC fractions across all sites, depths and managements were pooled to provide a wide range in SOC concentrations (5.6 to 47.7 mg C g^{-1} soil). The relationship between unprotected SOC and total SOC was best fitted to a linear function when pooled the 0 – 5 and 5 – 15 cm soil depths samples (R^2 = 0.87) (Table 9). However, for the physically and chemically protected SOC pools, both linear and saturation models showed similar regression coefficients (Table 9), and therefore they were indistinguishable. The biochemically protected SOC pool did not show significant regression either to a linear nor a saturation models (Table 9) and remained relatively similar and independently of the SOC content.

When considering the whole set of olive oil orchards differing in parent material and management (Study 2), the relationship between the pool of unprotected organic carbon and total SOC was best fitted a linear function (R^2 = 0.87, $P < 0.0001$) (Table 10 and figure 11a). However, a saturation type curve was also significantly fitted although with a lower regression coefficient (R^2 = 0.76, $P < 0.0001$). This was true even when soil samples corresponding to Mediterranean forest plots were removed from the regression analysis (data not shown).

Table 9. Regression coefficients for linear and saturation curves between soil organic carbon content in the fractions (mg C g^{-1} soil) and whole SOC (mg C g^{-1}). NA stands for no significant (P < 0.05) regression coefficient.

Fraction	Adjustment
Unprotected	Linear R^2 = 0.87 Saturation R^2 = 0.80
Physically protected	Linear R^2 = 0.78 Saturation R^2 = 0.77
Chemically protected	Linear R^2 = 0.63 Saturation R^2 = 0.61
Biochemically protected	Linear R^2 = NA Saturation R^2 = NA

The relationship between physically protected SOC and total SOC was best fitted to a saturation type curve (R^2 = 0.86, P < 0.0001) (Table 10 and figure 11b), but regression coefficient for the linear function was also very high and similar (R^2 = 0.82, P < 0.0001). SOC of the iPOM was linearly related to total SOC content, though significance of the saturation curve was only slightly lower than that achieved by the linear function (Table 10). On the other hand, the SOC of the silt and clay fraction within soil microaggregates showed a saturation relationship with total SOC, and this was especially true when only siliceous plots were considered (Table 10). The recalcitrant SOC within microaggregates was best fitted a linear function for the whole set of plots and also when siliceous and carbonated plots were considered separately (Table 10).

For the whole set of data, chemically protected SOC best fitted to a saturation type curve (Table 10 and figure 11c), however, this depended on the parent material. For carbonated soils, regression coefficient of the linear was slightly higher than that of the saturation model, whereas for soils of siliceous plots, a saturation type curve had higher regression coefficient (R^2 = 0.79; P < 0.0001) than that of the linear (R^2 = 0.69; P < 0.0001) (Table 10).

Best fitted curve between biochemically protected carbon and total SOC was linear (R^2 = 0.76; P< 0,001) (Table 10 and figure 11d) but this differed due to the type of mineralogy. For soils of siliceous mineralogy, linear and saturation curves yielded similar regression coefficients (Table 10), whereas for soils on calcareous parent material, a linear function was best fitted ($R^2 = 0.72$; $P < 0.0001$).

Table 10. Regression coefficient for the linear and saturation models between total SOC content and SOC of each isolated fractions, considering the whole set of plots and plots under different parent materials. Physically protected fraction is comprised of three sub-fractions: iPOM, chemically and biochemically protected within microaggregates.

Fraction/Sub-fraction	Whole set of plots		Siliceous		Carbonated	
	Linear	Saturation	Linear	Saturation	Linear	Saturation
Unprotected	0.87	0.76	-	-	-	-
Physically protected	0.82	0.86	-	-	-	-
iPOM	0.75	0.73	-	-	-	-
Chemically protected within microaggregates	0.26	0.49	0.72	0.79	0.63	0.65
Biochemically protected within microaggregates	0.75	0.66	0.87	0.82	0.73	0.66
Chemically protected	0.41	0.62	0.69	0.79	0.78	0.71
Biochemically protected	0.76	0.69	0.89	0.90	0.72	0.62

All the R^2 are significant at $p < 0.0001$ except the linear fit for the chemically protected within microaggregates considering the whole set of plots, which had a $P = 0.0004$.

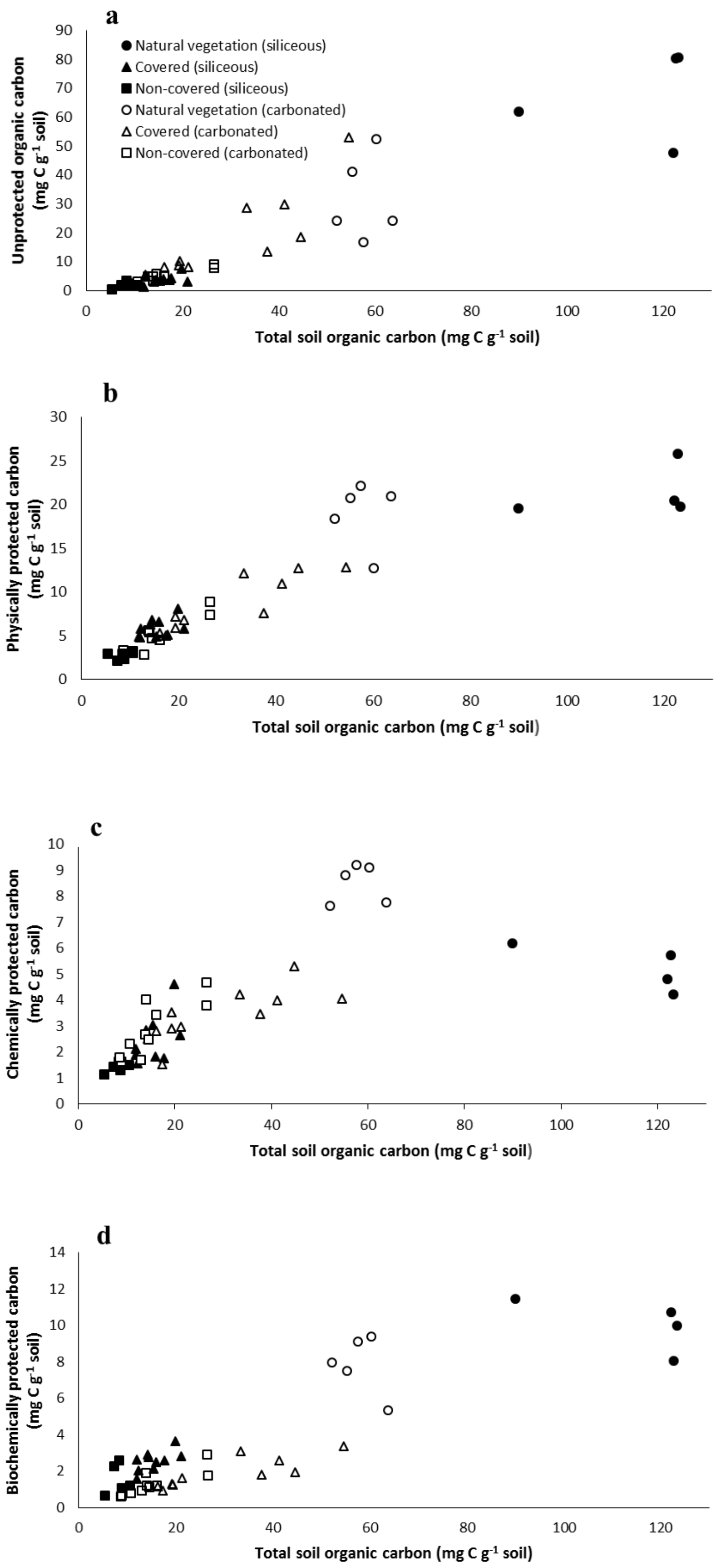


Figure 11. Relationships between soil organic carbon content and unprotected (a), physically (b), chemically (c) and biochemically (d) protected pools for the three managements (natural vegetation, weed-covered and non-covered) and the two parent materials (siliceous and carbonated).

5. Discussion

5.1 Effects of the presence of a spontaneous vegetation cover and soil mineralogy on SOC fractions

Soil organic C protection has been linked to physical soil properties (e.g. the amount, reactivity, and surface area of clay minerals). In addition, adsorption mechanisms have also been used to describe silt + clay SOC protection by Hassink and Whitmore (1997), who assessed the effect of soil texture on SOC accumulation by comparing three alternative models of physical protection. Finally, several researchers have proposed that the capacity of the soil to sequester C is based on more than just the chemical association with silt and clay, being attributable to aggregate protection and biochemical recalcitrance as well.

Each mineral soil matrix had a unique capacity to stabilize organic C depending not only on the presence of mineral surfaces capable of adsorbing organic materials (a protective capacity), but also the chemical nature of the soil mineral fraction, the presence of cations, and even the architecture of the soil matrix (Baldock and Skjemstad, 2000). Thus, both soil mineralogy and soil managements which are involved in net changes in organic C inputs and/or decomposition, might exert an important effect on SOC fractions.

In the study designed to evaluate the effect of soil mineralogy on SOC fractions, all SOC fractions of weed-covered plots, except chemically and physico-chemically protected pools, had higher SOC content than the non-covered plots (Table 2 and figure 8a). Nevertheless, the effects of the presence of weeds on these fractions were different in the carbonated and siliceous soils. The increase in the SOC in carbonated soils was mainly due to the unprotected and physically protected fractions, especially in the iPOM, and to a lesser extent to the chemically and biochemically protected fractions (Table 2, figures 7c and 7d). However, the increase in the siliceous soils was due especially in the physically protected fraction (Table 2, figures 7a and 7b). This could be due to expected higher decomposition rates in the siliceous soils due to the relatively high saturation deficit of the protected fractions, whereas in carbonated soils, with a higher total SOC content, the fresh OM remains in the unprotected pool. Moreover, part of this fresh OM feed the physically protected fraction as iPOM, explaining, at least partially, the fact that the unprotected and iPOM pools were higher under a weed-cover management. Table 2 shows that iPOM is the only fraction within microaggregates that is higher in weed-cover and also in carbonated plots, so we can assume that a great part of the "new" OM incorporated within microaggregates is POM.

A similar pattern was found in the fractions obtained in the study designed to evaluate the effects of the weed-cover on SOC fractions in five pair of weed-covered and non-covered olive orchards. Unprotected, and physically and chemically protected organic C fractions were significantly higher in soils covered with spontaneous vegetation (figure 5). Highest increase was achieved for the cPOM (coarse POM, SOC of > 250 µm, i.e. the main part of the unprotected pool) fraction due to an increase in the organic C concentration of this fraction (e.g. mg C g^{-1} of the fraction; between 2.5 to 7.3 times higher than that soils of the non-covered plots) (figure 6). This was not unexpected, as recently derived, partially decomposed spontaneous plant residues together with seeds and microbial debris, such as fungal hyphae and spores that are not closely associated with soil minerals constitute the unprotected SOC pools. On the other hand, physically protected C was from 1.8 to 10.8 higher in olive oil orchards covered with spontaneous herbaceous plant (Table 1).

The result of the unprotected fraction in both studies (in the five-paired plots and in that assessing the influence of the parent material) are in line with recently study by Cardinael *et al*. (2015), which studied the impact on SOC stocks of alley cropping agroforestry in southern France. They found that the increase in the incoming OC in plots with an herbaceous plant cover led to an increase in the most labile fractions of the topsoil, but did not in the SOC associated to the fine fractions. Results of this study are also in line with those of other studies which showed an increase in the unprotected SOC pool after the implementation of management practices which involved an increase in the input of organic C. This pool should not be considered as a pool of C sequestrated at the long term, as it is highly sensitive to management practices (Six *et al*., 1999; Six *et al*., 2002) and, consequently, highly influenced by future soil managements. Indeed, many early studies have found that the LF (ligh fraction, i.e. fine POM) and cPOM are relatively easily decomposable and are greatly depleted upon cultivation (e.g. Cambardella and Elliott, 1992; Six *et al*., 1999; Solomon *et al*., 2000), indicating their relatively unprotected (biochemical and physical) status.

The physical protection exerted by macro- and/or microaggregates on organic C is attributed to the compartmentalization of substrate and microbial biomass (Killham *et al*., 1993; van Veen and Kuikman, 1990), the reduced diffusion of oxygen into macro and especially microaggregates (Sexstone *et al*., 1985) resulting in a reduced activity within the aggregates (Sollins *et al*., 1996). Although in these two studies the amount of soil aggregates or soil aggregate stability were not measure, it is relatively well documented that plant residues serves, following the decomposition, as a binding agent to hold soil particles together forming aggregates (Jastrow *et al*., 1998). Recently, Garcia-Franco *et al.* (2015) showed after 4 years of green manuring in an almond orchard, that the formation of micro and macro aggregates were promoted. Therefore, the presence of a plant cover and the surface displacement of the plant residues increased the amount of SOC protected by physical means. In addition, the soil organic C of the silt+clay particles (< 53 µm) within micro aggregates (53 – 250 µm) were higher in the weed-covered soils (figure 5, and Table 2 and figure 8a), suggesting that the formation of microaggregates within macroaggregates is increased after the surface application of plant cover residues. The release of biogenic products and other binding agents, such as polysaccharides and root exudates (Puget and Drinkwater, 2001), during the incorporation and relatively-rapid decomposition of the residues of the plant cover may have promoted the solid-phase reaction between organic matter and clay and silt particles, leading to the formation of stable microaggregates (Golchin *et al*., 1994). The results of this study indicate that a significant part of the C stabilization is due to physico-chemical protection of OC by mineral particles (Krull *et al*., 2003; Bronick and Lal, 2005). This result is in line with those of Garcia-Franco *et al*. (2015) who found that the proportion of microaggregates within small macroaggregates increased after green manuring together with reduced tillage. The higher OC concentration found in the study of 5 paired weed-covered/bare soils comparison in both the free and occluded POM in the weed-covered plots, relative to bare plots, (figure 6) can be beneficial to long-term SOC sequestration because microaggregates have longer turnover times and higher stability than macroaggregates (Denef *et al*., 2007; Huang *et al*., 2010), indicating the potential of this management practice to promote SOC accrual and stabilization. SOC concentration of the silt+clay particles separated by wet sieving in soils covered by wild herbaceous plant community was on average 3.2 times higher than that of soils under non-cover management.

Nevertheless, the incorporation of plant residues as unprotected SOC is the first step in the C sequestration process. In a conceptual model of SOM dynamics with measurable pools given by Six *et al*. (2002), the SOC protected fractions (physically, chemically and biochemically) arise from the unprotected SOC pool and the proportion of the SOC that will be mineralized and protected in the other fractions will depend on the soil mineralogy properties and management practices.

Interestingly, in both studies, the content of the biochemically protected pool showed a direct relationship with the total SOC content (although these results were much clearer in the study comprising different parent materials) (figure 8a). These results question the accepted theory wich comprised these fractions into the "passive" pool (Leavitt *et al*. 1996; Trumbore, 1993). Other authors obtained similar results than ours. Balesdent *et al*. (1996) did not find evidence of the relationship between recalcitrance and hydrolysability. v. Lützow *et al*., (2006) in a deep revision of the SOC fractionation methods explained that acid hydrolysis with HCl 6N removes easily degradable materials (e.g. carbohydrates and proteins) by disruption of hydrolytic bonding and, thus, only biologically recalcitrant alkyl and aryl materials stay intact, such aromatic humified components and wax-derived long chain aliphatics (Paul *et al*. 1997). Paul *et al*. (2006) found a relationship between total SOC and the non-hydrolysable fraction and that "it can be rapidly lost or gained after changes in soil management, such as afforestation or cultivation", suggesting that other mechanisms besides recalcitrance would be involved in the persistence of this fraction. This theory is supported by v. Lützow *et al*., (2006) who affirm that the organic compounds accessible to microorganisms can be mineralized and, therefore, also the biochemically protected pool in the fine fraction. However these authors also suggest that sometimes, at the beginning, many different groups of microorganisms are necessary to decompose these compounds and, therefore, some recalcitrance is shown especially during the first steps of the decomposition. However, they also pointed out that it was necessary that these compounds were associated to some hydrophobic substances to keep away from the microorganisms and, thus, not to be mineralized. Nevertheless, results of our study show that the dynamics of the physico-biochemically and biochemically protected fractions are very similar (Tables 2 and 3), indicating that physical protection does not affect the dynamic of the non-hydrolysable fractions.

The magnitude of the SOC accumulation in the four fractions varied depending on soil mineralogy (Tables 2 and 3, figure 8c and d). Accumulation of SOC in the unprotected, physically and chemically protected fractions was greater in carbonated than in siliceous soils. This was probably due to a high mineralization of the SOM and a very low vegetation cover biomass production – or crop residues – in the siliceous site (Los Pedroches Valley) (Parras-Alcántara *et al*., 2014). This fact is supported by the higher E_4/E_6 values in carbonated soils (Table 6), thus suggesting that these are formed by higher proportion of labile substances compared to those of the siliceous soils. However, the SOC in the biochemically protected pool in the fine fraction (< 53 µm) and also that which was found within microaggregates (53 – 250 µm) were higher in the siliceous plots (Table 2). This pattern is of interest because siliceous soils had lower total SOC content than the carbonated soils (see chapter IV and figure 8a). The mechanism responsible of this pattern must be strong because typically the lower is the SOC content of the soil, the lower is the content of biochemically protected SOC. Moreover, this pattern occurred only in the weed-covered plots, where the incoming OM is presumably much higher in carbonated than in siliceous soils. As the biochemically protected fraction showed similar dynamics in terms of concentration and percentage when comparing siliceous and carbonated plots (Tables 2 and 3), this fact will be further discussed below.

Total SOC content in the top 5 cm was higher than in the 5 – 15 cm soil layer, mainly due to higher unprotected and physically protected SOC organic carbon. This was not the case for the chemically and biochemically protected pools (figure 2). The higher unprotected SOC content in the upper soil layer was not unexpected, since the upper layer has a higher fresh OM content and, as it was commented previously, the cPOM (unprotected pool) and iPOM (part of the physically protected pool) consist of fresh OM.

The presence of a weed-cover and the parent material not only had effects on SOC fractions, but also in the percentage contribution of these fraction to the whole SOC.

In the study aimed to assess for the effect of the presence of a weed-cover and soil mineralogy on SOC fraction, the contribution (%) of the chemically protected organic C was lower in the weed-covered plots that in bare soils (figure 8c and Table 3). Although the percentage of the physically protected organic carbon was not significantly different between managements, the sub-fractions comprising this pool were. The proportion of the iPOM was higher in the weed-covered plots (Table 3), whereas the proportion of the physico-chemically protected pool was higher in the non-covered (i.e. bare) plots (Table 3). The inverse effect of the presence of a weed-cover on these two sub-fractions resulted in the lack of significance in the proportion of the physically protected organic carbon (Table 3 and figure 8c). This result is likely due to the fact that iPOM is a sub-fraction sensitive to the weed-cover management as it was shown previously. Nevertheless, the percentage contribution to the whole SOC of the unprotected SOC, which is an organic C fraction sensitive to a weed-cover management, did not differed between managements (figure 8c). However, that percentage was higher in olive orchards under carbonated than under siliceous soils (figure 8d). Two facts main explain this result; i) The highest annual weed biomass production expected on carbonated soils respect to that of the low fertility siliceous soils together with the reported low soil disturbance of uncovered olive orchard in the siliceous soil, and ii) higher transformation rates from the unprotected pool to other protected fractions (e.g. biochemically protected organic carbon) of the siliceous soils as the saturation deficit is relatively low.

The proportional contribution of organic fractions to the whole SOC in the siliceous soils was not sensitive to a change in the management. Lozano-García and Parras-Alcántara (2013) did not found changes in SOC content in dehesas in our siliceous site when changing from conventional to organic farming, and they highlight the role of the soil disturbance in the SOC content changes. In this case the reduced tillage combined with herbicides in the siliceous plots might cause a low disturbance in the NC plots. The proportion of the biochemically protected pool did not differed between managements despite it accounted for more SOC in the weed-covered plots. Similar results were obtained by Silveira *et al*. (2008), who found that in soils with different total SOC content and non-hydrolysable (e.g. biochemically protected) organic carbon, that the percentage of this fraction was similar in both soils. Thus, they concluded that non hydrolyzable C pool did not depend upon the total C content, but possibly the quality and the chemical characteristics of the organic materials. A similar result was also found by Plante *et al*. (2006), who concluded that the percentage of the non-hydrolysable organic C did not depended on the initial SOC content and, therefore the percentage of this fraction is invariant with the management treatment. In addition to this, the proportion of the biochemically protected pool in the siliceous plots was about two times higher than that of the carbonated, and this difference was found for both managements (figure 7 and figure 8b). This might be related to the low quality of the weed residues under siliceous parent material and/or the enhancement of the poor studied complex processes that render biochemically protected organic carbon. Indeed, soil C-to-N ratio, which is a classical index to describe the recalcitrance of the SOM (Vallejo, 1993), under siliceous parent material was relatively high.

Nevertheless, it is necessary to analyse deeply the influence of the different variables on the proportion of the SOC fractions. For that purpose, a principal component analysis was carried out.

5.2 PCA as an overview of the influence of management and mineralogy on SOC and other soil properties

The role of the soil mineralogy in the soil organic C fractions and soil properties was clear as PC1 arranged the plots according to their mineralogy (figures 9 and 10, and Table 4). On the other hand, the effects of the presence of the weed-cover were also clear, especially for the calcareous soils, as PC2 distributed plots according to the annual inputs of organic carbon which was directly related to the presence of weed cover (i.e. higher total SOC content) (figure 9 and 10 and Table 4). Importantly, as the variance explained by PC1 and PC2 was similar, the contribution of both, soil mineralogy and the presence of weed-cover, was also similar.

As it was discussed above, the weed-cover management in the siliceous plots presumably produced much less annual biomass from the spontaneous resident vegetation cover than those of the carbonated plots. On contrary, differences between bare and weed-cover plots in the carbonated soils were clear.

PCA also shows that the proportion of the most labile fraction – the unprotected – is related to soils with a high total SOC content, whereas the proportion of the physically and chemically protected pools was related to soils with low total SOC content (figure 9 and Table 4). The proportion of the biochemically protected pool depends not only on soil properties but also on the amount of the incoming organic C. The proportion of this fraction is higher in acid soils with a lower total SOC content and higher mineralization rates than those of the carbonated soils (figure 9).

5.3 Total SOC, management and humic substances

The total SOC was highly related to the TEC and HA content, the HA/FA ratio and the organic C content in the humin (Table 7). This indicate that the higher is the SOC content the higher is the content of TEC and HA (Table 6 and 7). This direct relationship between HA/FA and SOC content was also found by Aranda *et al.* (2011) in olive grove soils in Sierra Mágina (Jaén) (calcareous parent material). These results are not surprising, since the HA/FA ratio is related to the aromaticy of the HA and the organic carbon content (Kononova, 1966). As a result of this fact, the relationship between the total SOC and the HI (proportion of non-humic to humic substances) was negative; the relationship with the DH (proportion of humic substances to TEC) was positive; and, finally, the relationship with the HR (proportion of humic substances to TEC) was also positive (Table 7). Therefore, these results suggest that higher SOC leads to higher proportion of humic substances (HA + FA), but the HA has a greater increase than the FA.

Furthermore, the mineralogy played an important role, since it affected the HA/FA results, especially in the case of the weed-cover management, which showed 1.34 times higher HA/FA values than that of the siliceous plots (Table 6).

The E_4/E_6 ratio is considered to be inversely related to the degree of condensation and aromaticity of the humic substances and to the level of humification of SOM (Stevenson *et al*., 1994; Senesi *et al*., 2003). E_4/E_6 ratio was positively correlated with total SOC (Table 7), and therefore soils with higher SOC showed lower levels of condensation and aromaticy. The explanation is the following. Soils of olive groves with high SOC content corresponded to those olive groves with a weed-cover management. The weed-cover management leads to a higher incoming fresh OM. This fresh OM input is made of aliphatic compounds, with lower aromaticy and, therefore, easily decomposable. Nevertheless, paradoxically, higher input of fresh organic matter also leads to higher total SOC and higher amounts of aromatic compounds. In this line, the correlation between the

HA+FA and the E_4/E_6 ratio is significantly high and positive ($R^2 = 0.852$; $P < 0.05$) (data not shown in the tables).

Therefore, in olive groves under a weed-cover management there is a high input of fresh OM which leads to a higher amounts of humic substances in the soil than the non-cover management. Indeed, the amount of the organic C in the humin and the biochemically protected C in the humin is higher with higher total SOC content (i.e. higher incoming organic C mainly through the resident vegetation cover) (Table 7). Nevertheless, part of this fresh OM is mineralized and released to the atmosphere. Therefore, the challenge of the weed-cover management is to minimize the mineralization of this incoming organic C.

5.4 Linking SOC fractions

TEC showed high correlation with the unprotected SOC fraction (Table 8). This is not surprising, since the TEC is high correlated with the TOC (Table 7). As it was shown previously, higher contents of total SOC leads to increase the content of the most labile fractions. In this line, the HA + FA content is high correlated with the iPOM (Table 8). As it has been commented, the iPOM increases with an increase in the unprotected pool.

Interestingly, there is a high correlation between the humic substances and the protected SOC (excluding from this analysis the iPOM fraction) (Table 8). It is well known that humic substances are substances resulting from high advanced degradation processes (e.g. Kononova, 1966). These substances might be those forming the SOC protected fractions, or part of them. However, it is very complicated to establish a clear association between fractions, as the humic substances fractionation is based on chemical features, whereas the fractionation from Six *et al*. (2002) is based on the dynamic of the SOC fractions, and it is possible that one fraction from the Six *et al*. (2002) fractionation is formed by substances with different chemical features.

Finally, the study of the relationship between the biochemically protected pool and the humin is also complicated. A high correlation between the organic C in the humin and the organic C content in the biochemically protected pool was found (Table 8). Humin is supposed to have high recalcitrant features so this relationship is very interesting.

However, the correlation is lower when assessing only the biochemically protected C in the humin with the biochemically protected pool. Neverhtles, in both cases the correlation was positive and significant (Table 8). However, in this sense, it is remarkable that the biochemically protected C in the humin in the weed-covered siliceous soils was zero (Table 6). This is surprising, since, as it was commented previously, the siliceous soils amounted higher levels of biochemically protected C. Therefore, according to these results, the organic C in the humin might not correspond to the recalcitrant C. Clearly, in this case is not possible to associate the organic C in the humin with the organic C in the biochemically protected pool.

These relationships represent only a first step towards a new model which should be proposed in the future, in which chemical and dynamical features will be combined in order to understand not only the dynamic of the different SOC fractions into the soil but also their chemical interactions with soil minerals through knowing their chemical structure.

5.5 Do SOC fractions show a saturation pattern?

We assumed that SOC concentration is a proxy for soil C input. Stewart *et al*. (2008a) showed mathematically the relationship between the C concentrations of individual soil fractions and total SOC concentration. This allows expressing C saturation as a function of SOC concentration rather than soil C input. Nevertheless, we acknowledge the limitations to this analysis imposed by using soils from different environments, which will vary in their approximation of steady-state conditions.

Under this assumption, a linear relationship between whole SOC concentration and SOC content of the different fractions indicates the lack of C saturation dynamic, whereas fractions exhibiting either an asymptotic or an exponential relationship are influenced by C saturation.

The range of SOC concentrations assessed for the saturation evaluation varied between 5.6 to 47.7 mg C g^{-1} and 5.4 to 123 mg C g^{-1} for the studies aimed to evaluate the effects of soil depth weed-cover management, and weed-cover management and soil mineralogy on SOC fractions, respectively.

Unprotected

In those olive orchard sites with SOC concentration ranging 5.6 to 47.7 mg C g^{-1}, the linear pattern between SOC and the unprotected pool for the whole set of data (R^2 = 0.87) (Table 9) indicates that this pool did not fit to the hypothesis of C saturation. The pattern was the same when the range of SOC was wider (5.4 to 123 mg C g^{-1}; R^2 = 0.87, Table 10 and figure 11a). This finding is in line with Stewart *et al*. (2008a), who found that in 100% of the soils collected from agroecosystems differing in annual C inputs, the cPOM was best fitted to a linear function, and this was true in the 75% of the sites for the LF. Numerous studies have demonstrated that the unprotected fraction is formed by easily decomposable SOC (e.g. Cambardella and Elliott, 1992; Six *et al*., 1999; Solomon *et al*., 2000) suggesting, therefore, that this fraction is not a protected pool.

Protected fractions

For the range of SOC of 5.6 to 47.7 mg C g^{-1}, relationship between whole SOC and concentration of organic C physically protected from microbial activity follows both a linear and a saturation type curve for the whole set of plots (Table 9).

However, when the range of SOC was 5.4 to 123 mg C g^{-1}, the physically protected pool was best fitted to a saturation curve (Table 10 and figure 11b). The organic C within microaggregates is not accessible to soil microorganisms, suggesting that a saturation level may exist, and thus the relationship between this fraction and SOC should be of a saturation type when a wide range of SOC is evaluated. Nevertheless, the iPOM was best fitted to a linear function, although difference with the R^2 of the saturation function was very small (Table 10). This finding is also in line with Stewart *et al*. (2008a) who suggested that the dynamic of the iPOM is similar than that of the unprotected pool. Values of the iPOM in non-covered and weed-covered plots respect to the maximum level achieved in Mediterranean forest site in the siliceous soils were between 3 and 23% and between 12 and 32%, respectively, in the carbonated site (data not shown). These relative low percentages respect to the forests site may indicate that this fraction does not show a saturation pattern. The chemically protected pool within microaggregates for the carbonated plots best fitted a saturation function (Table 10), but the R^2 was slightly higher than that of the linear. These results indicate that although this fraction could fit to a saturation curve (e.g. there is a limit on the amount of C protection), it follows also a linear function within the range of SOC considered. Furthermore, if we extrapolate this fitted saturation function (the saturation function of the chemically protected pool within microaggregates in calcareous soils) to higher total SOC values – in order to look for a saturation limit – for instance 100 mg C g^{-1} (which is about the maximum value reached in the soils under siliceous natural vegetation)[7], the SOC concentration in the fraction would be about 6.0 mg C g^{-1}. However, the current values of this fraction in the carbonated plots are 2.3 and 3.3 mg C g^{-1} for the non-cover and weed-cover soils, respectively, corresponding to the 55% and 38 % of the saturation values in weed-covered and bare soils. On the other hand, the chemically protected organic carbon within microaggregates in the siliceous plots showed a saturation pattern (Table 10), with a limit of 3 mg C g^{-1} for a total SOC of 120 mg C g^{-1}. This value is about half of that of the carbonated plots. Current values of the SOC in this fraction in the non-covered and covered soils which correspond to 23 and 43% of that of the soils under natural vegetation, respectively. These values are slightly lower than those for the carbonated plots. Stewart *et al.* (2008a) found that overall this fraction was best fitted to a saturation function, but it was only true for about a 55% of the sites analized, whereas the other sites best fitted to a linear function.

The dynamic of the biochemically protected pool within microaggregates and the magnitude of the differences respect to the Mediterranean forest soils were similar in the siliceous and carbonated plots (between 22 and 33%[7] of the SOC found in the Mediterranean forest for bare and weed-cover soils, respectively). These results were similar for the biochemically protected pool for the whole set of plots and for the fine fraction in the carbonated plots. Nevertheless, in the case of the siliceous plots the biochemically protected pool in the fine fraction the adjustment of the linear and

[7] For the carbonated plots, an upper limit of SOC content of 100 mg C g^{-1} has been selected in order to assess the saturation deficit and also to be comparable with those results obtained in the siliceous plots, where soil under natural vegetation cover reached this value.

saturation curves were similar (Table 10), but in any case the level of the biochemically protected pool in the fine fraction in non-covered and weed-covered soils would be by 16 and 24 % that of the soils under natural vegetation cover. Very similar results were found for the carbonated plots.

These results are in line with the results of the previous section, and also with Plante *et al.* (2006), showing that this fraction did not depend on the initial total SOC because the proportion of this fraction was similar in the non-covered and weed-covered managements and, therefore, there would not be a saturation behaviour. Nevertheless, Stewart *et al.* (2008a) found that 50% of the sites studied followed a linear function, whereas the other 50% were best fitted to a saturation function. This fraction depends on the quality of the vegetation (primary recalcitrance) and also on the soil properties which enhance complexation and condensation reactions (secondary recalcitrance) (v. Lützov *et al*., 2006), so the behaviour of this recalcitrant fraction may depend strongly on the site.

The biochemically protected fraction did not show any significant relationship with SOC when the range of SOC analized was 5.6 to 47.7 mg C g^{-1} (Table 9). The lack of relationship when SOC range was lower but the existence of relationship when SOC range was wider, highlights the high complexity and high site-specificity of the processes related to the biochemically protected pool

When the range of SOC was 5.4 to 123 mg C g^{-1}, the regression coefficients of the linear and saturation curves when relating the chemically or the physically protected organic carbon concentrations and SOC were indistinguishable (Table 10 and figure 11d). It has been theorised that the relationship between inputs of organic C and concentration of organic C chemically and physically protected should be of a saturation type. The content of silt+clay particles and the ability to form macro and microaggregates in a given soil are limited, and thus the amount of organic carbon protected throughout these mechanisms should be finite and a maximum should be achieved. When the protective capacity of organic carbon exerted by silt+clay particles and macro and microaggreates is exceeded, further C additions are not stabilized by these protective mechanisms and thus C accumulated in the various unprotected pools and relationship between concentration of organic C in the chemically and physically protected fractions and whole SOC should be of saturation. The facts that in the sites where SOC ranged 5.6 to 47.7 mg

C g^{-1} the physically and chemically protected organic C did not showed saturation (Table 9) were likely due to the relatively low range of whole SOC of compared to that of Stewart *et al*. (2007) and that of the study where SOC ranged 5.4 to 123 mg C g^{-1}. Typically (it depends of the parameters of the function) at the initial range of values, predicted values of a saturation type curve is rather similar than that of a linear function. Therefore, to achieve a clear saturation pattern, it is required a broad range of C inputs (or SOC). Indeed, in the eight long-term agroecosystem experiments of Stewart *et al*. (2007), the number of fractions fitting the C saturation model within each site was directly related to maximum SOC content. Only the two sites with the greatest SOC range showed a C saturation dynamic in the chemically and biochemically protected pools of organic carbon, as well as the chemically protected within the microaggregates (chemical-physically protected pool). Thus, SOC saturation of these fractions might occur but that it is not always apparent in agricultural field experiments since the range of C input levels is too small for showing a saturation pattern.

This become apparent in the study with wider range of SOC was wider. The chemically protected pool in the fine fraction showed a complex dynamic. Overall, this fraction and SOC was best fitted to a saturation function (Table10 and figure 11c), but when plots under calcareous and siliceous parent materials separated different patterns emerged. In the siliceous soils best fitted function was of a saturation whereas was linear for the carbonated plots (Table 10). The fact that the best function was linear for the calcareous soils was likely due to the relatively narrow range of SOC considered, mainly due to the fact that under calcareous parent material the forests was a patched shrubland with relatively high slope and with levels of SOM relatively low. This was not the case for the siliceous soils which showed a wider range of SOC, and a saturation pattern for this fraction, due to the Mediterranean forest consisting of a relatively close high tree density forest with predominance of holm oaks (*Quercus ilex* L.). If extrapolated for the chemically protected pool in the fine fraction in the carbonated soils, following the same methodology than that used for the chemically protected pool within micrograggregates, to higher values of total SOC content, values of SOC chemically protected in these soils would be by 34 and 41% of that level achieved for this fraction at a total SOC content of 100 mg C g^{-1} (about 8.6 mg C g^{-1} soil) in the non-covered and weed-covered soils, respectively. This upper limit in carbonated soils contrasted with that of the siliceous plots, which was about 5 mg C g^{-1} soil. C. Considering the current levels of the chemically protected pool in the fine fraction for non-covered and weed-covered soils, they would be by 25 and 50 % of that of the soils under natural vegetation.

This saturation pattern for the siliceous soils (and presumably also for the carbonated soils) is consistent with the hypothesis which suggests that this fraction consists of organic carbon associated to silt+clay (Six *et al*., 2002; Stewart *et al*., 2008a).

In summary, these results suggest that the dynamics of the SOC pools are very complex, and the saturation limit depends strongly on the parent material as this limit was about two times in carbonated than in siliceous plots in the chemically and physico-chemically protected pools. However, the saturation deficit – or the difference between current SOC content in the bare and weed-covered plots and the saturation level – was similar in the siliceous and carbonated plots (between 40 to 50% for the weed-covered plots and 25 and 40% for the bare plots). The biochemically protected pool of organic carbon within microaggregates and in the fine fraction showed, in general, a linear pattern, but the difference with the SOC levels of the forests plots were higher than those of the chemically protected fractions. The iPOM showed a linear dynamic despite it is considered a physically protected sub-fraction. Nevertheless, the differences between the iPOM in the bare and weed-covered soils and that of the forests plots were between 3 and 32%. That means lower values than those of the chemically protected fractions and within the range of those of the biochemically protected.

Therefore, summarizing all these results for the protected fractions (all fractions except the unprotected pool), the potential saturation deficit of Andalusian olive groves soils would be about 60% in soils of olive groves with a resident vegetation cover and about 75% in olive groves with a bare soil, independently of the parent material. This would be an empiric approximation to the potential saturation deficit. Nevertheless, according to these results the saturation level for the chemically protected pool within microaggregates and in the fine fraction would be in siliceous soils half of the values of the carbonated soils.

5.6 Towards a better knowledge of SOC fractions dynamics

Results of these two studies clearly show that soil properties and management affect the SOC fractions dynamics. On the other hand, the quality of the incoming organic C also have an important effect on the SOC accumulation throughout differential decomposition rate. Therefore, the long-term accumulation of SOC is dependent on many complex, non-linear processes which show multiple interaction.

Integrating all these processes is a challenge and conceptual models should be developed to understand the dynamics of the different SOC fractions and the effects of soil management and soil mineralogy on these dynamics.

Taking into account that soil mineralogy plays a key role in SOC sequestration, parent material would be an appropriate feature to distinguish conceptual models of SOC accumulation. Figure 13 (at the end of the chapter) shows a conceptual model considering the results obtained in this chapter and also in Chapter IV.

The biomass production of the carbonated plots is assumed to be higher than in the siliceous parent material. Thus, the amount of the incoming organic C in the siliceous plots would come mainly from the residues of the olives (mainly leaves) and root processes. For that reason there were not large differences in the amount of the unprotected pool in the siliceous plots between managements, as in the non-cover management in siliceous plots there is also some incoming organic C from olive leaves and roots. Nevertheless, in both parent materials the proportion of the unprotected C which moves to the physically protected pool would be higher than that going to the chemically protected pool. The reason is that the size of particles containing the unprotected pool of organic carbon is reduced from coarse POM to fine POM, a part of which moves within soil microaggregates as iPOM. Nevertheless, another part of the unprotected pool would go to the chemically protected pool. In the Table 5 it was shown that the physico-chemically (H-µSilt + µClay) protected and chemically protected pools (H-dSilt + dClay) were significantly positive correlated with the total carbonates content, pH and clay content, and the physico-chemically protected was negatively correlated with the sand and quartz content. Thus, the pattern of the SOC fractions associated to silt + clay content (H-µSilt + µClay and H-dSilt + dClay) was the opposite of the SOC more recalcitrant and non-associated to silt and clay content (NH-µSilt + µClay and NH-dSilt

+ dClay) (Table 5). This was likely due to the fact that recalcitrant SOC corresponds to organic matter which has no chemical interactions with soil minerals (e.g. clay minerals), whereas SOC in the H-µSilt + µClay and H-dSilt + dClay corresponds to those fractions of organic C linked to the clay minerals. One of the main mechanisms by which organic C and clay minerals are linked is trough cation bridging (Sposito *et al*., 1999) (figure 12). However, the negative charges in the edge points on the clay minerals depend on the pH. When soil pH is very low, clay minerals and soil organic matter are positively charged, so they cannot built a bridge of cations (pH-dependent charges, figure 12). This might be the reason why the organic C appears as recalcitrant SOC in soils with a low pH value (i.e. < 7) (siliceous soil). It is well known that soil pH is positively related to some mineralogical and geochemical properties of the soil such as CEC, carbonates content or the presence of limestone. On the other hand, pH level is negatively related to the content of quartz and sand. Therefore, these results suggest also that in clay minerals of Andalusian olive grove soils pH-dependent charges would predominate over the permanent charges.

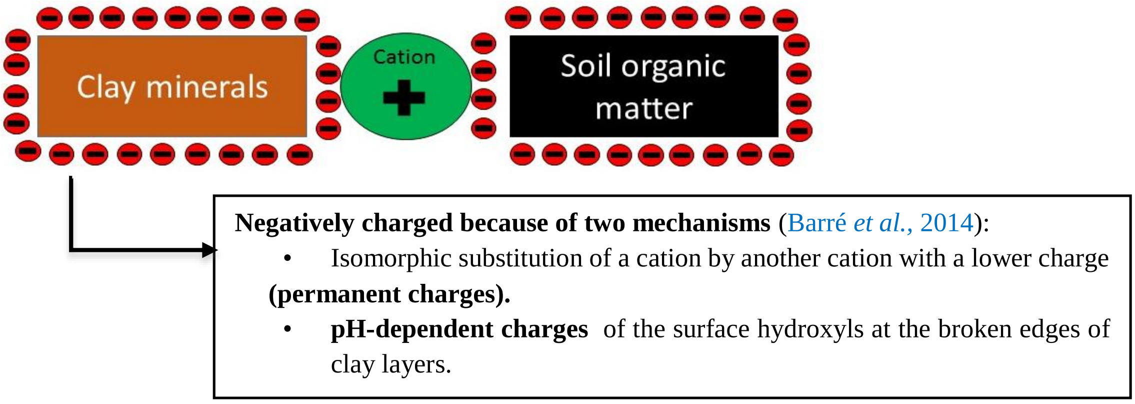


Figure 12. Scheme of the mechanism of “cation bridging”.

Therefore, the higher amount of the biochemically protected pool in siliceous Andalusian olive grove soils might be related to the low pH and low carbonates content. The organic matter at low pH cannot be linked to clay minerals, so it can be degraded and also be enriched in recalcitrant substances by condensation and complexation reactions. On contrary, the chemically protected SOC in the basic soils would be linked more easily to clay minerals and, therefore, it cannot be accesible to soil microorganisms and, thus, it cannot continue being degraded and enriched in recalcitrant substances.

However, this is a secondary recalcitrance, as it has been commented in others sections. There is also a primary recalcitrance corresponding to that unprotected organic C which have higher recalcitrance because its original biochemical composition (e.g. lignin, polyphenols…). Therefore, it is possible that the higher amount of the SOC in the biochemically protected pool corresponded also to this primary recalcitrance. With these results, it is possible that the secondary recalcitrance might predominate over the primary. Anyway, future studies should focus on the chemical features and dynamics of the biochemically protected pool (e.g. by using radioactive markers).

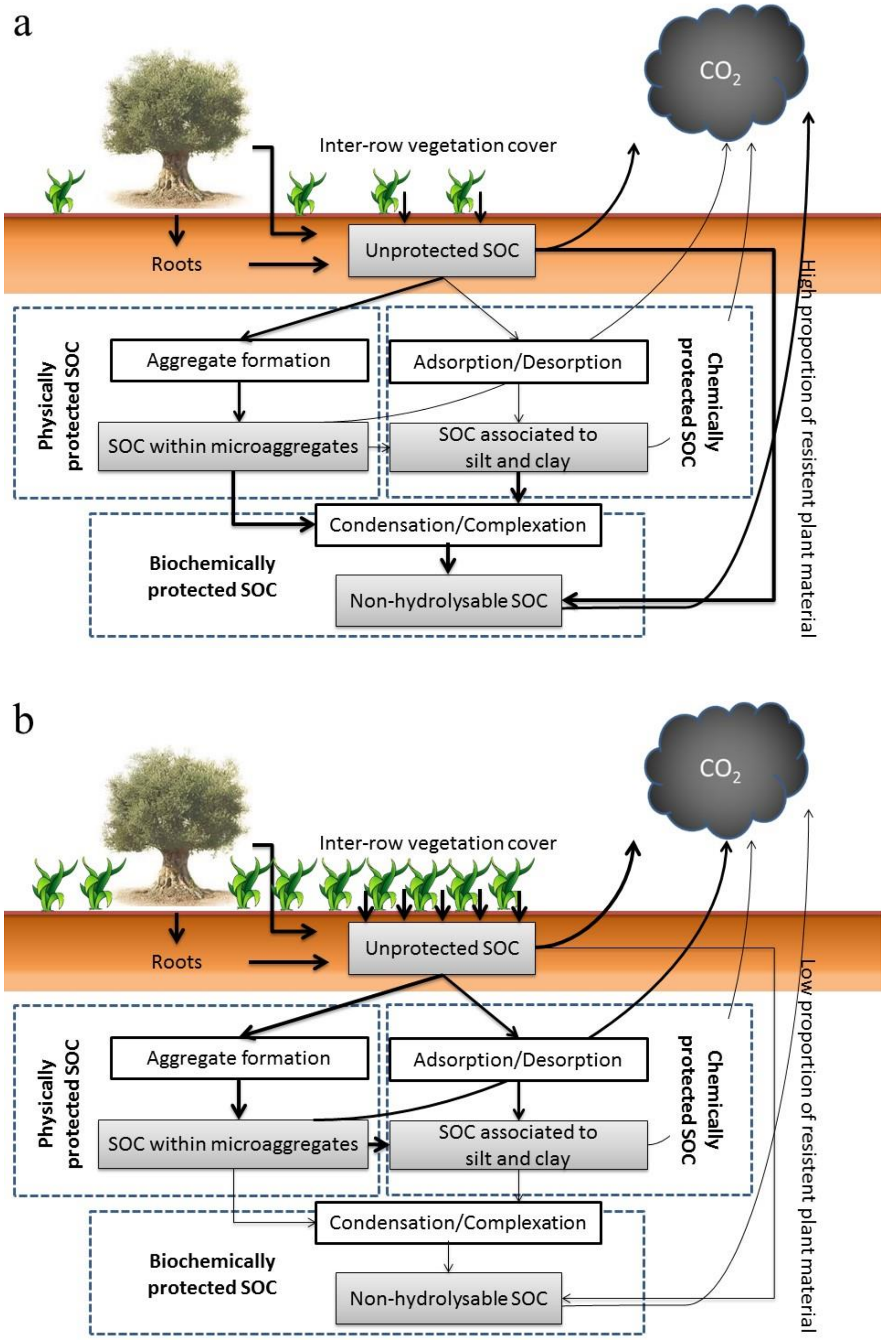


Figure 13. Conceptual model of the SOC fractions dynamics in the weed-cover management in olive grove soils under siliceous (a) and carbonated (b) plots. The thickness of the lines represents the amount of the different fractions. Note that in the carbonated plots biomass production of the weed cover is higher than in the siliceous plots. On the other hand, in the siliceous plots the amount of the biochemically protected pool is higher, whereas in the carbonated plots the amount of the physically and chemically protected pool is higher. In both cases there is a higher proportion of the unprotected pool which moves to the physically protected, mainly due to the iPOM.

6. Conclusions

The main conclusions of this chapter can be summarized as follows:

- The implementation of a spontaneous **vegetation cover** in the inter-row area in olive groves led to a **higher total SOC content in comparison to the non-covered soils.** This increase was **especially significant for the unprotected and physically protected pools**. The **dynamic of the chemically protected organic carbon depended on the soil mineralogy**, being higher in the weed-covered plots under carbonated parent material, whereas the biochemically protected pool increased a little or remained at similar levels in both managements.

- Overall, **the contribution of the SOC fractions to the total SOC was not affected by the presence of a spontaneous vegetation cover**. The relatively low disturbance of the soils of the non-covered olive orchard (reduced tillage combined with herbicides) might explain that.

- The depth strongly affected the amount of the different SOC fractions. **The amount of the unprotected and the physically protected pools were significantly higher in the upper layer**. The weed-cover management accumulated higher SOC content in comparison to the non-cover in top 5 cm and also in the 5 – 15 cm soil layer, mainly due to an increase in the unprotected and physically protected pools.

- Soil mineralogy had a significant effect on SOC fractions. **Total SOC in carbonated plots doubled that under siliceous.** This **increase was due to the unprotected and physically and chemically protected pools**. However, interestingly, the **biochemically protected pool was significantly higher in the siliceous plots.** As a consequence, the proportion of the unprotected pool was higher in the carbonated plots, whereas the proportion of the biochemically protected fraction was higher in the siliceous ones. This was likely due to the lower pH in the siliceous soils, which enhance complex processes of complexation and condensation.

- **Humic and fulvic acids content tended to be higher under a weed-cover management.** The **higher E_4/E_6 ratios of the humic acids of the weed-covered** olive orchards and the carbonated plots suggest that in these soils the proportion of the fresh organic matter was higher than in the non-covered and in the siliceous plots.

- The **unprotected and the iPOM of the physically protected pools showed clearly a non-saturation dynamic**. Both fractions are the most sensitive to a change in the management.
- **The chemically protected pool within microaggregates and in the fine fraction showed a clear saturation dynamic**, **being about at 50 and 30 % of the saturation level for the weed-covered and non-covered soils**, respectively. Nevertheless, there were differences between parent materials. In the siliceous plots the saturation limit seems to be much lower than in the carbonated plots (almost half of that the carbonated).
- **The dynamic of the biochemically protected pool was unclear**. This may be due to biochemical features of the incoming organic C. Neither the biochemically protected pool within microaggregates nor the pool in the fine fraction showed a clear dynamic. Nevertheless, sometimes they showed a non-saturation dynamic, thus suggesting that this pool, sometimes, should not be considered as a "real" protected pool.


Todas las cosas
están
relacionadas
entre sí como la
sangre que une
una familia.
Todo cuanto
haga con la
trama se lo hará
a sí mismo.
Cacique de los
indios Duwamish

CHAPTER VII

Patterns of SOC fractions loss due to erosion in olive groves under contrasted vegetation cover management

1. Introduction

Throughout this Thesis some of the advantages related to SOM and SOC of the presence of a vegetation cover in the inter-row of olive groves have been mentioned. However, one of the main advantages is the reduction on soil losses by water erosion (see 4.2 section in Chapter I) (e.g. Durán-Zuaro *et al*., 2009; Gómez *et al*., 2003, 2004, 2009, 2011).

The high slopes and the shallow soil depth of the Mediterranean cultivated soils for fruit orchards are one of the main environmental, economic and management limitations (Casalí *et al*., 2009). Thus, as it was highlighted by López-Vicente *et al*. (2016), there are some studies reporting economic benefits after the adoption of the vegetation-cover management (e.g. Taguas *et al*., 2012). Other authors did not fin differences on yield (e.g. Simoes *et al*., 2014), whereas other studies found a decrease in yield in the vegetation-cover management compared to the conventional tillage (e.g. Ferreira *et al*., 2013).

On the other hand, Kirkels *et al*. (2014) carried out a deep review about the three steps involved in the erosion process in agricultural lands: i) detachment, ii) transport associated with potential aggregate disruption and iii) deposition (Morgan, 2005; Berhe *et al*., 2007; Quinton *et al*., 2010) (figure 1).

First, the detachment of SOC (i.e. decrease on SOC stocks) takes place at eroding landscape positions and with a rate and extent related to the slope gradient and convexity (figure 1) (Gregorich *et al*., 1998; Liu *et al*., 2003; Ritchie *et al*., 2007). The SOC depletion in this step can be increased by two facts: i) an increase in the mineralization processes due to exposure to new environmental conditions (oxygen, moisture, temperature) and ii) soil losses might lead to a decrease in yield production, so less C is returned to the soil. (Kirkels *et al*., 2014; Gregorich *et al*., 1998; Jacinthe and Lal, 2001; Lal, 2003; Berhe *et al*., 2005; Quinton *et al*., 2010).

Second, the transport of the SOC along hillslopes can result in deposition on different sites (figure 1): eroded/intact places, aquatic environment or in mineralization, thus resulting in a net C flux to the atmosphere (Kirkels *et al*., 2014; Lal, 2003, 2010). Light fractions (e.g. labile SOC) would be preferentially transported over longer distances (Kirkels *et al*., 2014; Starr *et al*., 2000; Lal, 2003). The enrichment of SOC in sediments following water erosion would be higher at higher distances from the source area. Therefore, suspended sediments would have high SOC contents (Kirkels *et al*., 2014;

Quine and Van Oost, 2007; Kuhn *et al*., 2009; Wang *et al*., 2010). This enrichment would be positively related with the roughness of the landscape (Kuhn *et al*., 2009).

Finally, the third phase, the deposition can take place in different landscape positions (figure 1). These landscapes can have a potential storage capacity (e.g. colluvial, alluvial and lacustrine or riverine environments) (Stallard, 1998; Harden *et al*., 1999) or can be waterlogged sites (Smith *et al*., 2001; McCarty and Ritchie, 2002; Liu *et al*., 2003). Local redistribution and storage would be the most dominant process on the landscape scale (Smith *et al*., 2001; Liu *et al*., 2003). Nevertheless, Kirkels *et al*. (2014) remark that SOC dynamics on depositional areas (i.e. C budget) depend on various complex and competing processes (e.g. changed mineralization, biomass production or deep burial of SOC).

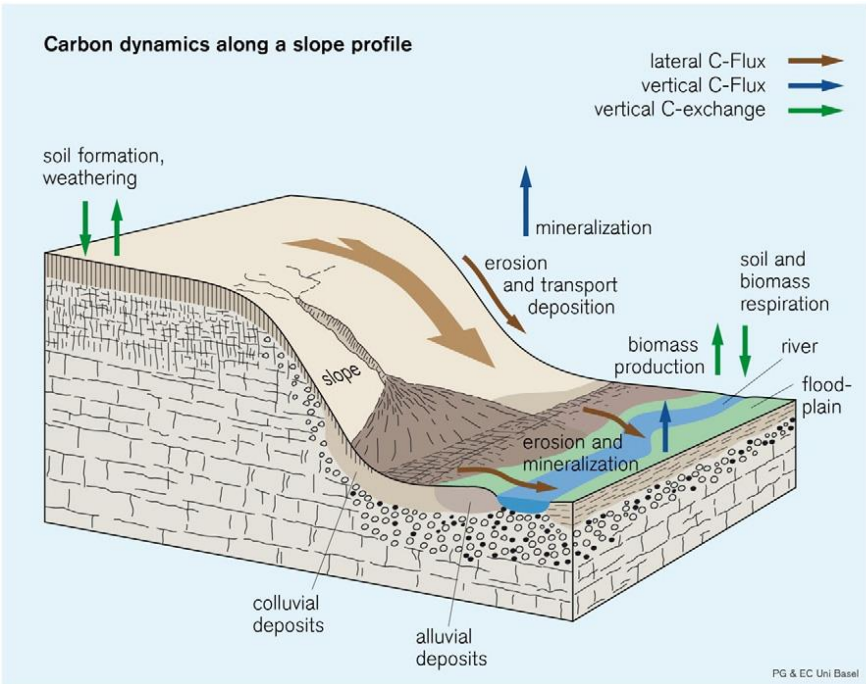


Figure 1. Carbon dynamics along a slope profile. Lateral and vertical carbon fluxes in a terrestrial system with a nearby aquatic system are a result of the interactions between biomass production, soil formation, erosion and deposition processes (Kirkels *et al*., 2014).

Thus, the impact of erosion depends on geomorphological properties, since the erosion would be negative for the eroding plots due to soil degradation and lower productivity and positive for depositional sites (Stallard, 1998; Harden *et al*., 1999; Van Oost *et al*., 2005; Quine and Van Oost, 2007; Ritchie *et al*., 2007). In this context, Aranda *et al*. (2011) found higher SOC and N contents, and higher CEC in olive groves under colluvial soils than in olive groves under marls in eroding areas under a conventional tillage.

Because of the complexity of these processes the contribution of erosion of arable lands to the CO_2 emissions is not yet quantified (Liu *et al*., 2003; Van Oost *et al*., 2005; Kirkels *et al*., 2014). Importantly, these latter authors highlight that the erosion, transport and deposition processes can imply transitions between the different SOC pools (e.g. aggregate disruption or deep burial), and these transitions can affect mineralization processes. Therefore, according to these authors, the question would be, therefore, whether redistribution of sediment and SOC in agricultural landscapes results in a carbon sink or a source.

As it was mentioned above, in olive groves (and generally in crops) the study of the effects of the erosion have been focused on quantifying the soil (or SOC) losses in a single plot. But these studies did not assess whether this SOC is mineralized or simply moved and finally deposited on a colluvial site.

On the other hand, these studies have been mainly designed to study the dynamic of the total SOC, but not distinguishing between the different SOC fractions. This is important, since, it is possible that after selective erosion processes (rain splash and interrill erosion) soils would be enriched in some specific SOC fractions and impoverishing other fractions (Kuhn *et al*., 2009; Wang *et al*., 2010).

Therefore, the study of the erosion process through evaluating the dynamics of the SOC fractions would be a suitable tool to assess the influence of the erosion processes on SOC sequestration.

2. Objectives

The objective of this study is to **assess the SOC losses in olive groves due to water erosion**. For that purpose it was studied not only the total amount of SOC lost but also **the amount of SOC lost associated to each SOC fraction** in order to find out the most sensitive fractions to the erosion and, therefore, the soil enrichment or impoverishment in some fractions.

3. Material and methods

Experimental and sediment collectors designs, on field set up and sampling of the eroded material was done by the Instituto de agricultura sostenible (CSIC, Córdoba) under the supervision of José Alfonso Gómez Calero. In this study only the eroded material was analized for SOC.

Details of the experimental design, on field set up of the collectors and sampling can be found in Gómez *et al*. (2009) and in Instituto de Agricultura Sostenible (CSIC) document. Some features of these are detailed next.

3.1 Experimental design

Site and location

The experimental site (Finca Santa Marta) is located near Benacazón (Sevilla) (figure 2). Total area is 21 ha, 7 of which are under irrigation and mean slope is of 11 %. Soil texture is sandy loam and with an organic matter content of 1.3 %. 25-y mean annual precipitation is 650 mm and typically it is concentrated at the end of autumn and winter.

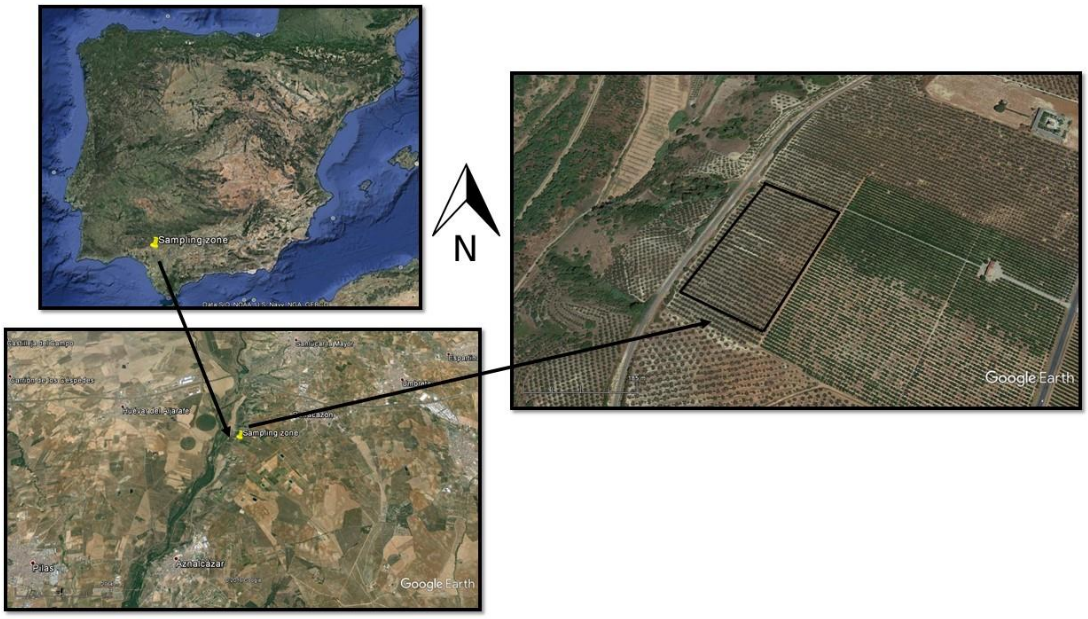


Figure 2. Location of the study area in the Finca Santa Ana.

Each erosion plot consisted of a rectangle of 60 m long and 8 m wide, resulting in a total area of 480 m^2. Plots have a sediment collector system to accumulate the water and sediments resulting from the erosion (figure 3).

Figure 3. Picture showing the tree-tanks systems installed in each of the plots to collect the water and sediment (Photograph of Instituto de Agricultura Sostenible, CSIC). Experimental design, collectors set up and sampling were performed by Instituto de Agricultura Sostenible, CSIC under the supervision of J. A, Gómez Calero) (Instituto de Agricultura Sostenible, CSIC).

The collector system of each plot consists of 3 fiberglass tanks connected in series, the two first tanks with 1 m^3 of capacity and the third one with 0.5 m^3. The system is over-dimensioned in a 32% over the most intense event predicted in order to avoid the collapse of the system.

3.2 Sources of variation

Weed management was the main source of variation. This source of variation consisted to two levels: inter-row covered by vegetation and non-covered (bare). In addition to this, another source of variation was the seasons as a full hydrological cycle was considered. Three periods were specially considered; pre-wet, wet and post-wet These were based on the rainfall intensity and thus on the magnitude of the soil losses due to erosion. It was hypothesised that SOC fractions in the eroded soils was highly dependent on the magnitude of the soil erosion.

Six 480-m^2 experimental plots were set up with three different managments: i) bare soil (plots 2 & 4), or ii) soil covered by a seeded *Lolium multiflorum* (plots 3 & 6) or ii) mixed covered of different arthropod fauna stimulating species (plots 1 & 5) (figure 4). A brief history of each plot is given below:

Plot 1

From 2005 to 2009 the inter-row area was covered with *Lolium rigidum* (planted by hand) after the first precipitations in autumn and was fertilized with inorganic fertilisers.

In November 2009 it was seeded with a mixture of different arthropod fauna stimulating species with an inorganic fertilization. This cover was successfully planted and in May of 2010 it was mowed and re-seeded in November of 2010 with the same mixture, density and methodology than in 2009.

Plot 2

Since September of 2005 this plot is monitored. The inter-row area was controlled mechanically with a plough with a depth between 0.1 – 0.15 m two or three times between the end of autumn and spring depending on the vegetation growth. In the year 2010-2011 the plot was tilled two times.

Plot 3

This plot was monitored since September 2003. The inter-row was covered with *Lolium rigidum* and managed in the same way as plot 1. In November 2009 was seeded with *Lolium multiflorum* with inorganic fertilisation. In November of 2010 it was re-seeded with the same mixture, density and methodology than in 2009.

Plot 4

The plot_was monitored since September 2003 and has been managed in the same way that plot 2, controlling mechanically the spontaneous vegetation of the inter-row area.

Plot 5

This plot was monitored since September 2005 and has been managed in the same way that plot 1, being seeded with a mixture of different arthropod fauna stimulating species.

Plot 6

The plot was monitored since September 2005 and its management has changed. During the year 2005-2006 the spontaneous resident vegetation was controlled mechanically in the inter-row area in the same way than plots 2 and 4. Since 2006-2007 a plant cover of *Lolium rigidum* was planted and was controlled chemically in spring. Since 2010-2011 it has been managed as in the plot 3.

Figure 4. The three managements in April of 2010. Planted with Lolium multiflorum, mechanical control and mixture (from left to right) (Instituto de Agricultura Sostenible, CSIC).

Table 1 shows results total SOC, SOC content of the different fractions and stratification ratio of the covered (1, 3, 5 and 6) and tilled (2, 4) soils.

Table 1. Average values of the unprotected (U), physically (PP), chemically (CP) and biochemically protected (BP) organic carbon, and of the total soil organic carbon content (mg C g^{-1}) in soils of plots from 1 to 6 (grouped by management: covered, C, and tillage, NC). Standard deviation is given in brackets. The last column corresponds to the stratification ratio (S.R) of the total soil organic carbon between the two depths sampled (0 – 5 and 5 – 15 cm). Results of the significance according to the management are given in the last row (P < 0.05).

Management	Depth	U	PP	CP	BP	Total SOC	S.R.
Covered	0-5	6.37 (1.79)	3.76 (0.90)	3.35 (0.63)	1.69 (0.57)	15.17 (2.61)	1.49 (0.31)
	5-15	3.62 (0.92)	2.82 (1.02)	2.35 (0.96)	1.74 (0.74)	10.52 (2.56)	
Non-covered	0-5	3.38 (0.70)	2.70 (0.84)	3.12 (0.61)	1.27 (0.44)	10.47 (0.71)	1.13 (0.14)
	5-15	3.15 (0.88)	1.68 (0.39)	3.38 (0.93)	1.08 (0.76)	9.30 (1.29)	
Management		C > NC	C > NC	C = NC	C = NC	C > NC	C > NC

The sediments were recovered during the hydrological year 2010-2011. This year was rainier (773 mm,) than the 10-y average (559 mm). Rainfall was concentrated during December and May. During May a heavy storm occurred and strongly affected the magnitude of the eroded material collected (figures 5 and 6).

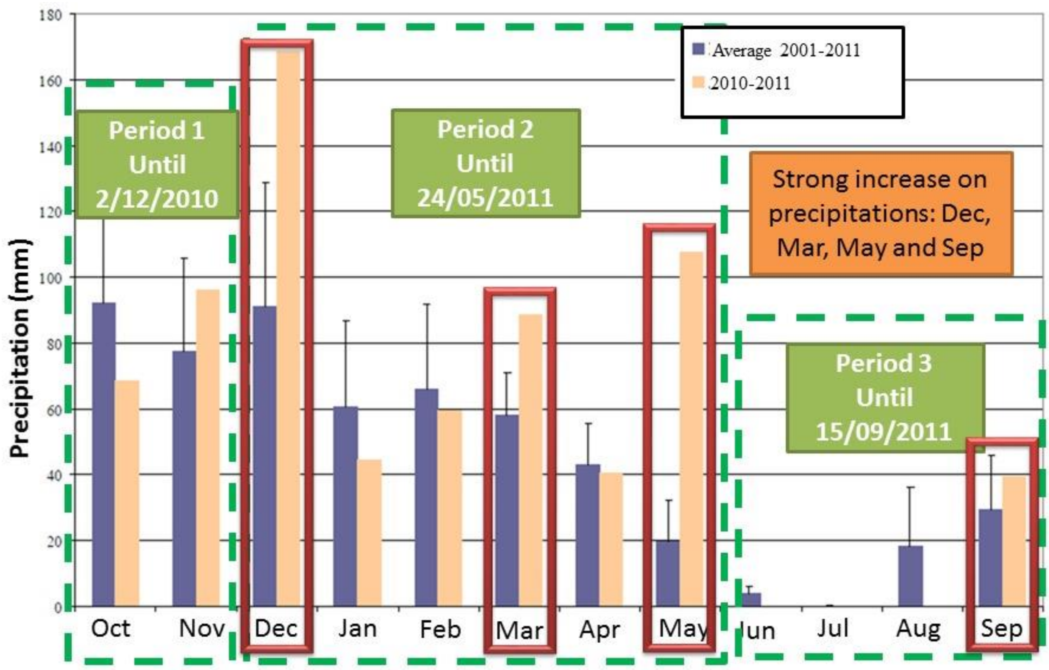


Figure 5. Monthly precipitations during the hydrological year of 2010-2011 compared with these of the average for the 2001-2011 period. Error bars show the 0.5 standard deviation. Green dashed lines group months with similar precipitations patterns. Red lines indicate months in which rainfall was above the 10-y average . Data obtained from Instituto de Agricultura Sostenible, CSIC.

Three different periods were distinguished based on different precipitation patterns (figure 5).

First, a period comprising October and November, where precipitations where relatively high but continuous. The second period starts in December, where precipitations were much higher than the 10-y average and were followed by some months (January and February) where precipitations were lower than the average and included May, which is the final month of this period, where precipitations were extremely higher, reaching a value higher than 100 mm. The third period corresponds to the summertime, where no precipitations were registered, but it includes September, where precipitations started again. Erodibility was relatively high during December and May (figure 6).

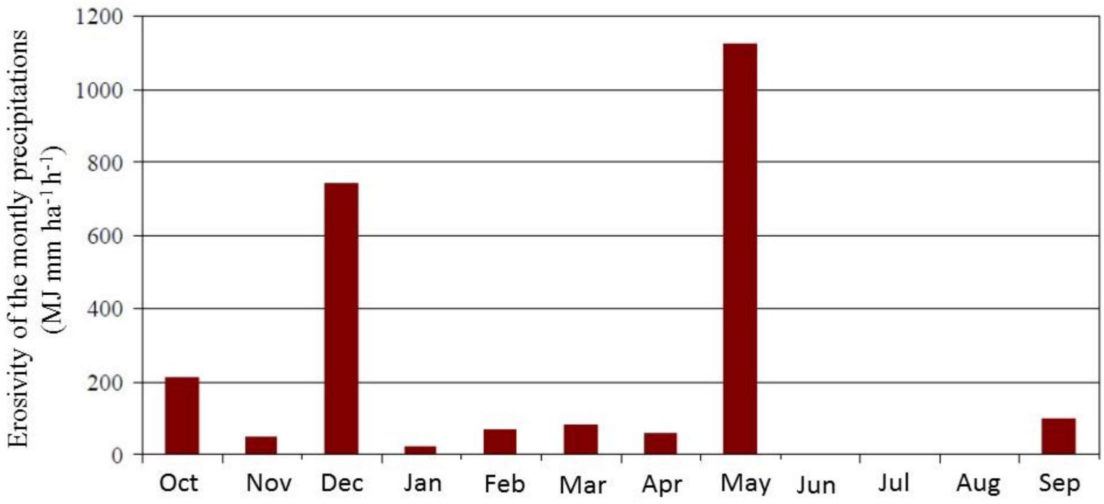


Figure 6. Monthly erosivity of the precipitations, (MJ mm ha^{-1} h^{-1}) from October 2010 to September 2011. Data from Instituto de Agricultura Sostenible (CSIC).

Figure 7 shows that December and January were the months with highest total soil losses, with relatively high values during March and May for the uncovered plots. Annual soil losses were higher in the bare soils than in the covered soils. The effect of the vegetation cover was especially remarkable during the vegetation cover growing period (i.e. spring).[8]

[8] More information about collected sediments, plots, environmental conditions and other variables can be found in the document of Insituto de Agricultura Sostenible (CSIC) (see References section)

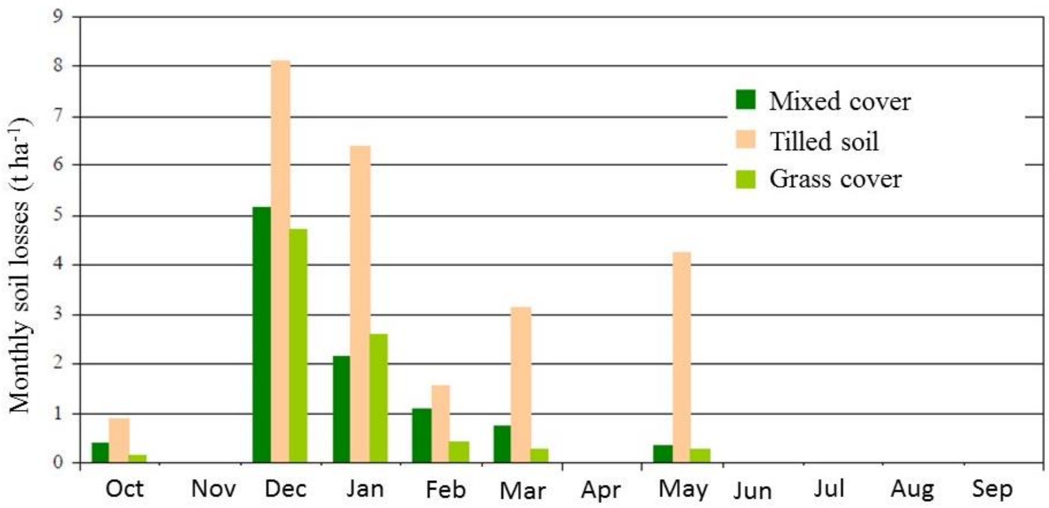


Figure 7. Monthly soil losses (t ha^{-1}) from October 2010 to September 2011 in the three treatments: with vegetation cover (grass and mixed cover) and in the treatment consisting on a bare soil due to the conventional tillage. Data from Instituto de Agricultura Sostenible (CSIC).

3.3 Dependent variables

The dependent variables are those related with the SOC:

- Total SOC content in the sediments (mg C g^{-1} sediment)
- SOC content of the different fractions in the sediments (mg C g^{-1} sediment)
- Organic C density of the fraction in the sediments (mg C g^{-1} fraction)
- Enrichment ratio:

$$Enrichment = \frac{SOC_{sediment}}{SOC_{soil}}$$

where, $SOC_{sediment}$ is the soil organic carbon content of the whole soil or the fraction in the sediments recovered, whereas the SOC_{soil} corresponds to the soil organic carbon content in the whole soil (i.e. soil not eroded). Thus, if the enrichment is > 1 the soil of the plot is being impoverished in the fraction or in the total soil organic C, whereas if opposite, the soil of the plot is being enriched.

Once the sediments are recovered, pre-treated and weighted, the total SOC is analized following the method described in the 2.2 section of Chapter II. After that, the SOC fractionation was carried out (method described in the 2.1 section of Chapter II).

3.4. Statistical analysis

The effects of the dependent variables (management, season) and their interactions on SOC losses were assessed using two-way ANOVA procedure, software STATISTICA (StatSoft, 2001).

4. Results and discussion

4.1 Dynamic and total SOC losses

Periods of higher total SOC losses were in line with the monthly precipitations, and this dynamic was generally similar for covered and non-covered plots (figure 8). SOC losses in the covered plots were lower than in the non-covered during late winter and spring, especially during the high rainfall intensity event of March and May. As it was detailed in Chapter V, the main growing season of the vegetation cover takes place during spring. Clearly, the presence of a vegetation cover had an important role in decreasing SOC losses. For instance, in March, about 29 Kg C ha^{-1} were lost in the non-covered whereas this value was less than half of that (12 Kg C ha^{-1}) in the covered soils.

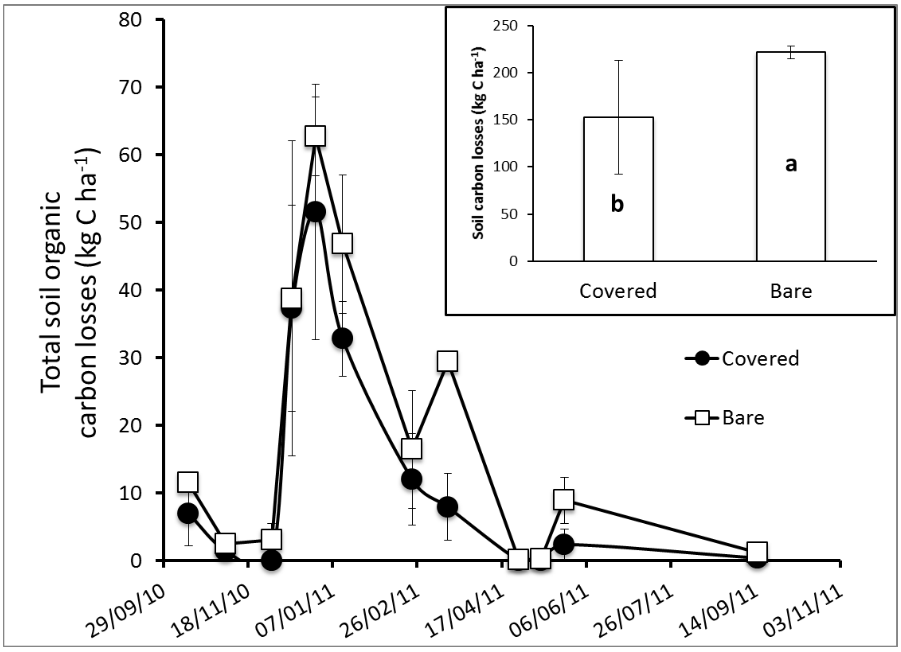


Figure 8. Total soil organic carbon losses in a hydrological year in plots with a vegetation cover management and in plots with non-covered (i.e. bare) soils. On the right corner, total annual soil organic carbon losses. Different letters mean significant differences ($P < 0.05$). Bars represent standard deviation.

A great percentage (about 80 %) of the total SOC losses were concentrated during January, February and March. For the whole hydrological year, total SOC losses were significantly lower in the plots with a vegetation cover (153 Kg C ha^{-1}) compared to the non-covered plots (222 Kg C ha^{-1}). These results are in line with those found by Gómez *et al*. (2004), who found a decrease of 3.3 times in soil losses in an olive grove under vegetation-cover compared to the conventional management (slope of 13%). Gómez *et al*. (2011), in a relatively rainy year, found soil losses of 2.7 t ha^{-1} yr^{-1} in a non-covered olive orchard, whereas this value was 15 times lower for olive orchard soils under vegetation-cover management (0.17 t ha^{-1} yr^{-1}). Nevertheless, they did not find significant differences in soil losses between managements in a very dry year. On the other hand, Gómez *et al*. (2003) using the revised equation of the USLE (RUSLE), estimated very different soil losses depending on the slope. They estimated for a slope of 20% in a plot under conventional tillage soil losses of about 70 t ha^{-1} yr^{-1}, whereas this value was about 40 t ha^{-1} yr^{-1} in the covered plots. Assuming a mean SOC content of about of 1.0 and 1.2 % for a non-covered and covered soils (according to Table 1), SOC losses estimated were about 700 Kg C ha^{-1} yr^{-1} and 480 Kg C ha^{-1} yr^{-1}, respectively. These values are higher than those found in our experiment, but it is not surprising, since in our case the slope was much lower (about 11 %). However the proportion of the SOC losses remained similar than in our case, about 1.5 times higher losses in the non-covered soils.

4.2 Dynamics of the SOC fractions in the eroded material

Amount of SOC losses

Although losses of unprotected organic C were slightly lower in the covered plots (48 Kg C ha^{-1}) compared to those of the bare soils (57 Kg C ha^{-1}) differences were not significant (figure 9a and Table 2).

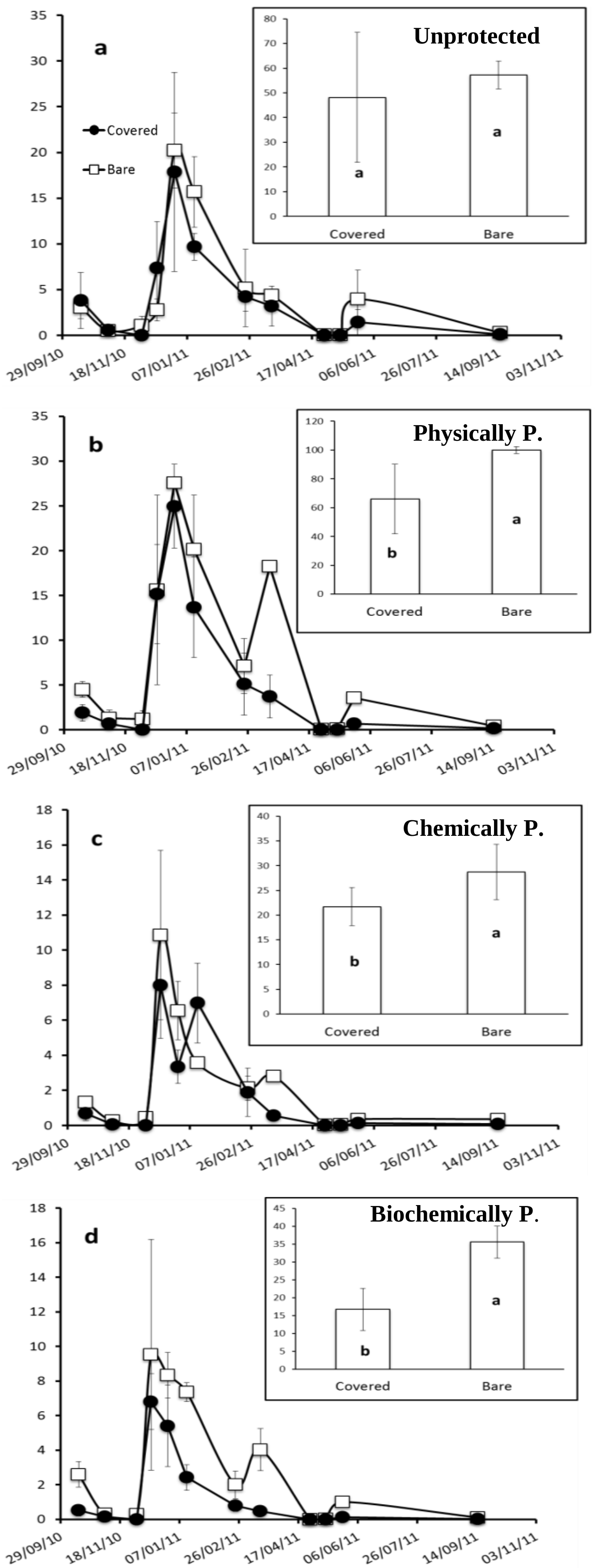


Figure 9. Soil organic carbon fractions losses (Kg C ha^{-1}) (a, unprotected; b, physically protected; c, chemically protected; d, biochemically protected) in a hydrological year in plots with a vegetation cover management and in plots with non-covered (i.e. bare) soils. On the right corner, annual soil organic carbon losses of each fraction are shown. Different letters mean significant differences (P < 0.05). Bars represent standard deviation.

Interestingly, the lost of physically protected organic C showed a similar pattern than that of total SOC, and annual losses of SOC of this protected fraction in the non-covered soils (100 Kg C ha^{-1}) were 50 % higher than that of the covered plots (66 kg C ha^{-1}) (figure 9b and Table 2). Highest differences between the two managements took place in spring, where the physically protected SOC lost were more than 3 times higher in the non-covered.

In the whole, the amount of the chemically protected organic carbon fraction lost was significantly lower in the cover management than in the non-covered soils (22 and 29 Kg C ha^{-1}, respectively) (figure 9c and Table 2).

Finally, the lost of biochemically protected pool in the covered soils was half of that of the non-covered soils (17 and 36 Kg C ha^{-1}) (figure 9c and Table 2). Thus, the largest differences, proportionally, of SOC losses between managements were achieved in the biochemically protected fraction.

Percentage contribution of the SOC fractions to the total SOC losses

The highest contribution to the total SOC losses was for the physically protected pool (Table 2). Indeed, about 45 % of the total SOC losses were due to this fraction, independently of the managements. The second highest contribution to the total SOC losses was due to the unprotected pool, 26 and 31 % for the bare and covered soils, respectively. For both managements, the contribution of the unprotected and physically protected pools to the total SOC losses was higher than 70 %. The contribution of the chemically and biochemically protected pools were similar and ranged 11 – 16 %.

Table 2. Mean annual soil organic carbon losses (Kg C ha^{-1}) and contribution (%) of each of the soil organic carbon fraction to the total soil organic carbon losses in the hydrological year 2010-2011. Different letters mean significant differences ($P < 0.05$). NC = non-covered plots, C = covered plots.

	Unprotected		**Physically protected**		**Chemically protected**		**Biochemically protected**	
	NC	**C**	**NC**	**C**	**NC**	**C**	**NC**	**C**
Kg C ha^{-1}	57a	48a	100a	66b	29a	22b	36a	17b
% of the total SOC	26	31	45	43	13	14	16	11

Interestingly, losses of unprotected pool of organic carbon in the covered plots represented up to 45 and 55 % of the total SOC lost in the pre and post-wet periods (figure 10a). This result suggest that in the first events after long period without rains (pre-wet) or in periods were rainfall intensity or erosivity is low (post-wet) unprotected C is preferentially eroded. This result is in line with those described by Starr *et al.* (2000) and Lal (2003).

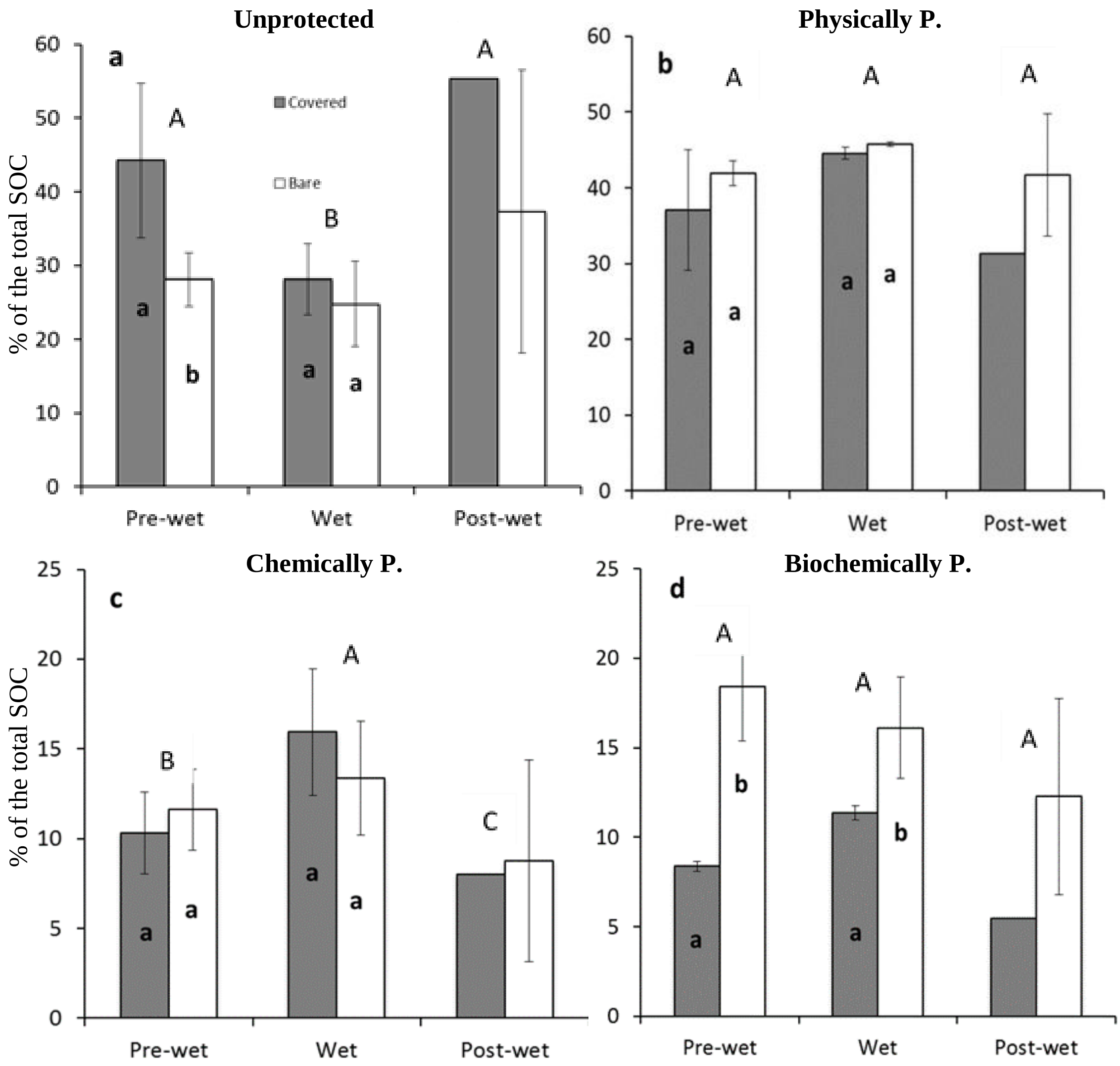


Figure 10. Contributions (%) of the unprotected (a), physically protected (b), chemically protected (c) and biochemically protected (d) pools of organic carbon to the total soil organic carbon losses of the three distinct periods: pre-wet, wet and post-wet. Different small letters mean significant differences in the average between covered and covered plots in each period, whereas capital letters mean significant differences between periods ($P < 0.05$). Error bars indicates standard deviations. Note that in the post-wet period there are not statistical results, due to the fact that there was not enough eroded material in collectors of the covered plots.

An opposite dynamic was found for the chemically (< 53 µm) protected pool, which in both treatments contributed the most during the wet period (figure 10c). These results indicate that in periods of high precipitations the smaller fractions (silt and clay particles) are preferentially eroded, probably due to the fact that the most labile pools were already eroded in the first rain events. On the other hand, the contribution of losses of the physically protected SOC did not differ neither among periods nor between managements in each period (figure 10b). The percentage contribution of losses of the biochemically protected organic carbon was higher in the non-covered plots respect to the covered for each period and no differences among period were found (figure 10d).

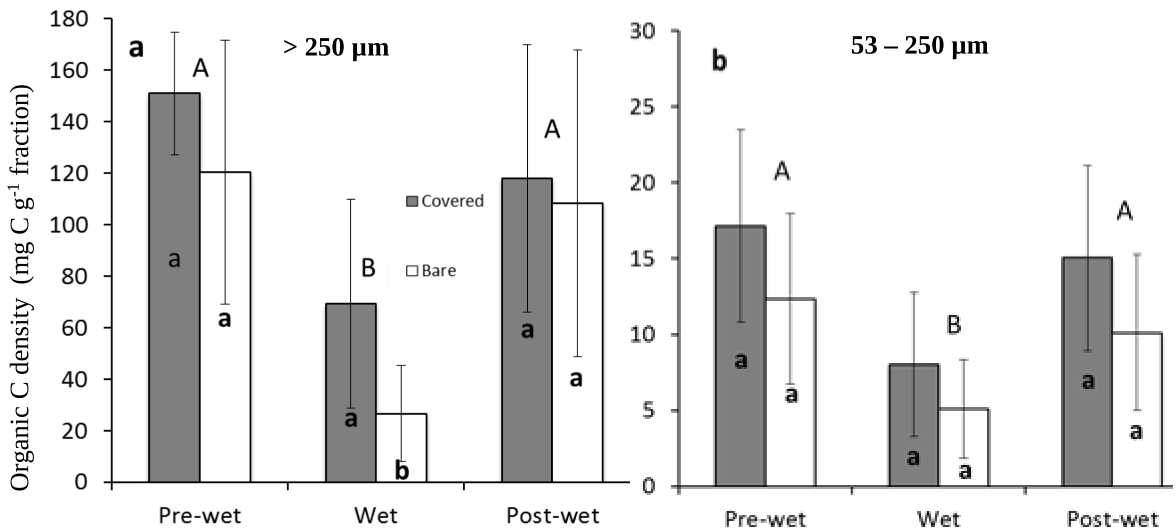
4.3 Organic C density of the different SOC fractions

The density of the organic C (e.g. mg C g^{-1} fraction) represents the content of the organic C per unit of weight of the fraction. As total SOC concentration was significantly higher in the covered plots (Table 1) the organic C density was also higher in the covered plots for the majority of the SOC pools (figure 11). Nevertheless, in some cases, the high variability of the results led to not to show significant differences between managements.

The figure 11a shows that the organic C concentration of the > 250 µm (i.e. mainly the unprotected pool) was significantly higher during the pre-wet period (typically about 100 mg C g^{-1} fraction), being about two times that of the wet period, whereas in the post-wet period, after the long period without precipitations the organic C density of this fraction increased after the precipitations. These values suggest that, initially, after the first precipitations, the fraction was enriched in fresh organic matter (low density organic material, such small pieces of plant residues), but over time the amount of the organic matter in the surface decreases and thus, its contribution to the > 250 µm. In the wet period this fraction would be formed mainly by mineral particules (e.g. sand). Therefore, although the amount of the collected sediment in the wet period was high the relatively low density of the organic C in the fraction < 250 µm did not lead to an increase in the SOC losses of this fraction.

This explanation can be completed with that described in Kirkels *et al*. (2014). They describe that, initially, the upper soil layer is enriched in organic C, but with continuous rains lead to a SOC depletion (quantitative change) and, thus, to an exposure of subsoil

C, which is mainly formed by more passive forms of organic C (i.e. slowly degradable pool) (quality change) (Liu *et al*., 2003; Kuhn *et al*., 2009) (figure 12). This effect is especially visible in the non-covered plots, where the organic C of the upper layer is low and formed mainly by olive leaves, easily erodible (although the high variability of the results sometimes lead to not to show significant differences compared to the covered plots).Thus, the eroded SOC is reduced over time (as precipitations continue), whereas sediment erosion rates remain constant. As a consequence of the differences in the quantity and quality of eroded SOC over time from eroding soils CO_2 emissions in highly eroded soils might be reduced. However, as these authors remark, this fact is valid only when soil and C erosion rates exceded C fixation.



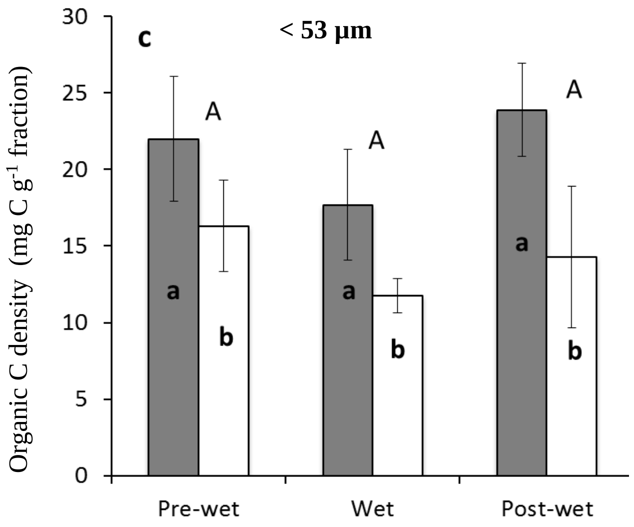


Figure 11. Organic carbon density (mg C g^{-1} fraction) of the > 250 µm (≈ unprotected) (a), 53 – 250 µm (≈ physically protected) (b), and < 53 µm (≈ chemically and biochemically protected) c) for the covered and non-covered plots in the three different periods: pre-wet, wet and post-wet. Different small letters means significant differences between managements, whereas different capital letters stands for significant differences among periods ($P < 0.05$). Error bars indicates standard deviations.

The intermediate fraction (53 – 250 µm), mainly formed by microaggregates, showed a similar pattern (figure 11b). Average values ranged 12 – 18 mg C g^{-1} fraction, almost one order of magnitude lower than that of the > 250 µm soil particles, during pre-wet and post-wet periods, by were lower than 10 mg C g^{-1} fraction during the wet period. For this fraction, it is likely that low-density microaggregates (e.g soil microaggregates with some organic matter within) were specially eroded after the first and lower intense precipitations, whereas in the period of high intense and longer precipitations higher-density microaggregates (e.g. with more content of mineral soil particles likely due to a mineralization of the most easily decomposable organic C within microoaggregates) were preferentially eroded. Interestingly, this fraction had the lowest SOC densities during the wet period, suggesting that this fraction is mainly composed of fine sand poor in organic carbon.

Lastly, no differences in the organic C density of the smaller fraction (< 53 µm) were observed (figure 11c). This is in line with the hypothesis that this fraction is mainly formed by organic C associated to silt and clay minerals and independently of the rainfall intensity there is no preferential loss of richer or poorer organic carbon silt-clay particles. Overall, soil organic carbon density of this fraction was higher in covered plots.

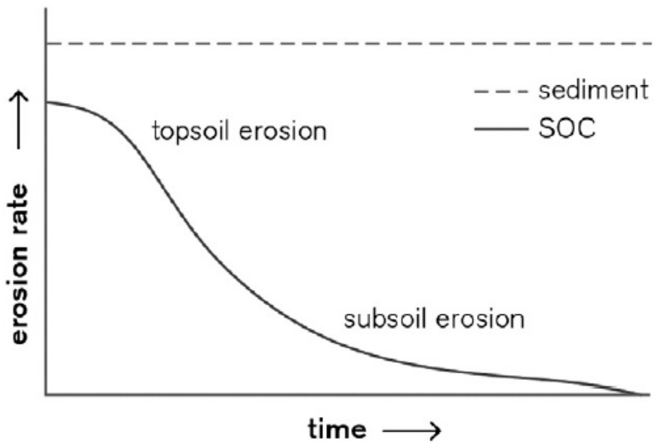


Figure 12. Conceptual model describing the discrepancy between sediment and SOC erosion on temporal scale. At the beginning of the rains the erosion rates of the topsoil and sediments are similar, but over time, as precipitations continue, the upper layer is eroded and suffers a depletion of its organic C content. At the same time, deeper layers are exposed to the environmental conditions, but this fractions have usually lower organic carbon concentrations and the organic C is usually protected, so this organic C is slowly degradable. This conditions are only valid when the soil organic carbon erosion is higher than the input of organic C. Image elaborated by Kirkels *et al*. (2014) from Liu *et al*. (2003) and Kuhn *et al*. (2009).

Therefore, the density of organic C found in the three different size classes is in line with the conceptual model elaborated by Kirkels *et al*. (2014). According to these authors, there is over time a SOC depletion of the most labile fractions (in our case, the unprotected pool), whereas there were not significant changes for the smallest fractions (< 53 µm). However, the physically protected pool showed a similar dynamic than the unprotected pool. As it was shown in Chapter VI, the physically protected pool is increased by the presence of a vegetation cover, indicating that, although it is a protected pool, this fraction response relatively fast to a change in management. Furthermore, in this that chapter it was found that iPOM was the fraction responsible to the increase in the SOC content of the physically protected pool. Therefore, erosion might disrupt soil microaggregates leading to the iPOM losses but maintaining the smaller size SOC of this fraction. In this sense, Kirkels *et al.* (2014) highlight the key role of the mineralization processes during transport. Thus, the detachment process causes disruption, slaking or breakdown of aggregates (Lal, 2003; Lal *et al*., 2004; Lal and Pimentel, 2008; Van Hemelryck *et al*., 2011), thus resulting in a more accessible organic C to the microorganisms (i.e. easier degradable). Nevertheless, there is some controversy on the importance of this mineralization process after aggregate disruption, as estimations vary from 20 to 100 % of the soil organic C within aggregates.

As a consequence of this, the density of the organic C in the physically protected pool would decrease with the continuous erosion events. Nevertheless, in the growing season of the covered management this fraction would increase, especially in the iPOM content

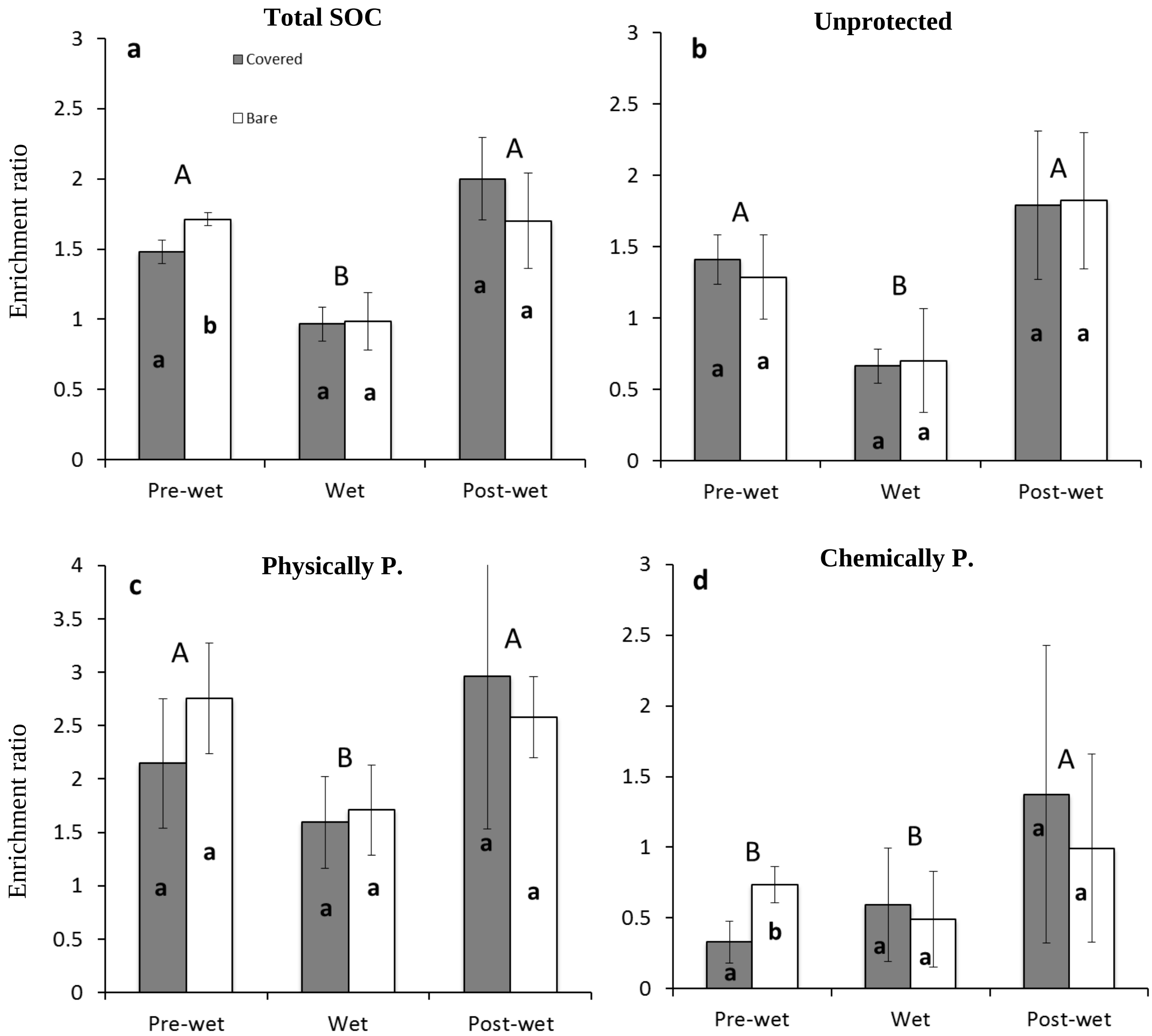
4.4 Enrichment ratio: in which fractions is soil being impoverished/enriched after water erosion?

Considering the total SOC content and the SOC content of the different fractions at the beginning of the measurements and those values of the collected sediments is possible to know if the soil is being enriched or impoverished in some SOC fractions after one year. Figure 13 shows the results for the enrichment ratio. When SOC enrichment ratio for total SOC or specific SOC fraction is higher than 1, means that the eroded sediment is enriched in SOC or in that specific fraction, whereas if it is < 1 then the eroded sediment is relatively impoverished in the fraction. Sediments recovered as a consequence of non-selective erosion processes are considered to have the same properties compared to the soils from which they derived (Van Oost *et al*., 2008; Kuhn *et al*., 2009). On the other hand, selective erosion, characterised by a preferential detachment of SOC, takes place in high intensity precipitation events with rain splash and interrill erosion, which might be important locally (Kuhn *et al*., 2009;Wang *et al*., 2010). Therefore, the enrichment ratio allows to know whether selective erosion is taking place.

Figure 13a shows that total organic C content in the eroded material was higher than that of the soil in the pre-wet and post-wet periods (e.g. SOC enriched ratio > 1), whereas in the wet period SOC content was similar in sediments than in the whole soil (e.g. SOC enriched ratio ≈ 1). These results suggest that independently of the management, SOC enriched particles are preferentially lost during first precipitations after summer. Similar pattern was found for the unprotected pool (figure 13b), but the enrichment ratio of this fraction was lower than one (relatively impoversished) during the wet period. These results are in line with those found on the percentage contribution of this fraction between different periods. Taking into account that about 50 % of the loss of organic carbon during the pre and post wet period corresponded to the unprotected fraction, overall these results indicate that during periods of relatively low rainfall intensity and preceded by periods with no rains, there is a marked preference to loss low-density relatively small fragments of organic matter.

Figure 13c shows the SOC enrichment ratio for the physically protected pool. Enrichment ratio was always higher than 1, and averaged more than 2 during pre and post wet periods and decreased significantly during the wet period, although never was below 1. Differences between managements were not significant. Clearly, this indicates that both, covered and non-covered plots are preferentially losing organic carbon within soil mircroaggregates.

These results suggest that soil is losing physically protected organic C during the whole period. That means clearly a soil degradation process, since as it was shown in Chapter I physically protected pool is the most important protected C, as it is really isolated from microorganisms' activity. The preferential lost of the physically protected C means a lost of physical and structural soil features, since microaggregates play a key role on soil physical structure (Six *et al*., 2004).



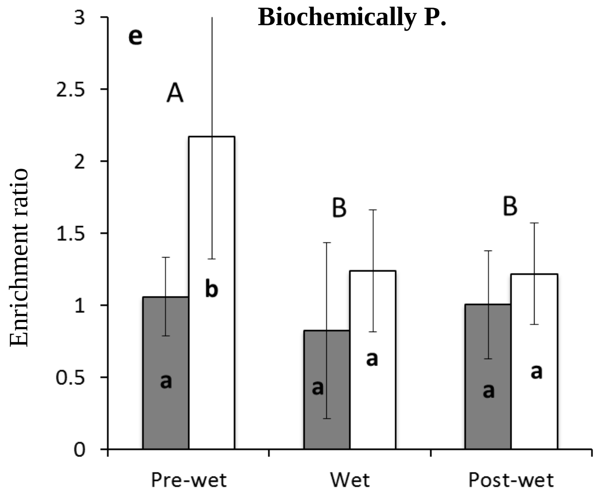


Figure 13. Organic carbon enrichment ratio of the total soil organic carbon (a), unprotected (b), physically protected (c), chemically protected (d) and biochemically protected (e) pools, in the three different periods: pre-wet, wet and post-wet and for the covered and bare soils. Different small letters mean significant differences in values for each fraction between each period, whereas capital letters mean significant differences between periods ($P < 0.05$). Error bars indicates standard deviations.

Enrichment ratio of the chemically protected organic C pool was typically lower than 1, and this was true for the pre-wet and wet periods (figure 13d). Only in the post-wet period values were slightly higher than one. In addition, enrichment ratio for this fraction was significantly higher in the non-covered plots during the pre-wet period, but differences between managements were not different in the other periods.

Finally, the enrichment ratio for the biochemically protected organic C pool showed different dynamics depending on the management. For the non-covered soils, the eroded material was enriched in this fraction, but this was not the case for the covered soils. During the wet and post-wet periods these values were around 1.0 (figure 13e).

In summary, these results suggest that in one year the soil lost organic C, and this loss was due mainly to losses of the unprotected and physically protected pools. In the case of the physically protected pool the impoverishment of the whole soil was strong and it suggests that the soil might be being degraded as a consequence of the water erosion.

4.5 Summarizing the dynamic of the SOC fractions in the context of water erosion processes

Unprotected pool

Figure 9a shows that the unprotected pool is mainly lost after the first intense events of rain. After this period, even in the covered plots (i.e. unprotected SOC entering through the biomass) in spring and summer unprotected organic C losses progressively decreased. Figure 10a suggests that the contribution of the unprotected organic C to the total SOC losses are especially higher at the beginning of the rainy periods, indicating that the unprotected pool would be preferentially lost in these first intense rainy events. This might be due to the higher density of organic C of this fraction in those periods (figure 11a). The explanation would be that the unprotected SOC is the most labile pool and, thus, the most easily erodible, and over time, as rains increase, the soil would be impoverished progressively in this fraction (figure 13b) and, afterwards, proportionally mineral particles and other fractions will be preferentially eroded.

Physically protected pool

For both managements, about a 45% of the SOC losses belonged to the physically protected pool (figure 10b). Furthermore, figure 9b shows that it is a clear difference in the amount of the physically protected SOC between managements in spring. During the growing season of the vegetation cover in the inter-row until it is mowed (from March to May) the precipitations eroded about 4 times higher physically protected C in the non-covered than in the covered plots. Therefore, the protective function of the biomass was clear in this period. Nevertheless, during the main part of the year physically protected organic C losses were lower or slightly lower in the covered than in the non-covered plots, even when there is no vegetation cover. This fact is probably due to the better properties characterizing soils with higher soil organic matter content (e.g. soil porosity, water infiltration…). However, the eroded sediments showed always enrichment ratios > 1, indicating that independently on the management there is a preferential lost of physically protected pool (figure 13c).

Chemically protected pool

As in the case of the other pools the total amount of chemically protected SOC lost was significantly higher in the non-covered than in the covered soils (figure 9c). This size fraction (< 53 µm) showed, overall, significantly higher organic carbon concentrations in the covered management (figure 11c). For that reason differences in the eroded chemically organic C were not usually very large. Despite this fact, as in the case of the physically protected pool, in spring SOC losses of this fraction were about 5 times lower in the covered plots. On the other hand, overall, SOC content of this fraction in the eroded soil was lower than in the whole soil, thus suggesting that after the erosion the whole soil is enriched in this fraction (figure 13d).

Biochemically protected pool

The differences on the organic C between managements were the highest in this fraction, being about two times higher in the bare soils. Again, as the figure 9d shows, the biochemically protected C lost by erosion was always higher in the bare soils, and the difference between managements were especially high in spring, being about 8.5 times higher in the non-covered plots. Nevertheless, as in the case of the chemically protected pool, the contribution of this fraction to the total SOC losses was relatively low (figure 10d). Overall, there were not large differences in the enrichment rations (figure 13e), so the organic carbon content of this fraction in the sediments and in the whole soil remained similar.

4.6 Towards a general scheme of the erosion process in olive grove soils. The key role of the inter-row vegetation cover

Figure 14 shows a scheme of the SOC fraction dynamics.

SOC losses of the different fractions are lower in the covered than in the bare soils. Only the unprotected pool showed similar dynamics in both managements, whereas the other two fractions (physically protected and < 53 µm) showed higher losses especially during spring in the bare soils.

Thus, in periods with high but not intense precipitations the vegetation cover acts as a protective cover and strongly reduces the SOC losses, especially those of the protected pools. During high intensity precipitations events (e.g. spring storms) in the covered soils SOC losses belonged mainly to the unprotected pool and were much lower than those of the non-covered plots.

As it was shown in the enrichment ratios (figure 13a), the soil sediments have higher total SOC content than the whole soil, and it was true for both managements, meaning that soil is losing organic C because of the water erosion. However, through the cover management there is an extra organic C input which can compensate these SOC losses and improve soil fertility properties (see Chapters IV and VI). On contrary, in the bare soils the incoming organic C compared to the covered-soils is much lower and also with poor fertility properties (see Chapters IV and VI) and, thus, soils under this management will be so degraded over time.

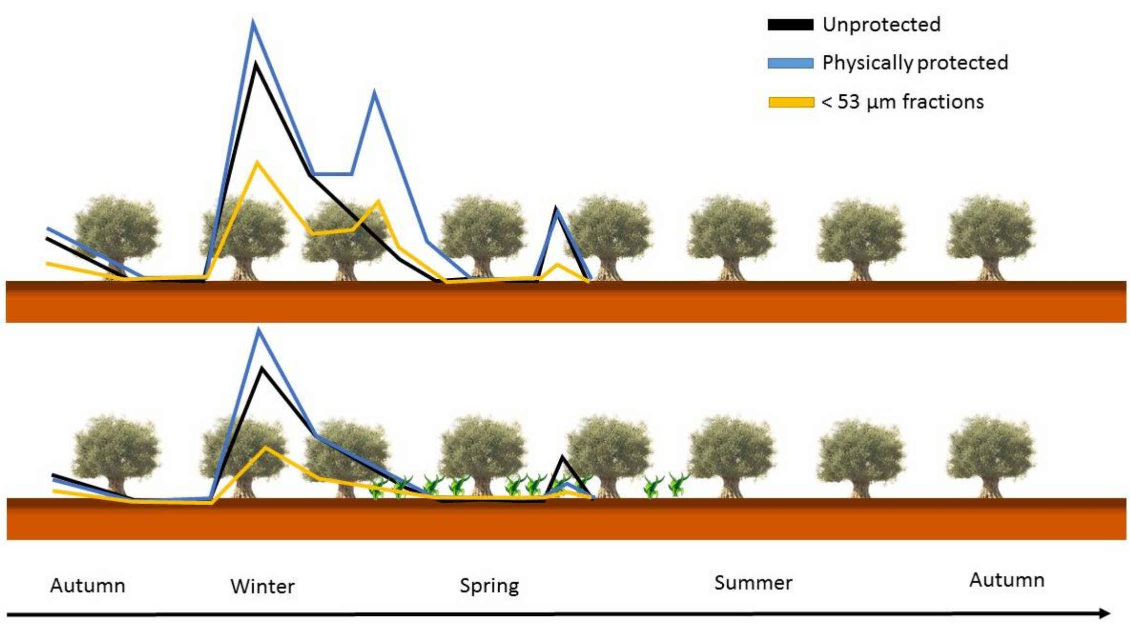


Figure 14. General scheme of the dynamic of the erosion of the different soil organic carbon fractions over time in an olive grove with a mean slope of 11%. Note that the chemically and biochemically protected pool are summarized in one single fraction (< 53 µm). The image above corresponds to an olive orchard with a conventional tillage (i.e. bare soil), whereas the image below corresponds to a vegetation-covered olive orchard.

4.6 Is the erosion a source or a sink of CO_2?

This is a key question and, unfortunately it depends on many complex variables. As Kirkels *et al*. (2014) highlight, there are three key factors which can provide a response to this question. i) The dynamic replacement by net primary production at eroding and/or depositional sites. In the covered plots, this was the case. Indeed, the eroded C is replaced by the annual income of plant-residues organic carbon. ii) The proportion of this fraction which is mineralized during transport and deposition processes. iii) rates of sediment and SOC erosion and deposition (Stallard, 1998; Harden *et al*., 1999; Berhe *et al*., 2007; Van Oost *et al*., 2007; Kuhn *et al*., 2009; Quinton *et al*., 2010).

As it was shown in Chapter IV the range of annual plant-aboveground organic C deposited in the soil was 0.2 – 1.0 t C ha^{-1}. Losses of soil organic C in the plant-covered olive orchards of this study were about 0.15 t C ha^{-1} yr^{-1}. Therefore, it is expected that in olive groves under a plant cover management, there is an effective SOC replacement. An opposite situation would be found in olive groves under non-cover management. In this case, the only organic C inputs would be those coming from the olive trees (e.g. olive leaves, olive fruits and those coming from the rhizodeposition processes). In this case, annual total SOC lost due to erosion were about 0.22 t C ha^{-1}. SOC "extra" losses were mainly due to the protected pools and thus, there is a continuous loss of potential to protect organic C, which may lead to a deterioration of soil properties (e.g. nutrient losses, degradation of soil structure and lower water retention capacity) (Starr *et al*., 2000; Jacinthe *et al*., 2001; Lal *et al*, 2004), and hence in potential productivity.

The second key point was related to the proportion of the SOC which is mineralized during the erosion processes. As it was commented before, the detachment of soil particles mainly in the non-covered soils lead to a disruption of the soil aggregates, thus facilitating the mineralization of the organic C which were protected within soil aggregates. The extent of this mineralization would be directly related with the distance from the eroded to the depositional sites.

The third point consists of the rates of sediment and SOC erosion and deposition. As it was detailed before, SOC losses might be higher after the first precipitations, when upper soil layer is still relatively enriched in organic C. Therefore, as a consequence of the erosion process soils might be a source of CO_2 in this first step and progressively they might change to a neutral (i.e. neither source nor sink) or a sink. In the case of the non-covered olive orchards, results show that protected SOC is highly lost during the spring period. As a consequence, the non-covered plots might be a source of CO_2 during the whole period of precipitations.

On the other hand, the eroded material was collected in different tanks which were interconnected, and the fate of the eroded SOC was not assessed. Therefore, it was not quantified the mineralization of the eroded SOC, which highly depended on the geomorphology features. For instance, Aranda *et al*. (2011) found better soil properties (e.g. SOC and N content) of colluvial than eroded soils in olive orchards under conventional management. The deposition site of the eroded SOC and the distance between the eroding and the depositional sites will lead to a higher or lower mineralization of the eroded organic C. For that purpose, eco-geomorphological models are a suitable tool and future researches should be focused on this field.

Independently on the fate of the eroded SOC, the results of this study reveal that the amount of the SOC lost in the non-covered plots was higher than in the covered plots and, moreover, this extra SOC losses are mainly due to the physically protected organic C losses. Thus, not only SOC is lost but also potential for protect SOC in case there is a change in management.

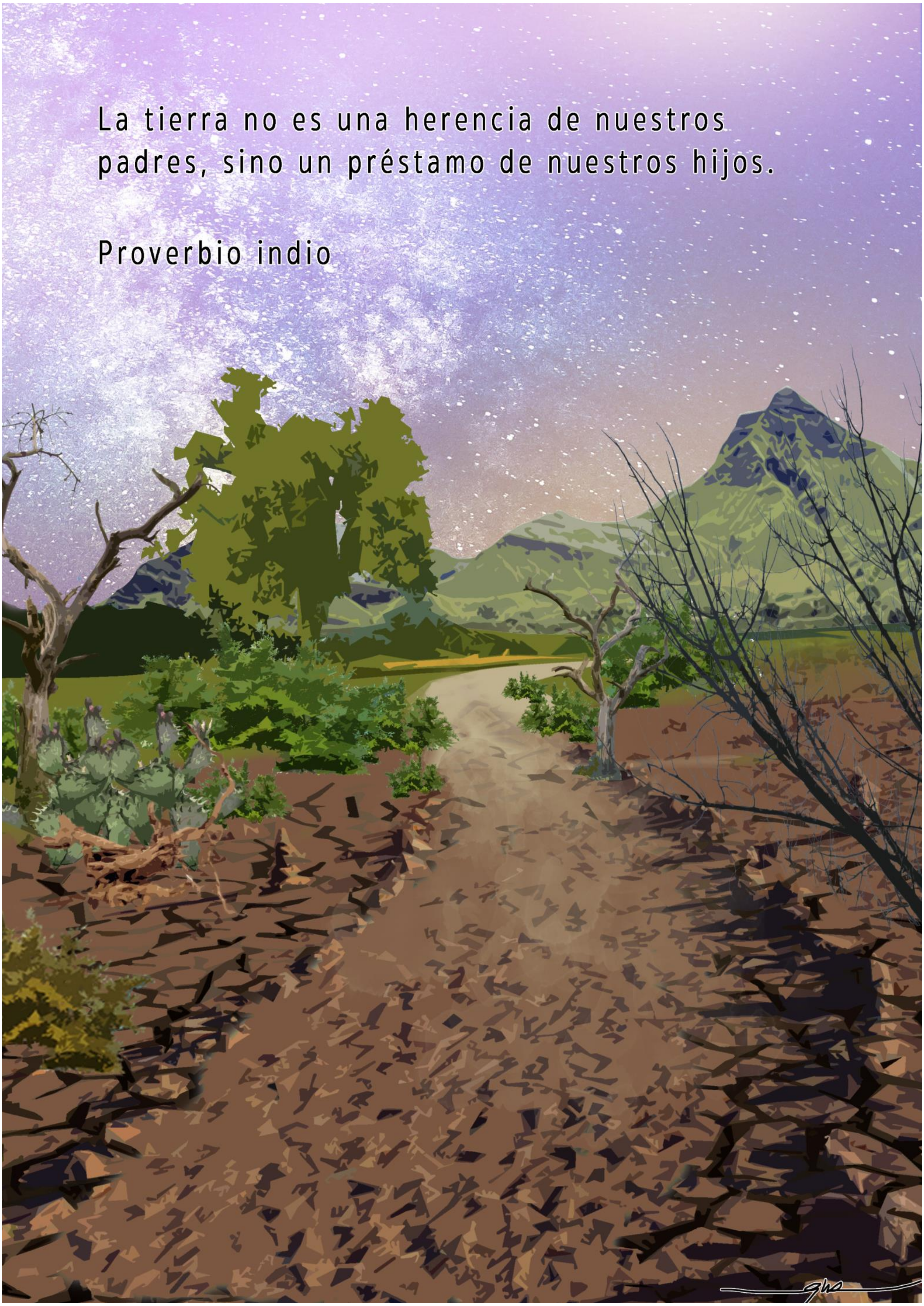
5. Conclusions

After the experiment described in this chapter, the following facts are remarkable:

- Total annual SOC losses in the **non-covered** olive orchards were **222 Kg C ha^{-1}**, whereas **153 Kg C ha^{-1}** were found in the **vegetation-covered** plots.
- Overall, total SOC content of eroded material was higher than that of the total SOC, and this was true independently of the management. This suggests that the **erosion process was selective and leads to a SOC depletion**. Nevertheless, taking into account **the input of organic C *via* plant residues in the covered plots, these SOC losses are compensated**. This was not the case for the non-covered olive orchards and may lead to a progressive decrease of the SOC content.
- Overall, losses of **unprotected** organic carbon showed similar pattern in both managements. Contribution of this fraction was **high during the pre-wet rain events and decreased during wetter months**. In general, the magnitude of unprotected SOC loss was higher or slightly higher in the bare soils.
- **Losses of physically protected organic carbon** were **higher in both managements at the beginning** of the intense rain events, and were, in general, higher or slightly higher in the bare soils. During the wet period losses of this fraction in the covered plots progressively decreased and only in very intense precipitations events were increased. On the other hand, losses **in the non-covered plots were more regular and higher in spring** and also after the very intense precipitations events.
- **Chemically and biochemically protected pools** showed, overall, a **similar pattern to that of the physically protected pool** (higher losses at the beginning

of the rain events and progressively decreased in the covered plots, and in the bare plots losses remained high in spring and after intense rain events).

- The **percentage contribution of the different fractions** to the total SOC did not differed between managements. Between **70 – 75 % of the total SOC losses were protected organic C**.
- According to the enrichment ratio, after water erosion processes **soils under both managements are impoverished in the physically protected pool** (values ranged 1.5 – 3.0), but **enriched in the chemically protected organic C.**
- The relative high losses of the unprotected pool, the low stratification ratio, and the absence of a vegetation cover protecting the soil and replacing the SOC lost by erosion under **non-cover management**, lead to a **vicious circle of soil degradation processes.**


La tierra no es una herencia de nuestros padres, sino un préstamo de nuestros hijos.
Proverbio indio

CHAPTER VIII

Long-term SOC sequestration under sustainable managements in olive groves: effects of weed-cover, olive tree pruning residues and olive mill pomace. Application of the RothC model

1. Introduction

As it was shown in the meta-analysis (Chapter III), usually, the studies focused on obtaining values of SOC sequestration do not take more than 10 years (e.g. García-Ruiz and Gómez-Muñoz, 2011; Lopez-Piñeiro *et al*., 2011). However, the efficiency of the SOC stabilization is not constant, but it reaches the highest values during the first years after the change in the management and these values decrease along time (Six *et al*. 2002) (see also results of the meta-analysis in Chapter III).

For that reason, new studies focused on calculating different SOC sequestration rates according to different periods of time are needed. Thus, models created to assess the changes in the SOC level at long-term periods after changes in the specific management practices become a good tool to achieve this objective. Some of these recommended management practices were shown in the meta-analysis (see Chapter III). In addition to the weed-cover management two other economically viable recommended managements can be also implemented: i) the application of shredded olive tree residues (e.g. Repullo *et al*., 2012a) and ii) the application of composted olive mill pomace (e.g. García-Ruiz *et al*., 2012, see also Chapter V).

There are many models used to assess the evolution of the SOC under specific conditions (SOMM, ITE, Verberne, RothC, CANDY, DNCC, CENTURY, DAISY, NC-SOIL). One of the most widely used is the RothC model (Coleman and Jenkinson, 1996; 1999). Among them, RothC stands out for its relatively easiness to be run and the relative low volume of the input data needed. Furthermore, it has been applied in a wide variety of environmental conditions. Nevertheless, at this moment there are some improvements which have to be done to achieve a better adjustment to drylands, including Mediterranean conditions (Farina *et al*., 2013).

RothC model has been used in more than 80 countries, in different crops and rotations (Studdert *et al*., 2011; Lugato and Berti, 2008), barely (Álvaro-Fuentes and Paustian, 2011), grasslands (Cerri *et al*., 2007; Liu *et al*., 2011; Francaviglia *et al*., 2012), vegetables (Stamati *et al*., 2013), olive orchards (Nieto *et al*., 2010), vineyards and scrublands (Francaviglia *et al*., 2012), and by applying different managements and under a wide variety of environmental conditions.

Furthermore, a high number of researches have been focused on validating the model by comparing the laboratory measures with the predicted values (e.g. Coleman *et al*., 1997; Falloon and Smith, 2002, Skjemstad *et al*., 2004; Peltre *et al*., 2012; Barančíková *et al*., 2010), finding that modelled SOC by RothcC were similar than real values.

Another advantages of the RothC consists of that it is possible to stablish a relationship between the fractions in which the RothC divided the SOC with those fractions which are obtained in the laboratory (unprotected and physically, chemically and biochemically protected) (Zimmerman *et al*., 2007). This fact allow to use the results of the fractionation in the laboratory in the model. This is very interesting, since the results of the different studies carried out in this Thesis could be used for the validation of the RothC in the future.

In summary RothC model is a widely used model and also validated in different conditions under different crops. Then, although with some cautions, RothC is a suitable model to study the SOC dynamic in olive grove soils after the implementation of three sustainable management practices: i) presence of weed cover, ii) application of shredded olive tree pruning residues and iii) application of composted olive mill pomace.

Models are useful to assess for patterns at long-term periods. This type of tools are complementary to field and laboratory studies. In the case of this Thesis the model has been used to obtain qualitative results which allow us to complete the results of the rest of the studies carried out. Therefore, with this last chapter, it has been completed the Thesis with the three main type of studies: laboratory, field and modelling. Combining these three it is possible to provide a complete analysis of SOC sequestration.

2. Objectives

The main objective of this chapter is:

Evaluate the long-term **influence of three sustainable economically viable management practices** (presence of **weed-cover**, and the application of: i) shredded **pruning residues** and ii) **composted olive mill pomace**, in olive groves **on SOC accumulation**.

3. Material and methods

3.1 Model description

RothC-26.3 (Coleman and Jenkinson, 1996;1999) is a model designed to predict the turnover of SOC in non-waterlogged topsoils that allows assessing the effects of soil type, temperature, moisture content and plant cover on the turnover processes.

There are 9 input variables to feed the model together with the initial SOC content. These are: monthly rainfall, monthly open pan evaporation, average monthly mean air temperature, clay content of the soil, an estimate of the decomposability of the incoming plant material (the DPM/RPM ratio; see below), the degree of soil cover, monthly input of plant residues, monthly input of farmyard manure and depth of the soil layer.

In this model, SOC is split into five pools: four active pools and the inert organic matter (IOM) pool. The four active compartments are: Decomposable Plant Material (DPM), Resistant Plant Material (RPM), Microbial Biomass (BIO) and Humified Organic Matter (HUM). Each of the active pools decomposes in the soil following a first-order process into CO_2, BIO and HUM (figure 1).

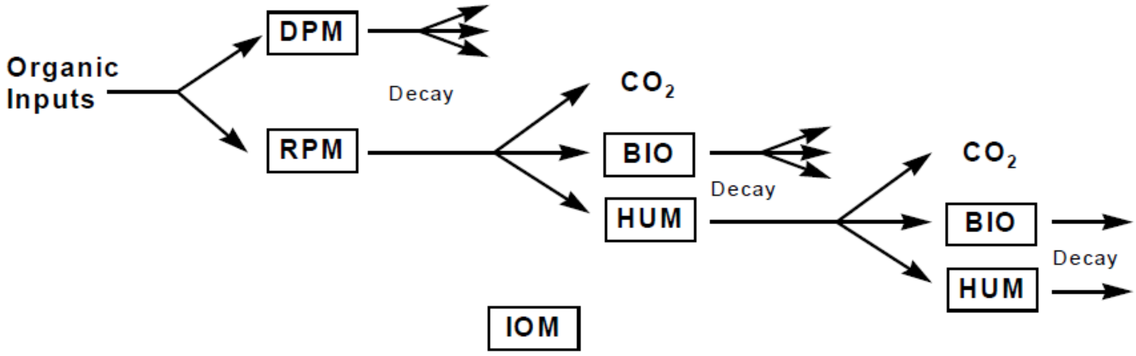


Figure 1. Main Structure of fate of the organic carbon in the RothC model (Coleman and Jenkinson, 1996).

3.2 Methodology of the application of the model

The steps for running forward the model are the following. The first step is to calculate the IOM fraction using the equation described by Falloon *et al.* (1998). Then, the model is run by modifying the annual organic C input until an equilibrium level of SOC is reached. The equilibrium level of SOC is that of the SOC amount of the conventionally managed olive grove, which was 13.7 t C ha^{-1} for the first 15 cm of soil. In addition to the initial total SOC content for the conventional management, the amount of the four active fractions are also calculated. Then, RothC is run in "short term" mode under different scenarios (see below). In this mode, we obtained ten run forward scenarios to predict the SOC accumulation using different levels of the organic C inputs in the weed-cover (PC), application of pruning debris (PD) and composted olive mill pomace (MP) managements (figure 2 and Table 5).

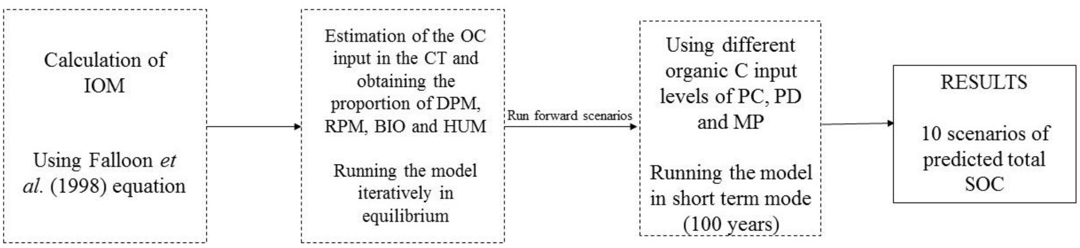


Figure 2. Scheme of the methodology of the RothC application to generate the run forward scenarios. PC, PD and MP stands for weed-covered, shredded olive tree pruning debris and composted olive mill pomace, respectively.

3.3 Inputs required to run forward the model

Climate

The selected climate features were these of Mancha Real (Jaén). This was based on the fact that weather at this location is representative of a high area of the Andalusian olive groves. Table 1 shows the 25-y monthly averages of temperature, rainfall and open pan evaporation at this meteorological station.

Table 1. 25-y monthly average temperature (Temp), precipitation (PP) and monthly average open pan evaporation (OPE).

	Temp (º C)	PP (mm)	OPE (mm)
Jan	8.7	25	48
Feb	10.0	174	63
Mar	12.4	72	105
Apr	17.3	17	166
May	19.4	18	229
Jun	24.1	23	259
Jul	30.6	0	332
Aug	30.0	18	289
Sep	24.1	27	196
Oct	16.1	37	112
Nov	10.4	97	52
Dec	9.1	165	47
Annual mean	**17.7**	**675**	**1899**

Soil data

Initial SOC and clay contents was that of the mean data for the first 15 cm of soil of five olive oil groves of Jaén and Granada (Andalusia, Spain) (Table 2).

Table 2. Initial soil organic carbon and clay contents in the five sites selected to obtain an average value (0 – 15 cm). Standard deviations are shown in brackets.

Sites	Initial SOC ($t\ C\ ha^{-1}$)	Clay (%)
Cambil 1	18.12	
	12.21	30
	14.37	
Cambil 2	13.45	
	19.97	43
	18.31	
Tobazo	8.25	
	10.69	38
	7.56	
Moraleda	16.34	
	16.54	17
	15.68	
Deifontes	11.76	
	11.52	21
	10.65	
Mean (sd)	**13.69 (3.74)**	**30 (11)**

The IOM was calculated using Falloon *et al*. (1998) equation:

$$IOM = 0.049 \times SOC^{1.139}$$

According to this equation, IOM was estimated to be 0.97 t C ha^{-1}.

Organic input data

Annual weed biomass production

Two levels, medium and high, of annual weed biomass production was considered to run the model. These two values were selected from the range of annual aboveground weed biomass production of the Chapter IV. Annual aboveground net organic C production ranged 0.24 – 1.0 t C ha^{-1} and averaged 0.54 t C ha^{-1}. The mean and highest annual organic C production was selected to run the RothC. Taking into account a root:shoot ratio of 0.8 estimated by Guzmán *et al*. (2014) for grassland, the total annual inputs of organic C (aerial + root biomass) was estimated to be 1.0 and 1.8 t C ha^{-1} for medium and high levels, respectively.

Shredded olive tree pruning residues

There are many tree performance variables which are related to annual olive tree pruning production. These include the age of the tree, annual olive fruit production and olive specie and/or variety. Three levels of annual organic C inputs due to shredded olive tree pruning were taking into account to run the model (Table 3). These three levels corresponded to olive oil groves with mean, high and very high annual olive fruit production. From these olive fruit annual production figures, the expected annual tree pruning production was estimated according to the model of Civantos and Olid (1985) which allow to transforms annual olive fruit production into annual olive pruning biomass.

Table 3. Levels of annual olive fruit production (Kg olive ha^{-1}) and those of olive tree pruning debris (Kg ha^{-1}) and organic C with the olive pruning debris (Kg C ha^{-1} yr^{-1}) selected to run the model. The organic C content of the olive tree pruning debris (46.2%) was that determined in this study.

Annual olive fruit production (Kg ha^{-1})	**Annual olive tree pruning production (Kg ha^{-1})**	**Annual organic carbon input due to the olive tree pruning (Kg C ha^{-1})**
3500	1738	802
5000	2521	1164
6500	3304	1526

Composted olive mill pomace

Selected annual organic C input due to the application of composted olive mill pomace was 0.92 t C ha^{-1}. This value was based on the mean organic C content of six composted olive mill pomace (collected from six olive mills which are currently producing composted olive mill pomace) and a mean of annual dose application by olive oil farmers (information from an own survey).

DPM/RPM ratios of the weed biomass, shredded olive tree pruning and composted olive mill pomace

To run RothC it is required that the proportion of the decomposable plant material (DPM) and resistant plant material (RPM) for each of organic inputs are estimated. RothC has a default value of 1.44 (which means that the easily decomposable organic C is 59% of the total weight of the biomass) for organic inputs. Nevertheless, the characteristics of the spontaneous resident vegetation cover, the shredded olive tree pruning and composted olive mill pomace are different than those of the sources of organic carbon originally used for RothC. For that reason, we calculated the specific DPM/RPM ratios for weed biomass, shredded olive tree pruning debris and composted olive mill pomace throughout a one-y laboratory experiment.

To estimate the DPM/RPM of the aerial biomass of the weed, the shredded olive tree pruning and the composted olive mill pomace, an equivalent amount of these materials (4 mg C g^{-1} of soil) was incubated with soils of contrasted texture and SOC content during one year at 25 ^{o}C and 60 % of WHC. Cumulative CO_2 production was followed during one year. A double exponential-four parametric function was fitted to the data ($r^2 > 0.83$; $P < 0.05$) and the labile and refractory organic C pool of these sources of organic carbon and their specific decomposition rate was calculated. DPM-to-RPM ratio was assumed to be Labile-to-refractory ratio. The mean for the assayed soils was used.

Calculated DPM/RPM ratios for the three different organic inputs are shown in Table 4.

Table 4. DPM/RPM ratios for aerial weed biomass. Shredded tree pruning remains and composted olive mill pomace.

	Aerial weed biomass	Shredded olive tree pruning debris	Composted olive mill pomace
DPM/RPM	0.66	0.19	0.12

DPM/RPM ratio for aerial biomass averaged 0.66. This value was very close to the default value given by the RothC for the unimproved grasslands (0.67). Values for shredded olive tree pruning remains and olive mill pomace were 3.5 and 5.5 times lower, respectively. The ratio for composted olive mill pomace are in line with the results shown in Chapter V. Indeed, only between 4 – 10 % of the organic C in the composted olive mill pomace was respired. Value of this ratio for shredded olive tree pruning remains are not surprising, since the C/N ratio and lignin contents are much higher than the weed biomass.

Scenarios

Ten scenarios were generated. Six of them correspond to a single organic input and four correspond to scenarios generated by mixing two different organic inputs. Table 5 shows the organic inputs, levels and descriptions (Table 6 shows the specific level of each input). Two levels for weed-cover management, three levels for olive pruning debris and one level for olive mill pomace. The remaining four scenarios were generated using the medium level of olive pruning debris and the level of olive mill pomace and combining them with the two levels of weed-cover. The baseline corresponds to an olive grove under conventional tillage (non-covered soil) (Table 5 and figure 3).

RothC data output

RothC predicts SOC content at different time periods and under different management involved in a change in magnitude and quality of the organic C input. Taking into account the initial SOC and the final SOC contents at different periods of time for each scenario, SOC accumulation rate can be inferred. In this study, SOC accumulation rates were calculated during the first 10, 50 and 100 years after the management change.

Table 5. The baseline scenario and the other ten scenarios, their organic carbon inputs and descriptions. Dotted lines separate the baseline scenario and the scenarios resulting from only one additional organic carbon input and a combination of different additional organic carbon inputs. In brackets in the scenario column levels of the organic inputs are named. Note that the scenarios 2 – 11 also include the internal inputs described in the baseline scenario.

Scenario	Input/s	Description
1. Baseline	Internal: olive leaves, organic C from the rhizodeposition processes…	Conventional tillage
2. Weed-cover (medium)	Spontaneous vegetation biomass	Weed-cover management with low organic C input
3. Weed-cover (high)	Spontaneous vegetation biomass	Weed-cover management with high organic C input
4. Olive pruning debris (low)	Shredded olive pruning debris and olive leaves	Application of low levels of shredded olive pruning debris
5. Olive pruning debris (medium)	Shredded olive pruning debris and olive leaves	Application of medium levels of shredded olive pruning debris
6. Olive pruning debris (high)	Shredded olive pruning debris and olive leaves	Application of high levels of shredded olive pruning debris
7. Olive mill pomace	Composted olive mill pomace	Application of composted olive mill pomace
8. Weed-cover (medium) + olive pruning debris (medium)	Spontaneous vegetation biomass Shredded olive pruning debris and olive leaves	Combination of a spontaneous vegetation cover with the application of pruning debris
9. Weed-cover (high) + olive pruning debris (medium)	Spontaneous vegetation biomass Shredded olive pruning debris and olive leaves	Combination of a spontaneous vegetation cover with the application of pruning debris
10. Weed-cover (medium) + olive mill pomace	Spontaneous vegetation biomass Composted olive mill pomace	Combination of a spontaneous vegetation cover with the application of composted olive mill pomace
11. Weed-cover (high) + olive mill pomace	Spontaneous vegetation biomass Composted olive mill pomace	Combination of a spontaneous vegetation cover with the application of composted olive mill pomace

4. Results and discussion

4.1 SOC accumulation rates under environmentally-friendly management in olive groves

Figure 3 shows the dynamics of SOC accumulation under the different scenarios. After 100 years, SOC contents under combined managements (25 – 40 t C ha^{-1}) were higher than these under single management (12 – 23 t C ha^{-1}). Typically, the higher was the annual organic C input, the greater was the SOC content, and for a similar annual organic C input, the lower was the DMP-to-RPM ratio, the higher was the SOC content after 100 years.

Highest SOC accumulation rates were achieved during the first 10 years after a change of management, and this was independently of the annual organic carbon input. 10-y SOC accumulation rates were between 2 to 2.5 times higher than these during the following 50 years and between 3 to 4 times higher if 100-y SOC accumulation rate is considered (figure 4 and Table 6).

This pattern has been well described. When SOC pool is increased by annual inputs of organic C, the magnitude of the decomposition, which is a *k* fraction of the SOC is increased and SOC accumulation rate is slow down until the organic carbon inputs is similar to the outputs (by CO_2 emission to the atmosphere), and SOC pool remains unchanged. Therefore, SOC is accumulated more efficiently during the first years after the change in the management and this is especially true for organic carbon sources with relatively low DMP-to-RPM ratio, such as shredded olive tree pruning and composted olive mill pomace. Despite this fact is well known (Six *et al*., 2002; Stewart *et al*., 2007; Stewart *et al*., 2008b, Stewart *et al*., 2009), it is often not considered when assessing the SOC accumulation rate of a given management (e.g. Lal, 2004b; Smith, 2004b; Franzluebbers, 2005; Smith *et al*., 2005; Hutchinson *et al*., 2007, Wang *et al*., 2010).

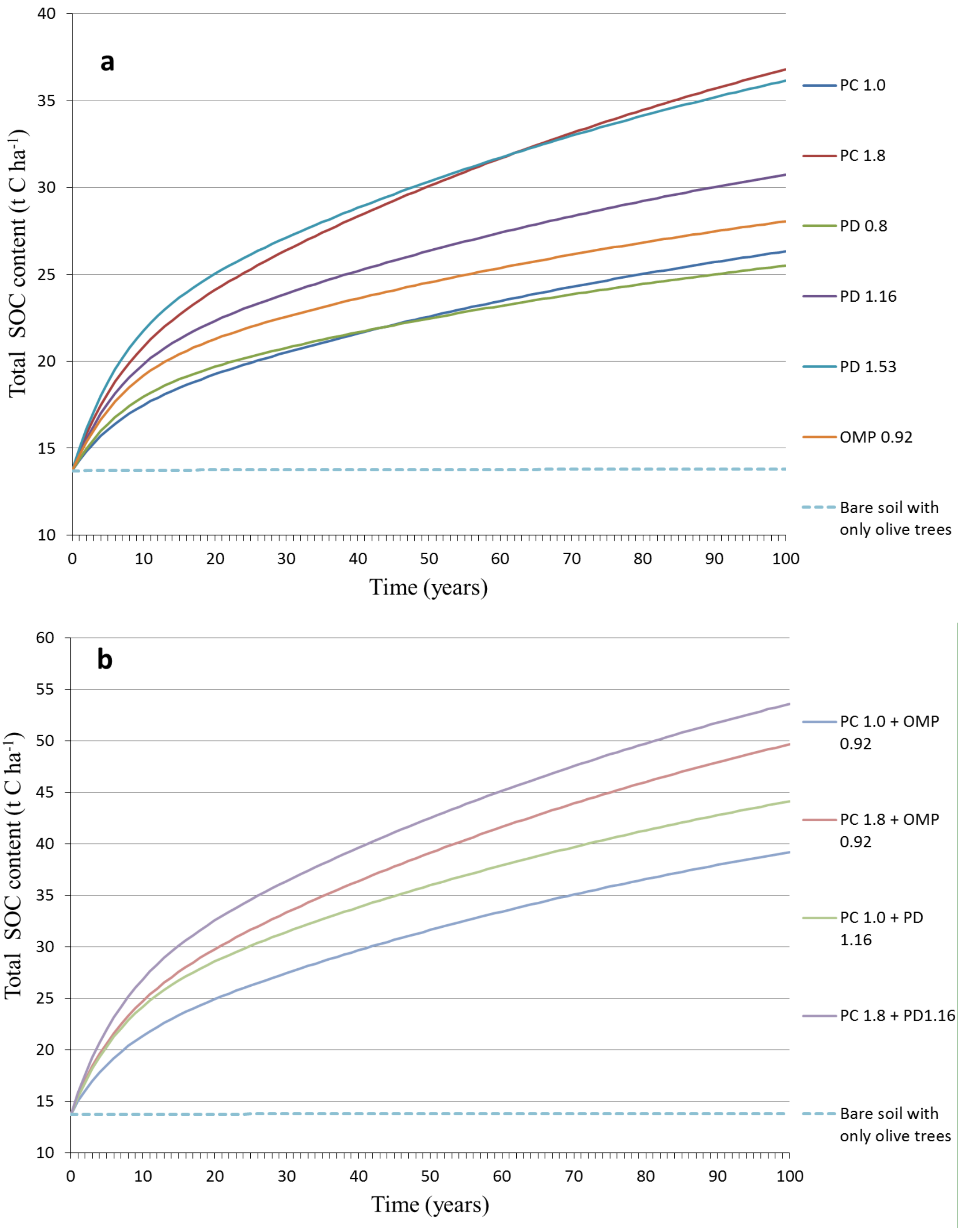


Figure 3. Dynamic of total soil organic carbon content (t C ha^{-1}) during 100 years after the change of single (a) and combined (b) managements (0 – 15 cm). Dynamic for the olive oil grove with bare soil without an annual input of organic carbon is also showed (dotted line).

Table 6. Annual organic carbon input (t C ha^{-1} yr^{-1}), initial and final soil organic carbon contents (t C ha^{-1}) and annual SOC accumulation rate (t C ha^{-1} yr^{-1}) for different periods (10 , 50 and 100 years) under the different single and combined managements.

Management	Annual O.C. input	Initial SOC	Final SOC	SOC accumulation	Annual SOC accumulation rate		
					10 years	50 years	100 years
Weed cover	1	13.70	26.32	12.62	0.38	0.18	0.13
	1.8	13.70	36.79	23.09	0.72	0.33	0.23
Olive pruning residues	0.8	13.70	25.50	11.80	0.43	0.18	0.12
	1.16	13.70	30.75	17.05	0.62	0.25	0.17
	1.53	13.70	36.15	22.45	0.81	0.33	0.22
Olive mill pomace	0.92	13.70	28.06	14.36	0.55	0.22	0.14
weed cover + olive pruning residues	2.16	13.70	44.14	30.44	1.05	0.45	0.30
	2.96	13.70	53.61	39.91	1.31	0.58	0.40
Weed cover + olive mill pomace	1.92	13.70	39.21	25.51	0.77	0.36	0.26
	2.72	13.70	49.68	35.98	1.10	0.51	0.36

For managements of single input, the highest SOC accumulation rate was obtained with the highest levels of annual organic inputs with a lower DPM/RPM. Thus, although the highest level of annual organic C input corresponded to weed-cover management (1.8 t C ha^{-1} yr^{-1}), the highest SOC accumulation rates occurred not only with the highest level of weeds (23.09 t C ha^{-1}) but also with the highest level of the pruning debris management (with 1.53 t C ha^{-1} yr^{-1} as incoming organic C input and a SOC accumulation of 22.45 t C ha^{-1}) due to a lower DPM/RPM than weed-cover management (figure 4 and Table 6).

10-y SOC accumulation rate for weed-cover management ranged 0.38 – 0.72 t C ha^{-1} yr^{-1} for an annual organic carbon input of 1.0 and 1.8 t C ha^{-1}, respectively. That means an efficiency in the annual C sequestration of between 38 and 40%. This finding is closed to the 34% found in the meta-analysis (Chapter III) and is also slightly higher to the SOC sequestration of 0.3 t C ha^{-1} yr^{-1} (15 cm of depth) reported by Castro *et al*. (2008) for an olive grove. Range of values were, however, lower than that of Repullo *et al*. (2012b) who estimated a SOC sequestration rate of 1.4 t C ha^{-1} yr^{-1} of (20 cm of depth) o that of Nieto *et al*. (2011) who found sequestration rate of between 2.6 and 3.5 in the first 30 cm in several Andalusian olive groves. Results of total SOC accumulation rates were also lower than that shown in Chapter IV (about 1.6 t C ha^{-1} yr^{-1}). Nevertheless, the differences on total SOC accumulated were similar than those found in Chapter IV (16 and 19 t C ha^{-1}) for the forst 15 cm of soil. 10-y SOC sequestration rate was lower than the average of 1.0 t C ha^{-1} yr^{-1} found for weed-cover olive cultivation in the meta-analysis of Chapter III.

Despite the risk in comparing studies performed in different pedoclimatic conditions, this could be due to an understimation of the amount of the incoming organic C through the biomass in the weed-cover management. As it was described in the M&M chapter (Chapter II, 1.2 section), usually only aerial biomass is used to estimate the amount of the organic C input by weed residues and thus annual input is underestimated, as belowground biomass may account for a great proportion of the total weed biomass. In addition, the role of the rhizodeposition *via* of organic carbon input is typically underestimated. Kuzyakov and Domanski (2000) estimated that rhizodepostition may contribute to the 50% of the organic C content of the incoming biomass. Therefore, one of the methods to consider rhizodeposition is by multiplying the total biomass with 1.5. Assuming similar C sequestration efficiency. By applying this factor, new figures of C sequestration rate emerge and values would range 0.6 – 1.1 t C ha^{-1} yr^{-1}. These new values are closer to the mean value of C sequestration rate for olive groves calculated in the meta-analysis. Therefore, these results suggest that for future studies, rhizodeposition should be considered.

The efficiency of the C sequestration in the first 10 years when shredded olive tree pruning residues were annually applied, was about 53%, a figure slightly lower than the 60% of the composted olive mill pomace, both higher than these found for the weed-cover management. However, these SOC sequestration efficiencies were much lower than those found (typically around 100 %) in the meta-analysis (see Chapter III). As it was commented in the meta-analysis, that fact might be related to synergies of the application of some amendments and also because of the infra-estimation of the inputs.

The highest SOC sequestration rate for combined managements was obtained when higher levels of organic C entered to the soil. Thus, the highest SOC accumulation rates

(1.31 t C ha^{-1} yr^{-1}) and total SOC accumulation (53.61 t C ha^{-1}) occurred with the combination of the highest level of weeds with a medium level of pruning residues (figure 4b and Table 6).

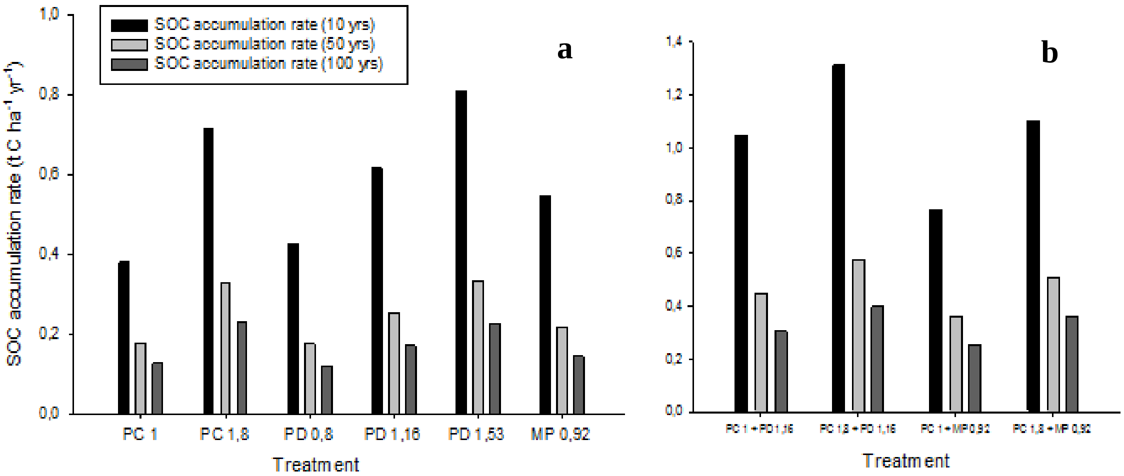


Figure 4. Soil organic carbon accumulation rate of the single (a) and combined (b) managements. The two first letters correspond to the treatment (PC = weed-cover, PD = application of pruning residues, MP = application of olive mill pomace) and the numbers indicate the annual amount of the organic C input.

Efficiencies of the C sequestration for the combination of weeds with shredded olive tree pruning residues ranged 44 – 49% and about 40% for the combination with the olive mill pomace. The explanation why these values are lower than the application of only pruning debris or olive mill pomace lies in that the mixture with the biomass, with a higher proportion of easily decomposable organic C, increases the proportion of the organic C that is mineralized in comparison to the single managements. These values were lower than the C sequestration efficiency found in the meta-analysis for the combined management practices in Chapter III), as it was the case for the single inputs.

RothC does not take into account the probable synergies when a sustainable management is implemented. For instance, olive groves are usually located on relatively high slopes (more than 8 %). Thus, the implementation of a weed-cover or an inert cover (mainly with shredded olive tree pruning residues) strongly decreases soil losses due to erosion (see Section 4.2 in Chapter I and results of Chapter VII). On the other hand, RothC might not be correctly adapted to Mediterranean conditions (especially to soil water content), thus overestimating the decomposition rate, thus needing an unrealistic high amount of the incoming organic C (Farina *et al*., 2013), therefore, underestimating SOC sequestration. Therefore, SOC sequestration predictions should be taken with caution.

Field studies involving a combination of sustainable managements and SOC sequestration in olive groves are not common as, usually, studies are focused on a single management. Regarding this fact and the high levels of SOC accumulation rates achieved by combining different sustainable managements, future studies should be focused on mixing managements and searching for positive synergies.Woody crops are more suitable to implement different sustainable managements than herbaceous crops. As it was highlighted in Chapter III (see 4.2 section) in the meta-analysis, a much wider inter-row area allows the implementation of the sustainable managements. Therefore, the potential of woody crops to combine sustainable managements leads to a higher SOC potential of SOC sequestration.

4.2 What would be the best scenario to accumulate SOC in Andalusian olive groves?

Best scenario to accumulate SOC in olive groves needs to be economically and technically viable and at the same time should show the highest SOC sequestration rate.

Considering the results shown in Table 6, combining the weed-cover and shredded olive tree pruning residues managements, and weed-cover and olive mill pomace managements were the best treatments to accumulate SOC, with 10-y annual SOC accumulation rates of 0.77 and 1.3 t C ha^{-1} yr^{-1}. Implementation of a weed cover is relatively easy for farmers, and most efforts are concentrated during one or two occasions when weeds are cleared. Therefore, efforts should be focused on analysing the easiest ways of applying pruning debris and composted olive mill pomace. While olive pruning debris are produced by the farm itself and can be shredded and applied *in situ*, composted olive mill pomace is produced as a result of the olive oil press process and after that it must be treated in a composting process before it is incorporated to soil. Composting is done in the olive mills. Thus, an olive oil farm is not self-sufficient to produce the amount of olive mill pomace necessary to be considered as an important management involving SOC accumulation.

Furthermore, as it was commented in Chapter I (4.2 section), olive pruning debris are often eliminated by burning them, thus emitting an "extra" CO_2. Therefore, a management involving the application of pruning residues in olive orchards would avoid these CO_2 emissions, as it was assessed by Nieto *et al*. (2010). Therefore, a combination of weed-cover and pruning debris management would be the best choice considering the amount of SOC accumulated after a hundred years of the management and the easiness of its implantation by olive farmers.

On the other hand, the combination of pruning debris (with a low DPM/RPM) and the weed-cover management (where biomass has a higher DPM/RPM) leads to a suitable mixture, since the higher decomposition rate of the biomass carries to a rapid incorporation of the organic matter to the soil, whereas the lower decomposition rate of the pruning debris leads to a slower incorporation of the organic matter and, therefore, increasing the SOC stratification rate, thus improving some soil physical properties of the upper layer, making the soil more resilient.

5. Conclusions

- Typically, the higher is the annual input of organic C, the highest is the SOC accumulation. **Highest total SOC accumulation rates** were found when **combining a weed-cover** with the application of **pruning debris** and **composted olive mill pomace.**

- **The highest SOC sequestration were reached during the first years after implementing the sustainable management**. This decrease in the SOC accumulation rate is not linear, so **the positive effects** of the sustainable managements on SOC accumulation **would be much more important during the first years**.

- Considering all these results and the economic and technical viability of implementing the different sustainable managements, the **combination of a weed-cover** with the **application of olive pruning debris would be a soundness strategy to increase SOC levels of the Andalusian olive groves**.

CHAPTER IX

Final considerations

1. Mediterranean conditions and the difficulty of predicting biomass production of the weed cover.

Mediterranean soils are characterized by low SOC content and long periods of water scarcity, especially in summer. This, together with high temperatures leads to very challenging conditions for soil microorganisms functioning.

These conditions contrast with those of others regions such as of other European countries. While in Central and Northern Europe the main limiting factor of the decomposition of the SOM is the very low temperatures during a great part of the year, in Mediterranean conditions is mainly the low soil water content (moisture) during 3 – 4 months (Vleeshouwers and Verhagen, 2001). As it was shown in Chapter V, autumn and spring were periods with greater decomposition of the SOM (i.e. soil respiration).

On the other hand, Mediterranean climate is also characterized for having very irregular conditions. The high inter-annual variability of the precipitations makes very difficult the prediction of soil and ecological variables. In this Thesis (Chapter IV) it has been highlighted the difficulty of providing an average value of the biomass production of the resident vegetation cover. Nevertheless, in this Thesis annual aboveground production of weed biomass residues has been taken from different parts of east Andalusian region and also in one plot during two consecutive years, obtaining a relative wide range of values (0.2 – 1 t C ha^{-1} yr^{-1}).

If this biomass is mowed (preferably from late March or early April) and left on soil surface, soil fertility conditions are improved at the long term (Chapter IV and Chapter VI). Indeed, one of these variables related to soil fertility, the SOC, was significantly increased and thus, it is necessary to give an outlook on the main benefits related to the increase of SOC content after implementing sustainable managements in olive groves.

2. Benefits of the implementation of sustainable managements in olive groves: the vegetation cover and other complementary managements

2.1 Weed-cover management

This thesis has been focused especially on studying the effects of the presence of cover in the inter-row area composed of resident vegetation. Nevertheless, other managements, such the application of the composted olive mill pomace, shredded olive pruning debris or a combination of these two with the presence of a vegetation cover are also sustainable managements which can be relatively easy implemented. There are two main benefits of implanting these sustainable managements: i) the increase in the SOC content mainly due to the increase in the incoming organic C input, and ii) the decrease in SOC lost due to a reduced soil erosion.

The meta-analysis of the Chapter III showed that in soils with the presence of a seeded or unseeded vegetation cover in olive groves SOC accumulation increases at about 1 t C ha^{-1} yr^{-1}. This figure of annual SOC accumulation rate is higher than the average of annual biomass production found in this study. The discrepancy between the two figures can be due to other unaccounted organic C inputs such as: i) the belowground biomass, and ii) rhizodeposition. The calculation of the rhizodeposition processes is very difficult and varies a lot depending on the study. Some studies show that the organic C in the belowground biomass could be up to 80 % of the aerial, and the organic C as a consequence of rhizodeposition processes about 50 % of the total biomass. However, these values are given high uncertainty.

In any case, results showed in Chapter IV clearly showed an increase in the SOC content in the weed-covered soils, which were about two times than of the non-covered soils for the top-15 cm of soil. Furthermore, this increase was not equally distributed among depth, as it was greater in the upper layer than in the deeper layer.

SOC sequestration efficiency varied over time after the change from a conventional to a weed-cover management. Efficiency was higher during the first years and declines thereafter. Thus, it is not fully correct to provide a single value of SOC sequestration for a given RMP. Different values for different periods of time are more realistic. This was the objective of using RothC model in Chapter VIII. It was shown that in the first 10 years, SOC sequestration rates were between 2 and 2.5 times higher than in the first 50 years, and these values represented between 3 and 4 times those found for the whole 100-y period.

Therefore, it is possible to estimate the SOC sequestration rates for the different periods taking the average value resulting from the meta-analysis for olive groves with the weed-cover management (1 t C ha^{-1} yr^{-1}). Applying these factors, the mean value for the first 50 years would be between 0.4 and 0.5 t C ha^{-1} yr^{-1}, and 0.25 – 0.33 t C ha^{-1} yr^{-1} for a period of 100 years .

Other benefits of the implementation of a weed-cover management is the decrease on SOC losses. It has been demonstrated in Chapter VII, a reduction of 70 Kg C ha^{-1} in an olive grove with an average slope of 11 % in a hydrological year. Moreover, it is necessary to remark that a great part of these “extra” losses were due to protected SOC losses, especially to the physically protected pool.

Thus, it is rather likely that the SOC sequestration might be higher than the predicted because of the important decrease on soil losses. Nevertheless, the extent to which the reduction of soil erosion contributes to SOC sequestration is highly depend on the geomorphological properties (slope, orientation, stoniness…). Therefore, future studies should be focused on applying mixed models (i.e. eco-geomorphological models) combining the study of the SOC fractions dynamic and erosion processes.

2.2 Other complementary managements: application of olive pruning debris and olive mill pomace

In Chapter VIII it has been shown that combining the weed-cover management with the application of shredded olive pruning residues or olive mill pomace leads to a higher SOC accumulation rates (0.8 – 1.3 t C ha^{-1} yr^{-1}) than a single management. This is not surprising. However, the quality of the incoming organic input should be considered when assessing the SOC sequestration. In Chapter V it was shown that the proportion of the respired labile C after the application of the composted olive mill pomace ranged 4 – 8 %, whereas for the biomass of the weed-cover ranged 30 – 60 %. Although pruning debris were not evaluated the similar values obtained for the DPM/RPM ratios for pruning debris and composted olive mill pomace in Chapter VIII suggest that the results of the mineralization of the organic C after the application of pruning debris and composted olive mill pomace might be similar.

However, although the composted olive mill pomace led to much higher SOC accumulation than the biomass residues, results of the PCA analysis shown in Chapter VI suggest that the weed-cover management is related not only to a higher SOC content but also a higher total N content. Therefore, the combination of the two managements (the weed-cover + pruning debris or compost) would be a suitable solution not only to increase the SOC content but also to improve soil fertility properties.

3. SOC dynamics and accumulation

3.1 Different managements and parent materials influence the SOC fractions dynamics

According to the results shown in Chapter VI, SOC fractions dynamic are related to the management and to the mineralogical and geochemical soil properties. The weed-cover management increased significantly the amount of the SOC of almost all SOC fractions, especially the unprotected and the physically protected (mainly due to the increase in the iPOM). However, the proportion of each SOC fraction was the similar under the two managements. This result is very important, since the physically protected pool was supposed to be affected negatively by the conventional management because of the breakup of the soil aggregates. However, the results suggest that this fact does not occur in some olive grove soils under the conventional tillage. The explanation might be related to the reduced tillage combined with the application of herbicides in the conventional tillage. The water scarcity in the inter-row area during part of the year, combined with the influence of the temperature leads to a growth of the biomass of the weed-cover basically in spring. The rest of the year the soil can be free of weeds and it is necessary to apply pre-emergence herbicides only before the growing season. As a consequence of this fact, the conventional tillage accumulates lower amount of SOC but in the same proportion that the weed-cover management.

Mineralogical and geochemical properties of the soil do affect not only the amount of the different SOC fractions, but also their proportion. In terms of amount of SOC, carbonated soils accumulated much greater SOC in all fractions except the biochemically protected, which was higher in the siliceous soils. In terms of proportion, the most relevant conclusion was that the proportion of the biochemically protected pool was higher in the siliceous soils and the unprotected pool was higher in the carbonated plots.

The explanation for the unprotected pool is relatively straightforward. Probably, the higher biomass production in the carbonated weed-covered soils led to much higher amounts of the unprotected and physically protected pools (especially the iPOM).

While the iPOM and the chemically protected pools within microaggregates were higher in the carbonated plots, the amount of the biochemically protected pool was higher in the siliceous plots. Similar patterns were found for the chemically and biochemically protected pool in the fine fraction. As it was proposed in the Chapter VI, the explanation might be related to the key role of the pH and the presence of carbonates. Soils with a pH of > 7 usually contains higher carbonated content (i.e. higher amount of Ca^{2+} and other cations) and with a medium or high clay content. On contrary, acid soils are typically richer on quartz and often with a relatively high content of sand (see PCA in Chapter VI). In carbonated soils the organic matter and clay minerals (i.e. Phyllosilicates) are negatively charged, so cations like Ca^{2+} can act as a bridge between the organic matter and the phyllosilicates (cation bridges formation). This SOC would be, thus, chemically protected. On contrary, in siliceous soils the low pH leads that the SOM and clays would not be as high negatively charged as those of the carbonated soils and, therefore, the SOC continues evolving towards enriching in recalcitrant substances (i.e. biochemically protection). Furthermore, the similar dynamics of the pools within and out of the microaggregates suggests that this mechanism is not dependent on the physical protection.

Nevertheless, the physically protected pool is a very complex fraction. While the chemically and biochemically protected pool have opposite dynamics depending on the parent material, the iPOM is highly dependent on the amount of the incoming organic C. Therefore, according to the results of this Thesis the physically protected pool should be always split into the three sub-fractions, since sometimes the opposite dynamics of the sub-fractions lead to a lack of significance for the whole fraction because of the opposite pattern of the different sub-fractions.

3.2 The existence of a SOC saturation limit

As it was shown in Chapter I, the saturation limit could be called *potential saturation limit* (Ingram and Fernandes, 2001), but it would be reached only in soils of natural ecosystems (e.g. forests). Therefore, a new concept is needed, the *effective stabilization capacity* (Stewart *et al*., 2007) or, in other words, an *attainable* SOC level (Ingram and Fernandes, 2001). This level would be a realistic SOC limit.

When a soil under natural vegetation cover is cultivated, some features related to soil fertility (SOC content, bulk density, aeration…) use to immediately worsen. Thus, the aim of the RMPs would be that the SOC level at the steady state is as close as possible to that of the natural vegetation. Thus, agroecosystems with a high net primary production (NPP), low disturbance or low residue removal will make closer the SOC level at the steady state to that of a natural ecosystem (Ingram and Fernandes, 2001).

Results of the Chapter VI showed that for the unprotected pool there would not be a saturation limit. A similar dynamic was found for the iPOM. The biochemically protected pools (both within and out of the microaggregates) showed no clear dynamic, probably because this fraction depends on the complex interaction of many variables (pH, carbonates content, texture, biochemical features of the biomass). On the other hand, the chemically protected pools (within microaggregates and in the fine fraction) best fitted a saturation dynamic.

However, although some fractions might not follow a saturation dynamic, the levels at the natural vegetation could be considered as the potential saturation limit. As it was shown in Chapter VI, current values of all fractions in woody crop soils under both managements might be far from those values under natural vegetation mature ecosystems. The challenge henceforth is to create agroecosystems being as close as possible to a natural ecosystem. Applying agroecology techniques would be a suitable solution.

4. The application of agroecology techniques: a suitable tool to improve olive grove agroecosystem

Agroecology techniques have been developed especially in Latin American countries (Altieri and Toledo, 2011). Briefly, agroecology searches for agroecosystems as close as possible to a natural ecosystem and with a high capacity of self-regulation. This is achieved by increasing the complexity of the agroecosystems (crop diversification, agroforestry systems, smaller plots…) and by integrating livestock in the agroecosystems (Altieri, 1999).

Currently, the mainstream organic agriculture consists of the substitution of inputs (inorganic by organic inputs) (Guzmán *et al*., 2000). However, the monoculture is basically the same (sometimes with a few more species because of the resident vegetation cover): great areas of monocultures and agroecosystems of low complexity, fossil-fuel dependence and also a strong dependence of the capital (Altieri and Rosset, 1995). This kind of agriculture is not focused on changing the management of the natural resources and does not solve the real causes of the ecological imbalance (Rosset, 1997).

Therefore, the current organic agriculture is far from being a complex agroecosystem. The result is that, as it was shown in Chapter VI. SOC content of the olive grove soils under spontaneous resident vegetation cover would be far from the SOC level of the natural ecosystems. Thus, there is a big scope to increase the SOC content of the Andalusian agroecosystems.

The benefit of applying agroecology techniques is not only to increase the SOC content. The higher diversity of plant species leads to a higher diversity of insects and other organisms who can prevent from the plagues (biological control), thus increasing the self-regulation aptitudes of the agroecosystems (Altieri, 1999). The diversity of crops and the integration of them with livestock lead to a higher diversity of products which can be obtained, thus reducing the importation of products and so reducing the CO_2 emissions of the transport. Therefore, the creation of agroecosystems with a high complexity leads not only to an increase in the SOC content but also to a decrease in CO_2 emissions from the transport. Direct and indirect CO_2 emissions should be considered when assessing the management practices in agriculture.

Translating these facts to the olive groves in Andalusia, future researches should be focused on studying the effects of the crop diversification on biogeochemical cycles. New crops should be introduced and mixed with olive groves. For example, pistachio groves mixed with olive groves are being planted since the last years in some areas of Jaén (Diario Jaén, 2015).

According to Rosset (1997) there are three main phases in the conversion from the conventional to an agroecological management. The first step is the reduction of the use of the agrochemical products (integrated management), thus increasing the efficiency of the use of these supplies. The second phase consists of substituting the inorganic by organic inputs. This management corresponds to the organic farming. But this is not the last phase. The last phase would be the redesign of the agroecosystems. That means to apply, locally inside the agroecosystem as a functional unit the three principles of the agroecology for the sustainable management of the natural resources: biodiversity, soil management and the promotion of the biological control (figure 1) (Altieri and Nicholls, 1999).

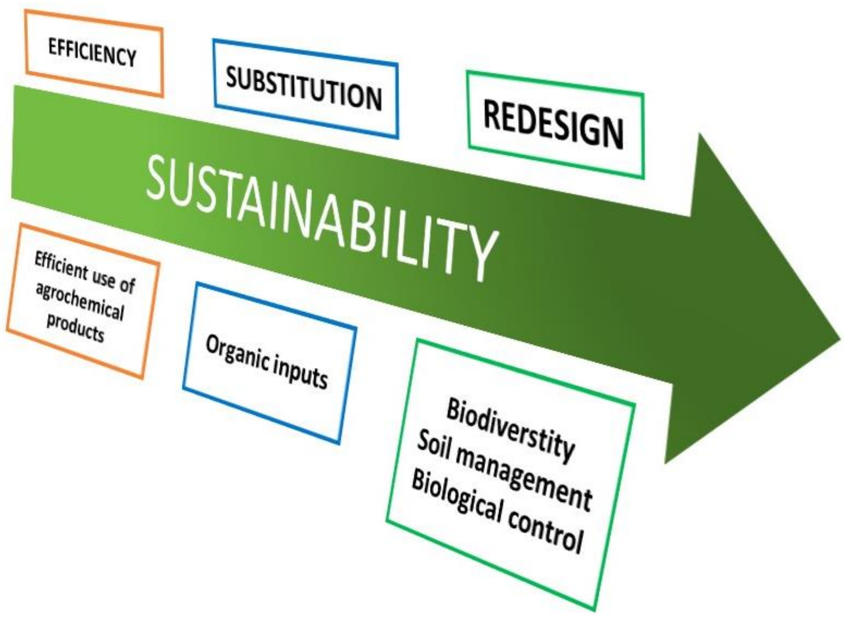


Figure 1. The three phases of the change from a conventional to an agroecological management. Rosset (1997), Altieri and Nicholls (1999), Sánchez-Escudero (2004).

Therefore, a final step is needed to achieve real sustainable agroecosystems. Redesign the organic farms should be the challenge for the next years and decades. Nevertheless, the agroecology science involves not only the environmental sustainability but also the social system. The redesign of the current agroecosystems could not be implemented without considering the collaboration of the society.

Despite this Thesis, and other similar studies, has clearly shown that RMPs lead to an increase on the SOC content, only a third of the Andalusian olive farmers maintain a spontaneous resident vegetation cover. This is clearly insufficient, since the weather conditions and the geomorphology make olive grove soils in general susceptible to degradation and desertification.

Therefore, from my point of view two different measurements are needed to increase the proportion of farmers applying RMPs: i) agro-environmental education, and ii) economic incentives.

4.1 Agro-environmental education

The work of scientists is not worth if we are not able to communicate and explain our results. Olive farmers do not usually have enough knowledge about environmental and agronomic processes. The result is that farmers (local communities) are losing the control of the lands, since the ancient knowledge has been substituted by external inputs which farmers do not have enough knowledge about, thus requiring more and more capital, energy and non-renewable resources (Sánchez-Escudero, 2004).

In order to counteract this situation plans and programs of agro-environmental education should be created as soon as possible. These plans and programs should explain the negative impacts on the environment and future harvests of tilling and applying agrochemical products. The objective of these programs would not be that farmers stop doing the conventional tillage, but to be aware of what they are doing when they till or apply an agrochemical product. And, finally, the final decision would be theirs. Thus, applying RMPs would not be an imposition, but their choice based on knowing as much as possible about it.

As the aim of this Thesis is not to create educational or divulgation programs I encourage people working on these social topics to take the results of this Thesis and to create these instruments to spread this knowledge.

Nevertheless, a transdisciplinary collaboration would be necessary. Agronomic, environmental, social and economic scientists should collaborate together. Currently, there are a new field of science, the "post-normal science" (Funtowicz and Ravetz, 1993) which tries to apply the knowledge of the scientists to solve problems of the society. By implementing workshops and other strategies these post-normal scientists are the link between the society, scientists and policymakers. With this collaboration, it would be possible to create opportunities for dialogue between the various parties involved.

However, unfortunately, these strategies are necessary but not sufficient to convince farmers to apply RPMs. Economic incentives are necessary too.

4.2 Economic incentives

Even if farmers had all the information about the RMPs and their benefits, often they need an economic incentive to implement them. Sometimes because they need to buy some machinery or they have to spend more time on the different field tasks, or simply because they think that the future harvests will be lower. In summary, they need a stimulus to change the management. This stimulus very commonly is an economic stimulus. However, two different types of economic stimulus could be applied: i) financial instruments (CO_2 markets) and ii) subsidies.

CO_2 markets

There are one good example of this kind of instruments: the European Union Emissions Trading System (EU ETS). This market was created in 2005 to reduce EU emissions in order to accomplish the Kyoto Protocol. This market does not include all industries and the wrong rules led to create a non-efficient market. For example, in January of 2008, the price of the tone of CO_2 was about 22 €, and at the beginning of October 2016 the price was about 5 € (http://www.sendeco2.com/es/). According to a working paper of the International Monetary Fund (Parry *et al*., 2014), the efficient price of the CO_2 tone for Spain would be about 50 $ (about 45 €). This is almost 10 times the current price. Therefore, these data shows clearly that the current CO_2 market is inefficient.

In the case of the agriculture the solution could be integrate the agriculture into the industry market of CO_2 emissions or create a new market only for the agriculture. However, the economic complexity of this financial instrument and the bad results of the existing market lead this solution to not to be adequate and to try to find other better solutions.

Subsidies

The main subsidy related to the agricultural system is the Common Agricultural Policy (CAP). The conditions of the CAP vary a lot depending on the country. While some European countries have already introduced some conditions to promote the environmental-friendly managements, Spain distributes the CAP money according to the crop surface. This, clearly, is contrary to the environmental preservation and it especially benefits those farmers having big monocultures, whereas farmers implementing RMPs are not incentivized.

In this context, Spain should reform the CAP (operative until 2020) as soon as possible and introduce some criteria related to environmental-friendly management practices leading to increase SOC content not only in olive groves, but also in the rest of the crops in Spain. I would be very glad to contribute with the results of this Thesis to help policymakers to achieve it.

5. Towards a sustainable agricultural system

I wish that this Thesis could help to the development of a new paradigm of the Andalusian agricultural system. We live in a world where it is produced enough food to feed more than the total Earth's population. However, hunger affects about 800 million people (FAO, 2015) and only the agricultural system would emits between 15 – 20 % of the total CO_2 (without considering livestock emissions). Furthermore, this agricultural system leads to a soil nutrient depletion, high soil erosion, soil and water pollution, natural resource depletion, and produces a high dependence of the agrochemical products. Consequently, this agricultural system increases hunger, environmental degradation and climate change and, furthermore, it is related to an increase in the number of wars, migrants, refugees, inequality and other social and economic problems (FAO, 2016a; 2016b; 2016c). As José Graziano da Siva, FAO Director-General, said, "peace begins in the countryside; there will be no peace without food security, and there will be no food security without peace". Thus, all these negative impacts are produced by this unsustainable system, in which the agriculture plays a key role. Therefore, change the agricultural system would be a first step not only to build a fairer System but also to preserve the Planet as we know it.

Nos damos cuenta de que somos muchos más de los que nos habían dicho que éramos; que anhelamos más y que, en ese anhelo, estamos más acompañados de lo que jamás habíamos imaginado.

Naomi Klein

CHAPTER X

Conclusions

The main conclusions of this work are summarized as follows:

1. According to the results of the meta-analysis, 10-y annual C sequestration rate for a resident vegetation covered olive grove is of about 1.0 t C ha^{-1}. However C sequestration efficiency tended to decrease along the time. The efficiency of the C sequestration rate would be about 30 %, although with high uncertainty.

2. C sequestration rates for the managements involving an external or internal organic amendment in olive groves (e.g. olive mill pomace and pruning residues) tended to be higher than that of the weed-cover management but they depended strongly on the amount of the input.

3. The annual production of aboveground biomass of resident vegetation cover was highly variable ranging from 0.25 to 1.0 t C ha^{-1}, and averaging 0.54 t C ha^{-1} yr^{-1}.

4. Weed-cover management increased up to two times the amount of SOC in comparison to the non-covered soils. Nevertheless, this increase was higher in the 0 – 5 cm than in the 5 – 15 cm soil layer. On contrary, in non-covered soils the SOC content in the two layers was similar. The existence of a stratification ratio in the weed-covered soils affects positively the soil physical properties in the upper layer, making the weed-covered soils less susceptible to the erosion.

5. Increasing the dose of the incoming organic C, the cumulative C-CO_2 respired was increased. However the higher was the dose, the lower the percentage of the cumulative respired C-CO_2 was. Therefore, higher proportion of the incoming organic C remains into the soil at higher doses of the input. This effect is stronger when the material is formed by a relatively high proportion of labile C (e.g. weed biomass).

6. The very low cumulative respired C-CO_2 after the application of composted olive mill pomace, compare to that found for the biomass from the weed cover, suggests that it is formed mainly by recalcitrant compounds, whereas the spontaneous resident vegetation cover would be formed by higher proportion of labile C. Therefore, the combination of these two inputs would be a suitable management in order to increase SOC sequestration and at the same time increase soil fertility properties.

7. The weed-cover management increased the organic C content especially of the unprotected and physically protected pools compared to the non-covered managements. That was true independently on the depth (0 – 5 and 5 – 15 cm), but it depended on the parent material.

8. Thus, parent material influences SOC content. Carbonated soils had double total SOC content in the first 15 cm compared to the siliceous soils. This was due especially to the increase on the most labile organic C fractions.

9. However, the biochemically protected organic C was higher under a siliceous parent material. This might be due to the low pH (typically about 6) of these soils, leading to a lower organic C linked to silt and clay minerals, thus allowing the organic C to evolve towards acquiring higher recalcitrant features through complexation and condensation reactions.

10. Only chemically protected pools (in the fine fraction and within microaggregates) showed a saturation dynamic, although this dynamic was only clear when a wide range of total SOC is considered. Our results suggest that both fractions for both parent materials would be saturated at about 50 % in the weed-covered plots and 30 % in the non-covered soils.

11. The unprotected and physically protected pools were more sensitive to the changes in the management. In the case of the physically protected pool, the results suggest that this fact was due to the iPOM, since it is the most labile sub-fraction forming the physically protected pool.

12. After one year, the presence of a vegetation cover in olive orchards decreased total SOC losses due to water erosion from 222 (bare soils) to 153 (covered soils) Kg C ha^{-1}. Non-covered plots lost especially protected organic C, mainly physically protected.

13. The different SOC fractions showed different dynamics during erosion events. The unprotected pool was mainly eroded after the first intense rains in both managements (covered and bare soils), whereas the protected pool losses were also higher in the non-covered soils during the precipitations in spring.

14. SOC sequestration rates obtained during the first 10 years were between 2 and 2.5 times higher than those for the first 50 years, and between 3 and 4 times those for

the whole period of 100 years. This decrease on the C stabilization efficiency means that over time the SOC will reach the new steady state.

15. According to the results obtained with the RothC model, the mean predicted SOC sequestration rate during the first 10 years after the combination of the weed-cover management and the application of olive pruning debris and olive mill pomace is between 1.1 – 1.3 and 0.8 – 1.1 t C ha^{-1} yr^{-1}, respectively. Assuming these data, the amount of the sequestered organic C in the upper 15 cm of soil under these managements is predicted to be 30.4 – 39.9 and 25.5 – 36.0 t C ha^{-1} after 100 years, respectively.

CHAPTER X

Conclusiones

Las principales conclusiones de este trabajo pueden resumirse en los siguientes puntos:

1. De acuerdo con los resultados del meta-análisis, el valor medio de tasa de secuestro de C para olivar con cubierta vegetal durante los primeros 10 años sería de aproximadamente 1 t C ha^{-1} $año^{-1}$. Sin embargo, la eficiencia en el secuestro de C descendería a lo largo del tiempo. En promedio, la eficiencia en el secuestro de C en el manejo con cubierta se situaría alrededor del 30%, aunque este valor ha sido calculado con un amplio rango de error.

2. Las tasas de secuestro de C para los manejos que implican entradas de C orgánico, internas o externas en olivar (ej. alpeorujo compostado y restos de poda) tendieron a ser más altas que las tasas calculadas para el manejo con cubierta vegetal, aunque dependieron fuertemente de la tasa anual de entrada de C orgánico.

3. La producción de biomasa aérea procedente de la cubierta vegetal fue muy variable, obteniendo valores entre 0.25 y 1 t C ha^{-1} $año^{-1}$, y promediando 0.54 t C ha^{-1} $año^{-1}$.

4. El manejo con cubierta vegetal incrementó el contenido en carbono orgánico en el suelo (COS) en alrededor de dos veces en comparación con las parcelas sin cubierta. No obstante, este incremento fue mayor en los primeros 5 cm que en el intervalo 5 – 15 cm. Por el contrario, en las parcelas sin cubierta vegetal el contenido en COS fue similar a lo largo de las distintas profundidades. Los valores de la razón de estratificación fueron superiores a la unidad en los suelos con cubierta, afectando positivamente a las propiedades físicas de la capa superficial del suelo y haciendo que éste sea menos susceptible a la erosión.

5. El incremento de la dosis de C orgánico aumentó la respiración acumulada de C-CO_2. Sin embargo, cuanto mayor fue la dosis menor fue el porcentaje de C-CO_2 que se respiró. Por tanto, a mayores dosis del input mayor fue la proporción de C orgánico que permaneció en el suelo. Este efecto es mayor cuando el material incorporado está formado por una relativa elevada proporción de C orgánico lábil (ej. biomasa de la cubierta vegetal).

6. La baja respiración acumulada de C-CO_2 tras la aplicación de alpeorujo compostado en relación con la encontrada para la biomasa de la cubierta sugiere que éste está formado principalmente por sustancias de carácter recalcitrante,

mientras que la biomasa estaría formada por mayor proporción de sustancias lábiles. Por tanto, la combinación de estos dos inputs constituiría un manejo adecuado con el fin de incrementar el secuestro de COS y, al mismo tiempo, mejorar la fertilidad del suelo

7. El manejo con cubierta vegetal incrementó especialmente el contenido en C orgánico de las fracciones no protegida y físicamente protegida en comparación con el manejo sin cubierta vegetal. Esto fue así independientemente de la profundidad (0 – 5 y 5 – 15 cm), pero dependió de la mineralogía.

8. La mineralogía afectó, por tanto, al contenido en COS. Los suelos carbonatados acumularon aproximadamente el doble de contenido en COS en los primeros 15 cm de suelo en comparación con los suelos silíceos. Esto fue debido especialmente al incremento en el contenido en C orgánico de las fracciones más lábiles.

9. Sin embargo, los suelos silíceos acumularon valores más elevados de contenido en C orgánico en la fracción bioquímicamente protegida en comparación con los carbonatados. Esto podría ser debido al bajo pH (típicamente alrededor de 6) en estos suelos, conllevando esto un menor C orgánico enlazado con el limo y los minerales de la arcilla, permitiendo así a este C evolucionar a través de reacciones de complejación y de condensación hacia formas de C orgánico más recalcitrantes.

10. Únicamente la fracción químicamente protegida (la del interior de los microagregados y la correspondiente a la fracción fina) mostró una dinámica de saturación, aunque ésta fue clara solo cuando se consideró un amplio rango de contenido total en COS. Nuestros resultados sugieren que ambas fracciones estarían saturadas en alrededor de un 50 % para los suelos bajo cubierta vegetal y en un 30 % para los suelos sin cubierta.

11. Las fracciones no protegida y físicamente protegida tendieron a ser las más sensibles a los cambios en los manejos. En el caso de la físicamente protegida nuestros resutlados sugieren que esto es debido a la iPOM (i.e. materia orgánica particulada en el interior de los microagregados del suelo), ya que es la sub-fracción físicamente protegida con mayor labilidad.

12. Tras un año, la presencia de cubierta vegetal en el olivar redujo las pérdidas de COS por erosión hídrica de 222 a 153 Kg C ha^{-1}. Los suelos sin cubierta perdieron especialmente C orgánico físcamente protegido.

13. Las diferentes fracciones de COS mostraron diferentes dinámicas durante los eventos de erosión. La fracción no protegida fue principalmente erosionada durante los primeros eventos importantes de lluvia en ambos manejos, mientras que las pérdidas de las fracciones protegidas permanecieron elevadas también en los suelos sin cubierta durante las precipitaciones de primavera.

14. Las tasas de secuestro de COS obtenidas para los primeros 10 años tras la aplicación del modelo RothC fueron entre 2 y 2.5 veces mayores que las alcanzadas para los primeros 50 años, y entre 3 y 4 veces mayores que las obtenidas para el período completo de 100 años. Esto supone un descenso en la eficiencia en la estabilización del C orgánico a medida que se aproxima al nivel del nuevo estado estacionario.

15. De acuerdo con los resultados obtenidos tras la aplicación del modelo RothC, la tasa media de secuestro de C en los primeros 10 años tras el cambio de un manejo convencional a uno con cubierta y la aplicación de restos de poda triturados y a un manejo combinando la cubierta con la aplicación de alpeorujo compostado sería de alrededor de 1.1 – 1.3 y 0.8 – 1.1 t C ha^{-1} $año^{-1}$, respectivamente. Esto se traduciría en un secuestro de COS tras 100 años de manejo en los primeros 15 cm de suelo de alrededor de 30.4 – 39.9 y 25.5 – 36.0 t C ha^{-1}, respectivamente.

CHAPTER XI

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ANNEX I

International stays

During this Thesis, the Ph.D student has done two international stays:

- From 15/09/2014 to 15/12/2014 in the "**Institute of Biological & Environmental Sciences**", University of Aberdeen, Aberdeen (Scotland, UK), under the supervision of the **Professor Dr. Pete Smith**.
- From 15/01/2016 to 15/04/2014 in the "**Centro di Ricerca per lo Studio delle Relazioni tra Pianta e Suolo (RPS)**", Consiglio per la Ricerca in Agricoltura e l'analisi dell'economia Agraria (CREA), Roma (Italy), under the supervision of the **Senior Researcher Dr. Rosa Francaviglia**.

Documents certifying these international stays are shown below.

UNIVERSITY OF ABERDEEN

Institute of Biological & Environmental Sciences
School of Biological Sciences
University of Aberdeen
23 St Machar Drive
Aberdeen AB24 3UU
Scotland, UK
Tel: +44 (0)1224 272702
Fax: +44 (0)1224 272703
Email: pete.smith@abdn.ac.uk

2nd February 2015

José Luis Vicente Vicente
Universidad de Jaén
Spain

Dear José Luis,

Re: José Luis Vicente Vicente visit to University of Aberdeen, September-December 2014

I am writing to confirm that you were a visiting student in my laboratory last year, between 15/09/2014 to 15/12/2014, in the Institute of Biological & Environmental Sciences, University of Aberdeen. You worked hard and were a great asset to the team when you were here. I would be very happy for you to visit again at any time, and I look forward to future collaborations with you.

Yours sincerely,

Professor Pete Smith, FSB, FRSE
Professor of Soils & Global Change
Science Director of Scotland's ClimateXChange
Director-Food Systems of Scottish Food Security Alliance-Crops
Theme Leader for cross-University Theme on Environment and Food Security

PROGRAMA ESTATAL DE PROMOCIÓN DEL TALENTO Y SU EMPLEABILIDAD

CERTIFICADO DEL CENTRO RECEPTOR TRAS LA ESTANCIA BREVE O TRASLADO TEMPORAL

CERTIFICATE OF STAY IN A FOREING INSTITUTION

1. Beneficiario/ *Applicant:*

Nombre y apellidos/ *Name: José Luis Vicente Vicente*

D.N.I./ *National identity Card:70899042r*

Centro de adscripción de la beca/ *Home Institución: Universidad de Jaén*

2. Centro en el que se ha realizado la estancia/ *Host institution:*

Nombre/ *Name: Centro di ricerca per lo studio delle relazioni tra pianta e suolo - Sede (RPS)*

Dirección/ *Adress: Via della Navicella 2-4. 00184*

Localidad/ *Country: Roma*

3. Investigador responsable en el centro de la estancia/ *Responsable person in the Host*

Institución/ *Institution: Centro di ricerca per lo studio delle relazioni tra pianta e suolo - Sede (RPS)*

Nombre/ *Name: Rosa Francaviglia*

Cargo/ *Post: Senior researcher*

CERTIFICO:
que el becario arriba mencionado ha realizado una estancia en este centro en las siguientes fechas: desde 15 / 01 / 2016 hasta 15 / 04/ 2016

THIS IS TO CERTIFY:
that the above mentioned person has performed a stay in this Institution in the following dates: From: 15 / 01 / 2016 To: 15 / 04 / 2016

Lugar y fecha: Roma, 15 de abril de 2016
City and date: Rome, 15th April, 2016

Firma y Sello/ *Signature & Stamp*
The Director
Dr. Anna Benedetti

crea

ANNEX II

International conferences

During the whole period of his Ph.D study, the student has gone to the following international conferences as a **first author**.

- **I International Meeting of CEICambio in Panamá**. Oral communication: Secuestro de carbono en olivar tras la implementación de practices respetuosas con el medio ambiente. Author: José Luis Vicente-Vicente. From 14/07/2015 to 18/07/2015. Ciudad de Panamá (Panamá).

- **5th Internations Symposium of Soil Organic Matter**. Poster: Soil organic carbon accumulation predicted by RothC model in Andalusian olive grove with three management practices: natural plant cover, olive pruning debris and composted olive mill pomace. Authors: José Luis Vicente-Vicente, Pete Smith, Marta Dondini, Roberto García-Ruiz. From 20/09/2015 to 24/09/2015. Göttingen (Germany).
- **5th Internations Symposium of Soil Organic Matter**. Poster: Influence of soil mineralogy and geochemical properties on soil organic carbon fraction in soils of contrasted mineralogy. Authors: José Luis Vicente-Vicente, Julio Calero, Roberto García-Ruiz, Víctor Aranda. From 20/09/2015 to 24/09/2015. Göttingen (Germany).

- **Seminar in the Centro di Ricerca per lo Studio delle Relazioni tra Pianta e Suolo (RPS)**. Oral presentation: Soil organic carbon research in Mediterranean agroecosystems. Author: José Luis Vicente-Vicente. 21/03/2016. Roma (Italy).

- **European Geosciences Union, General Asssembly 2016 (EGU)**. Oral presentation: Dynamics of soil organic carbon fractions in olive groves in Andalusia (Southern Spain) in soils with contrasted parent material and under different management practices. Authors: José Luis Vicente-Vicente, Roberto García-Ruiz, Julio Calero, Víctor Aranda. From 17/03/2016 to 11/03/2016. Wien (Austria).

ANNEX III

Reports of the External Assessments

Universidad de Jaén Escuela de Doctorado	**PhD DISSERTATION EXTERNAL ASSESSMENT (INTERNATIONAL MENTION)**

DOCTORAL STUDENT AND PhD DISSERTATION DATA	
Ph.D. Student name and surname:	José Luis Vicente Vicente
Title of the Thesis	*Soil organic carbon sequestration in olive groves in Andalusia: effect of the managements on soil organic carbon dynamics*

EXPERT/EXAMINER DATA	
Name and surname:	Beatriz Gómez Muñoz
Position:	Assistant Professor
University/Research Center Address and Country:	Department of Plant and Environmental Science Section of Plant and Soil Science University of Copenhagen – Faculty of Science

DISSERTATION ASSESSMENT	**Excellent**	**Very Good**	**Good**	**Pass**	**Fail**
Originality	X				
Goals	X				
Methodology	X				
Results relevance/significance	X				
Discussion and conclusions	X				

Please, mark with an X your evaluation

FREE FORMAT REPORT (USE AS MANY PAGES AS YOU NEED)

Hereby I inform that I have come to know the Thesis work entitled "*Soil organic carbon sequestration in olive groves in Andalusia: effect of the managements on soil organic carbon dynamics*", which has been carried out by José Luis Vicente Vicente under the supervision of Dr. Roberto García Ruiz at the University of Jaén.

The topic of this thesis is highly interesting and relevant, both from scientific and societal points of view since the global warming is one of the main environmental problem nowadays. Moreover, the agriculture has been attributed to be responsible of 15-20% of the greenhouses gases emission.

Spain is one of the main olive oil producers. In general the management of olive oil crops includes practices as tillage, herbicides and mineral fertilizers, but the combination of these practices with the erosion as result of high-slope plots, result in a dramatic reduction of soil organic carbon. The implementation of sustainable management practices i.e. the use of cover crops, incorporation of plant residues or compost application could improve the soil fertility and physicochemical properties and reducing the CO_2 emission from agricultural soils.

The study includes an extensive field sampling and lab work designed to quantify the different carbon pools in soil with different protection degrees. Moreover, the RothC model has been used to predict the influence of the presence of cover crops, the incorporation of plant residues and the fertilization with compost on soils organic carbon accumulation. Finally, a meta-analysis to evaluate the influence of different management practices on soil organic carbon content in three woody crops.

The results obtained in this study show that the presence of weed-cover crops increase the soil organic

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carbon compared to conventional tillage crops. Similarly, the addition of composted olive mill pomace showed very low CO_2 emissions due to the high proportion of recalcitrant substance in this material. Soil organic carbon accumulation is not only influenced by management practices, the soil physical and chemical properties also affects the amount of carbon accumulated in soil. Finally this Thesis reveals that there is a huge potential to increase soil organic carbon and, thus, helping climate change mitigation in olive crops by using sustainable management practices.
All these relevant results have the enough quality to be published in a one of the top refereed journals in this area, as for example the paper: "Soil carbon sequestration rates under Mediterranean woody crops using recommended management practices: A meta-analysis", which has been accepted in Agriculture, Ecosystems and Environment.
I also known José Luis Vicente Vicente has carried two short-stays abroad during his PhD formation, in important research institutions such as University of Aberdeen, supervised by Pete Smith, and the second one in Centro di Ricerca per lo Studio delle Relazioni tra Pianta e Suolo (RPS)", Consiglio per la Ricerca in Agricoltura e l'analisi dell'economia Agraria (CREA), Roma (Italy), whith the supervision of the Senior Researcher Dr. Rosa Francaviglia.
The literature listed at the end indicates that the candidate has studied also the most recent and relevant literature.
This PhD Thesis is a significant contribution to the knowledge of the soil organic carbon sequestration in olive crops. The PhD thesis is of good quality and certainly meets the standards of universities in the EU countries. For this reasons, I consider that this manuscript is appropriate for being presented for its defence and suitable for the "European Doctorate" mention at University of Jaén.

Date: 1-11-2016	Signature:

Universidad de Jaén Escuela de Doctorado	**PhD DISSERTATION EXTERNAL ASSESSMENT (INTERNATIONAL MENTION)**

DOCTORAL STUDENT AND PhD DISSERTATION DATA	
Ph.D. Student name and surname:	José Luis Vicente Vicente
Title of the Thesis	*Soil organic carbon sequestration in olive groves in Andalusia: effect of the managements on soil organic carbon dynamics*

EXPERT/EXAMINER DATA	
Name and surname:	Rosa Francaviglia
Position:	Senior researcher
University/Research Center Address and Country:	Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di ricerca per lo studio delle relazioni tra pianta e suolo Via della Navicella 2-4, 00184 Rome, Italy

DISSERTATION ASSESSMENT	Excellent	Very Good	Good	Pass	Fail
Originality		X			
Goals	X				
Methodology	X				
Results relevance/significance	X				
Discussion and conclusions	X				

Please, mark with an X your evaluation

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The thesis follows an excellent logic theoretical framework. Important findings are outlined in all chapters, with relevant and significant contributions derived from field and laboratory measurements with traditional and innovative techniques, and field studies with different managements of olive groves in a variety of conditions. A meta-analysis of the existing literature and a modeling exercise with the application of the RothC model are also provided. Discussion and conclusions are sound and fully supported by the experimental findings. Based on the overall assessment of the thesis, and the main contents summarized briefly in the following pages, I express a very positive judgment for the degree of Doctor from the University of Jaen "International Doctorate Mention".

CHAPTER I. Introduction. The chapter describes the importance of agricultural soils in the mitigation of climate change, providing details about the importance of soil organic carbon (SOC) and its relevance on soil functions, including the characterization of the different SOC fractions, the effect of different management practices, an in depth analysis of SOC sequestration, and introduces the concept of SOC saturation level. The introduction properly describes the importance of olive orchards, providing the state of the art of their management in Andalusia. Lastly a clear outline of the general and specific objectives of the thesis is provided.

CHAPTER II. Materials and methods. Sampling and pre-processing of soil and biomass, soil analyses (SOC fractionation, organic carbon and physico-chemical parameters), and statistical analysis are properly and thoroughly described.

CHAPTER III. SOC sequestration in woody crops: A meta-analysis. This chapter of the thesis deals with the effectiveness of Recommended Management Practices (RMPs) on SOC content, carbon sequestration and efficiency in comparison with the conventional management of woody crops. A total of 51 studies were selected during a literature review resulting in 144 independent comparisons between the RMP and the CONV managements in Mediterranean conditions. RPM categories were OA (organic amendment: compost, manure, crop residues, sewage sludge, other), CC (cover crops, resident vegetation cover), CC + OA = CMP (combined management practices). Additional variables considered in the chapter were woody crop specie (olive groves, vineyards, almonds), duration (years), soil depth and Mediterranean Sub-climates according to Köppen-

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Geiger classification. The influence of RMPs on SOC using data from CONV management as the reference was assessed. The main conclusion was that recommended management practices (RPMs) increased C sequestration in Mediterranean olive and almond orchards and vineyards compared to conventionally-managed cropping systems. Nevertheless, soil C sequestration was highest when applying organic amendments due to the relatively high annual doses of organic material applied, especially in olive orchards (e.g. pruning debris, composted olive mill pomace). However, the plant cover management, used as green manure, amounted to lower values of SOC sequestration rates, but the importance of this management is that it is relatively easy to be implemented by farmers at a relative low cost. Therefore, a combination of a plant cover in the inter-row of orchards with the application of external organic amendments (e.g. compost) or crop residues (e.g. pruning debris) would be a suitable management. Soil C sequestration rates were greatest during the first years after the change in management and progressively decreased, and lower soil C sequestration rates were achieved in semiarid and arid Mediterranean conditions.

CHAPTER IV. Influence of the spontaneous vegetation cover on total SOC content in olive groves. The chapter is based on three field studies in Andalusia with different objectives. Study 1: Quantification of the variability of the annual aboveground biomass production of spontaneous resident vegetation cover of olive orchards paired with a nearby and comparable olive orchard under conventional management. The objective was to assess the effects of spontaneous resident vegetation cover on total SOC content and considering different soil sampling depths. Study 2. Two contrasted-mineralogy sites (siliceous and carbonated, i.e. acid soils under granitic parent material, basic soils under marls) were selected. The objectives were to assess the effects of spontaneous resident vegetation cover on total SOC content, and evaluate the influence of some mineralogical properties on total SOC content. In each site, olive orchards with bare soil, with spontaneous natural vegetation and a forest were compared. The diffractograms of the acid and the basic soils are also provided. Study 3. Change in management in two nearby olive orchards (from bared to vegetation covered soil) to evaluate the short term changes of some soil properties. The objective was the quantification of the variability of the annual aboveground biomass production of spontaneous resident vegetation cover of olive orchards.
Considering the three studies the independent variables were: management (covered vs. non-covered soils), sampling depth (0 – 5 and 5 – 15 cm) and parent material (acid vs. basic soils). The dependent variables were aboveground annual biomass production, total SOC content, soil properties related to soil fertility, and mineralogical soil properties. The main conclusions were: Soils of olive orchards with a weed-cover had higher SOC both in the top 5 cm and in the 5–15 cm soil layer compared with bare soils. SOC stratification ratio was higher under a weed-cover management, indicating a better soil quality/fertility and higher resistance to soil erosion and other degradation processes. There was not relationship between annual biomass production of the weeds and SOC, likely due to other variables affecting the SOC dynamics. Soil parent material affected total SOC content, as under the same management, SOC in the soil soils was half of that of the basic soils.

CHAPTER V. CO_2 emissions and organic C inputs in olive grove soils in laboratory and field conditions. The objectives are: to assess the influence of a resident vegetation cover and environmental conditions (temperature and rainfall) on CO_2 emissions in field conditions; to evaluate the role of organic carbon quality on CO_2 emissions and organic carbon retention in laboratory conditions; to assess, in laboratory conditions, the influence of the application of different doses of the two types of organic inputs (pruning residues PR, and olive mill pomace COMP) on CO_2 emissions and organic carbon retention; to evaluate, in laboratory conditions, the influence of SOC saturation deficit on CO_2 emissions and organic carbon retention. CO_2 emission, cumulative CO_2 emission, and decomposition rate and the pools of labile and recalcitrant organic carbon were measured. In the case of the field study, only direct CO_2 emissions from soil were measured.
The main conclusions were: Soils with higher SOC content (i.e. lower saturation deficit) accumulated higher respired C-CO_2, but only in soils with no addition of organic input. Higher amounts of organic inputs leaded to lower cumulative respiration rates. Soils amended with PR respired much more CO_2 than those amended with COMP, due to the much faster incorporation to the soil of the PR than the COMP, since the COMP is formed by a high proportion of recalcitrant substances and PR is formed mainly by labile substances. A management combining PR and COMP could be suitable respectively to increase relatively fast the SOC content, and to add an organic C input with a slow incorporation rate to the soil. The field study showed that the respiration with a weed-cover management was higher in comparison with the non-covered soils, but only when warm temperatures and relatively high precipitations overlap (spring and autumn). Nevertheless, the total annual balance suggests that the C-CO_2 captured by the whole agroecosystem is higher in the weed-cover management than in the non-cover due to the higher uptake of CO_2 by the weeds.

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CHAPTER VI. SOC fractions dynamics: influence of management, mineralogy and soil depth. Mechanisms of SOC sequestration in Andalusian olive grove soils. The main objective of this chapter is to assess the influence of i) the presence of a spontaneous resident vegetation cover in the inter-row of olive groves, and ii) the mineralogical and chemical properties of the soil, on SOC fractions with different level of protection. Different depths were considered in some cases. The hypothesis of SOC saturation was also tested. Two fractionation methods of SOC were applied: the traditional SOC fractionation method (TEC, HA, FA and humin) that allows to calculate the Humification Index (HI), the Degree of Humification (DH), and the Humification Ratio (HR); the measurement of the E4/E6 ratio of the HA fraction, inversely related to the degree of condensation and aromaticity of the humic substances and to the degree of humification. The experimental design is described in the studies 1 and 2 of Chapter IV.
The main conclusions are: The implementation of a spontaneous vegetation cover in the inter-row area in olive groves led to a higher total SOC content in comparison to the non-covered soils. This increase was especially significant for the unprotected and physically protected pools. The contribution of the SOC fractions to the total SOC was not affected by the presence of a spontaneous vegetation cover. The depth strongly affected the amount of the different SOC fractions. The amount of the unprotected and the physically protected pools were significantly higher in the upper layer. The weed-cover management accumulated higher SOC content in comparison to the non-cover in top 5 cm and also in the 5–15 cm soil layer, mainly due to an increase in the unprotected and physically protected pools. Soil mineralogy had a significant effect on SOC fractions. Total SOC in carbonated plots doubled that under siliceous. HA and FA content tended to be higher under a weed-cover management. The higher E4/E6 ratios of HA of the weed-covered olive orchards and the carbonated plots suggest that in these soils the proportion of the fresh organic matter was higher than in the non-covered and in the siliceous plots. The unprotected and the iPOM of the physically protected pools showed clearly a non-saturation dynamic. Both fractions are the most sensitive to a change in the management. The chemically protected pool within micro-aggregates and in the fine fraction showed a clear saturation dynamic. Nevertheless, there were differences between parent materials. In the siliceous plots the saturation limit seems to be much lower than in the carbonated plots. The dynamic of the biochemically protected pool was unclear. Neither the biochemically protected pool within micro-aggregates nor the pool in the fine fraction showed a clear dynamic. Nevertheless, sometimes they showed a non-saturation dynamic, thus suggesting that this pool, sometimes, should not be considered as a "real" protected pool.

CHAPTER VII. SOC fractions dynamic during erosion processes in olive grove soils. The objective of this chapter is to assess the SOC losses in olive groves due to water erosion. For that purpose the total amount of SOC lost and the amount of SOC lost associated to each SOC fraction were studied in order to find out the most sensitive fractions to the erosion and, therefore, the soil enrichment or depletion in some fractions. Six plots and three managements were studied (planted with Lolium multiflorum, mechanical control and a mixture).
The main conclusions are: The non-covered management in olive orchards led to 222 Kg C ha^{-1} of total SOC losses in one hydrological year, and 153 Kg C ha^{-1} in the vegetation-covered plots. This value represents a significant increase of about 1.5 times on the total SOC losses in soils under conventional tillage. Total SOC content of the sediments recovered was higher compared to that of the whole soil for the different plots, thus suggesting that the erosion process leads to a SOC depletion. Unprotected pool losses showed similar dynamics in both managements. In general values of unprotected SOC losses were higher or slightly higher in the bare soils. Physically protected pool losses were higher in both managements at the beginning of the intense rain events, although in general these losses were higher or slightly higher in the bare soils. Chemically and biochemically protected pools showed, overall, a similar dynamic compared to the physically protected pool. The percentage contribution of the different fractions to the total SOC did not differed between managements. The physically protected pool was the fraction with the highest contribution (about 45% of the total SOC losses). The unprotected pool amounted between 25 – 30 %, whereas the < 53 µm fractions contributed about a 15 % each. Therefore, about 70–75 % of the total SOC losses belong to protected organic C. The enrichment ratio results show that the soil is depleted in the physically protected pool (values ranged 1.5–3) and is being enriched in the chemically protected pool. The relative high losses of the unprotected pool, the low stratification ratio, and the absence of a vegetation cover protecting the soil and replacing the SOC lost by erosion, lead soils under non-cover management to degradation processes that can be stopped only by changing the management to a vegetation-cover.

CHAPTER VIII. Long-term SOC sequestration under sustainable managements in olive groves: effects of weed-cover, olive tree pruning residues and olive mill pomace. Application of RothC model. The main objective are: Evaluate the long-term influence of three sustainable economically viable management practices (presence of weed-cover PC, application of shredded pruning debris PD, and composted olive mill pomace) in olive groves on SOC accumulation. Different scenarios of C input were used: Baseline (mainly olive leaves with conventional tillage), Weed-cover (medium and high, with spontaneous

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vegetation biomass), Olive pruning debris (low, medium and high with shredded olive pruning debris and olive leaves), Olive mill pomace (with composted olive mill pomace), Weed-cover (medium) + olive pruning debris (medium), Weed-cover (high) + olive pruning debris (medium), Weed-cover (medium) + olive mill pomace, Weed-cover (high) + olive mill pomace. Typically, the higher is the annual input of organic C, the highest is the SOC accumulation.

The highest total SOC accumulation rates were found when combining a weed-cover with the application of pruning debris and composted olive mill pomace. The highest SOC sequestration were reached during the first years after implementing the sustainable management, so the positive effects of the sustainable managements on SOC accumulation would be much more important during the first years. Considering the results and the economic and technical viability of implementing the different sustainable managements, the combination of a weed-cover with the application of olive pruning debris would be a soundness strategy to increase SOC levels of the Andalusian olive groves.

CHAPTER IX. General discussion. This chapter covers the following topics: Mediterranean conditions and the difficulty of predicting soil and agroecological variables; Benefits of the implementation of sustainable managements in olive groves: the vegetation cover and other complementary managements (application of olive pruning debris and olive mill pomace); SOC dynamics and SOC sequestration(managements and parent materials influence on SOC fractions dynamics; SOC saturation limit); The application of agroecology techniques: a suitable tool; SOC sequestration needs to focus on social and economic variables as well (Agro-environmental education, Economic incentives).

CHAPTER X. General conclusions. This chapter summarize briefly the findings of all the previous chapters.

Date: 3 November 2016	Signature: Rosa Francaviglia

Universidad de Jaén Escuela de Doctorado	**PhD DISSERTATION EXTERNAL ASSESSMENT (INTERNATIONAL MENTION)**

DOCTORAL STUDENT AND PhD DISSERTATION DATA	
Ph.D. Student name and surname:	José Luis Vicente Vicente
Title of the Thesis	*Soil organic carbon sequestration in olive groves in Andalusia: effect of the managements on soil organic carbon dynamics*

EXPERT/EXAMINER DATA	
Name and surname:	Jennifer Soong
Position:	Post doctoral researcher, PhD
University/Research Center Address and Country:	University of Antwerp Universiteitsplein 1, Wilrijk 2610 Belgium

DISSERTATION ASSESSMENT					
	Excellent	Very Good	Good	Pass	Fail
Originality		X			
Goals		X			
Methodology		X			
Results relevance/significance		X			
Discussion and conclusions		X			

Please, mark with an X your evaluation

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This dissertation on soil carbon dynamics is a strong body of research that would also qualify at the international level for PhD dissertations. It represents an original and novel piece of research and is coherently structured and clearly presented. Results therein are in concordance with current research topics and represent an important advance in ecological knowledge. This work demonstrates the ability of the candidate to carry out individual research, to formulate hypotheses and to appropriately use the methodological and statistical tools required to test these hypotheses. I express my sincerest hope that the corresponding organism at University of Granada will allow to Jose Luis Vicente Vicente to defend his dissertation.

Date:	Signature:
04/11/16	[signature]

Universidad de Jaén Escuela de Doctorado	**PhD DISSERTATION EXTERNAL ASSESSMENT (INTERNATIONAL MENTION)**

DOCTORAL STUDENT AND PhD DISSERTATION DATA	
Ph.D. Student name and surname:	José Luis Vicente Vicente
Title of the Thesis	*Soil organic carbon sequestration in olive groves in Andalusia: effect of the managements on soil organic carbon dynamics*

EXPERT/EXAMINER DATA	
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DISSERTATION ASSESSMENT	Excellent	Very Good	Good	Pass	Fail
Originality		X			
Goals	X				
Methodology	X				
Results relevance/significance	X				
Discussion and conclusions	X				

Please, mark with an X your evaluation

FREE FORMAT REPORT (USE AS MANY PAGES AS YOU NEED)

I consider the Thesis presented by Mr. José Luis Vicente Vicente is of great interest and quality. The topic addressed is interesting not only to researchers and staff from the Academic sector but also to policy makers and the general public. This Thesis provides very valuable insights on the effect of conservation practices based on plant covers in olive orchards and other Mediterranean crops on carbon sequestration and dynamics of SOC, which is crucial to understand the ultimate effect that these practices have on soil quality, crop productivity and climate change mitigation. The methodology proposed is exhaustive and robust, combining both modeling and experimental approaches, and the results are novel and relevant.

Mr. Vicente Vicente adequately tackles the topic of the Thesis through the establishment of a series of general and specific objectives, which are successfully achieved in the different Chapters. In Chapter III, a meta-analysis is applied to examine the effect of management practices on the SOC sequestration capacity of different Mediterranean woody crops (olive and almond orchards and vineyards). In Chapter IV, it is quantified annual aboveground biomass of plant covers and its effect on SOC content in olive groves, as well as the effect of mineralogy on SOC content, while Chapter VI examines the influence of plant covers and mineralogy on SOC fractions. In Chapter V, it is analysed the influence of organic carbon quality on CO_2 emissions. Chapter VII evaluates not only total SOC losses by water erosion in olive orchards but also losses associated to each SOC fraction. Finally, in Chapter VIII the long-term effect of different management practices on carbon sequestration in olive orchards is analysed with the RothC model.
The results shown in the different chapters drive to the conclusion of the importance of plant covers and other recommended management practices in the increase of SOC content in olive groves, and the higher potential of Mediterranean woody crops for carbon sequestration if sustainable practices are applied. The use of plant covers not only affects total SOC content but also the different SOC fractions. However, its effect varies depending on mineralogy.

Universidad de Jaén Escuela de Doctorado	**PhD DISSERTATION EXTERNAL ASSESSMENT (INTERNATIONAL MENTION)**

As a summary, the Thesis of Mr. Vicente Vicente clearly fulfills the quality standards for PhD theses on an international level. The studies were conducted on a highly-relevant topic and comprised well-established laboratory and field measurements and development of analytical and modeling approaches. Thus, the candidate proofed his eligibility for an internationally awarded PhD title.

Date: 05/11/2016	Signature:

ANNEX IV

Publications

Although the majority of the publications related with this Thesis are still now being prepared (and most of them have alredy been published in different Spanish conferences), some publications are already published or near to be published. In this sense, three publications must be highlighted:

- **Soil carbon sequestration rates under Mediterranean woody crops using recommended management practices: A meta-analysis**. Vicente-Vicente, J.L., García-Ruiz, R., Francaviglia, R., Aguilera, E., Smith, P., 2016. Agriculture, Ecosystems and Environment, 235:204 – 214.

Journal Metrics

Source Normalized Impact per Paper (SNIP): **1.674**

SCImago Journal Rank (SJR): **1.879**

Impact Factor: **3.564**

5-Year Impact Factor: **4.233**

- **Net ecosystem CO_2 exchange in an irrigated olive orchard of SE Spain: influence of weed cover.** Chamizo, S., Serrano-Ortiz, P., López-Ballesteros, A., Sánchez-Cañete, E.P., Vicente-Vicente, J.L., Kowalski, A.S., 2017. Submitted.

- **Temporal stability and patterns of runoff and runon with different cover crops in an olive orchard (SW Andalusia, Spain).** López-Vicente, M., García-Ruiz, R., Guzmán, G., Vicente-Vicente, J.L., Van Wesemael, B., Gómez, J.A., 2016. Catena, 147:125–137.

Journal Metrics

Source Normalized Impact per Paper (SNIP): **1.573**

SCImago Journal Rank (SJR): **1.191**

Impact Factor: **2.612**

5-Year Impact Factor: **3.097**

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Soil carbon sequestration rates under Mediterranean woody crops using recommended management practices: A meta-analysis

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ABSTRACT

Mediterranean woody crops, such as olive and almond farming, and vineyards are usually cultivated in soils low in organic matter, with limited water availability and frequently on medium to steep slopes. Therefore, when conventionally cultivated, soils of these cropping systems are net sources of CO_2 (throughout soil erosion and organic carbon mineralization). A promising option to sequester carbon (C) in these cropping systems is the implementation of recommended management practices (RMPs), which include plant cover in the inter-row area, minimum or no tillage and off- and on-farm organic matter amendments. However, the effects of RMPs on soil organic carbon (SOC) stocks in these cropping systems are widely overlooked, despite the critical importance of estimating their contribution on CO_2 emissions for policy decisions in the agriculture sector in Mediterranean regions. We therefore conducted a meta-analysis to derive a C response ratio, soil C sequestration rate and soil C sequestration efficiency under RMPs, compared to conventional management of olive and almond orchards, and vineyards (144 data sets from 51 references). RMPs included organic amendments (OA), plant cover (CC) and a combination of the two (CMP). The highest soil C sequestration rate (5.3 t C ha^{-1} yr^{-1}) was observed following the application OA in olive orchards (especially after olive mill pomace application), whereas CC management achieved the lowest C sequestration rates (1.1, 0.78 and 2.0 t C ha^{-1} yr^{-1}, for olive orchards, vineyards and almond orchards, respectively). Efficiency of soil C sequestration was greater than 100% after OA and CMP managements, indicating that: i) some of the organic C inputs were unaccounted for, and ii) a positive feedback effect of the application of these amendments on SOC retention (e.g. reduction of soil erosion) and on protective mechanisms of the SOC which reduce CO_2 emissions. Soil C sequestration rate tended to be highest during the first years after the change of the management and progressively decreased. Studies performed in Mediterranean sub-climates of low annual precipitation had lower values of soil C sequestration rate, likely due to a lower biomass production of the crop and other plant cover. Soil C sequestration rates in olive farming were much higher than that of vineyards, mainly due to the application of higher annual doses of organic amendments. The relatively high sequestration rate combined with the relative large spatial extent of these cropping system areas suggests that the adoption of RMPs is a sustainable and efficient measure to mitigate climate change.

1. Introduction

In terrestrial ecosystems, soil organic carbon (SOC) is by far the largest pool of organic carbon and globally contains over 1550 Pg C, followed by the soil inorganic carbon (SIC) pool (750–950 Pg C) and terrestrial vegetation (600 Pg C) (Schimel, 1995). Therefore, the soil

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C pool (SOC plus SIC) is about four times larger than the terrestrial vegetation and three times larger than the atmospheric carbon (C) pools. The net annual increase in atmospheric CO_2-C is estimated to be about 4.3 Pg yr^{-1} (Ciais et al., 2013). Consequently, even a small annual percent change in the amount of C stored or released from SOC stocks could easily affect the net change in atmospheric-CO_2 (Smith, 2012).

Forests and grasslands contain high stocks of C and are considered as net sink of C, while croplands often act as net sources of CO_2 due to soil disturbance which enhance soil organic carbon decomposition and to field management involving direct (e.g., diesel fuel for machinery) or indirect (e.g., chemicals) emissions of fossil fuels (Ceschia et al., 2010). Indeed, agriculture and land use change are together responsible for 21–24% of global anthropogenic greenhouse gas emissions (Smith et al., 2014; Tubiello et al., 2015).

Finding low-cost methods to sequester C in agricultural systems is emerging as a major international policy goal in the context of increasing concerns about global climate change. Among the methods that may reduce agricultural CO_2-derived greenhouse gas emissions, there is the adoption of recommended management practices (RMPs), which involves an accumulation of organic C in the soil without compromising crop production. In agricultural systems, the gain or loss of C over time due to cultivation (e.g. net ecosystem C balance) depends on the amount of C entering (for example through organic amendments or cover crops residues) and on that leaving the system (e.g. harvest of products, soil and plant respiration). In terms of SOC balance, RMPs reduce the oxidation of SOC and increase organic C inputs (Six et al., 2004). A reduction of the SOC oxidation can be achieved by changing the tillage type from conventional tillage (CT) to reduced tillage (RT) or no-tillage (NT). The increase in organic C input on farm can be achieved by the use of a cover crop (CC) in the rotations, or allowing the growth of wild vegetation in the inter-row of perennial orchard-type crops. Off-farm organic inputs can also be used for this purpose, such as manure, compost, or agro-industrial and urban wastes. Lal (2004) estimated a potential C sequestration for croplands by adopting RPMs in the range of 0.4–0.8 Pg C yr^{-1}, with similar, but at the lower end, estimates from IPCC (Smith et al., 2008, 2014)

Fruit orchards, such as olive groves and almond, and vineyards, are usually cultivated where soil fertility is relatively low and water availability limited, and therefore they are relatively well adapted to Mediterranean climates. These perennial crops represent about 16% of the agricultural land in the Mediterranean area (FAO data, 1998) and are of a great economic importance (Olesen and Bindi, 2002).

In comparison with annual crops (Smaje, 2015), fruit orchards have some structural features allowing them to potentially sequester significant quantities of atmospheric C. Their long life cycle allows them to accumulate C in permanent organs such as trunk, branches, and roots and in the soil (e.g. rhizodeposition). In addition, the massive and deep-rooted systems in these perennial woody crops allow direct transfer of SOC into the subsoil, making it less prone to mineralization. However, some conventional management of these cropping systems might lead to significant losses of SOC. Usually, conventional management involves bare soil in the inter-canopy area of the orchards, through regular tillage and/or pre- and post-emergence herbicides, leading to SOC losses not only because of the higher mineralization rates but also because of higher erosion rates. For example, Gómez et al. (2004) measured annual rates of soil losses in a conventional olive grove (4.0 t ha^{-1} yr^{-1}) which is 3.3 times higher than in a comparable plot where the soil was covered with spontaneous resident vegetation (1.2 t ha^{-1} yr^{-1}). Since the Mediterranean climate is characterized by relatively frequent, extreme, short-lasting rainfall events, erosion represents a problem, especially in high slope areas that can be solved – or minimized – by implementing RMPs. The relevance of SOC changes to the net greenhouse gases balance of cropping systems can be very large, particularly in the specific case of Mediterranean woody crops. In a life-cycle assessment study under Mediterranean conditions in Spain, Aguilera et al. (2015) found that soil C sequestration in organic olive orchards was equivalent to all other emissions combined, resulting in C-neutral crop production.

Soil C accumulation in these fruit orchards can be achieved relatively easily, both economically and technically, through the adoption of RPMs which include: i) reduced or zero soil tillage, which preserves soil organic matter from mineralization; ii) the frequent presence of herbaceous vegetation in the alleys, which can contribute to the build-up of soil organic matter, and iii) the inputs of external (e.g. manure) and internal (e.g. pruning debris) sources of organic matter. In addition, some fruit orchard crops have relatively low yields with a tendency to partition less C to the fruits than high-yielding ones and, therefore, some of the C fixed by photosynthesis enters the detritus cycle. In addition, improving soil resilience through increased SOC may positively impact the whole fruit tree industry. Increased knowledge of atmosphere-soil C fluxes mechanisms may facilitate interventions capable of enhancing C capture (Marland et al., 2004).

In spite of the strategic role of orchards and vineyards in Mediterranean regions (Olesen and Bindi, 2002), the role of RPMs on C fixation potential has only partially been explored. In recent years, the C budget of fruit tree plantations has received increasing attention with studies conducted in olive (Nardino et al., 2013; Sofo et al., 2005), palm (Navarro et al., 2008), apple (Zanotelli et al., 2015), peach (Sofo et al., 2005), and pear (Zhang et al., 2013). However, unlike other systems such as croplands (Ceschia et al., 2010; Smith, 2004), grasslands (Derner and Schuman, 2007; O'Mara, 2012) and forests (Barr et al., 2002; Vogt, 1991), there are no published large studies or meta-analysis comparing the ability of perennial fruit tree plantations to fix atmospheric C into the soil under RPMs in Mediterranean conditions.

Some recent meta-analyses have provided insight on the role of specific or grouped management practices on SOC. For instance, Poeplau and Don (2015) assessed the influence of cover crops on SOC stocks, Tuomisto et al. (2012) analysed the impacts of the organic farming in Europe on SOC content, nutrient losses, energy requirements or land use, Tian et al. (2015) assessed the influence on SOC changes of the addition of different fertilizers and crop residues in paddy soils in China, and Zhao et al. (2015) identified the management practices that lead to an increase in the SOC content in China. However, these studies do not distinguish between herbaceous and woody crops, and usually only herbaceous crops are considered. Aguilera et al. (2013) performed the first meta-analysis of SOC sequestration in Mediterranean crops, with 174 data sets from 79 different publications. The results of this study showed a high response of SOC to management changes under Mediterranean conditions. However, this work did not specifically focus on woody crops, and most of the studies were focussed on annual herbaceous crops, so the effects of specific RMPs designed only for woody crops were not specifically evaluated. In addition, the C sequestration efficiency and the effects of sub-climates, and the duration of implementation of the RMPs on SOC sequestration, were not assessed in this study. The influence of specific RMPs for woody crops on SOC sequestration and C sequestration efficiency is essential for estimating the contribution of the woody crops subjected to different management practices on CO_2 emissions, which is of critical importance for policy decisions in the agriculture sector in Mediterranean regions.

The aim of this study was to evaluate the influence of specific RMPs on SOC content of three common Mediterranean woody crops (olive orchards, vineyards and almond orchards), through a meta-analysis. We compared SOC in: i) Conventionally managed (used as control) without vegetation cover in the inter-row of the orchards and without any organic amendments; ii) Farms with a vegetation cover in the inter-row, iii) Farms with organic amendment inputs, and iv) Combined management practices.

2. Materials and methods

2.1. Literature review and data selection

A literature search was conducted for articles reporting comparisons between RMPs and conventional management in three typical Mediterranean woody crops: olive and almond orchards and vineyards. All the studies were carried out in areas under a Mediterranean climate type. Laboratory studies were excluded and only studies under field conditions were selected. We did not distinguish between irrigation and no irrigation, since irrigation in Mediterranean woody crops is usually done under the tree canopy, near the trunk and soil samples are usually taken in the inter-row area. When more than one study included data from the same experiment, the longest study was selected. If the duration of the study was the same in both cases, the study with most information was included in the analysis.

The studies included in the analysis were those cited in Scopus until January of 2016. Two fields where used for the search in the title, abstract or keywords of the article. The first field was the crop type; using the following words: "olive" or "vineyard" or "almond" or "*Olea europea*" or "*Vitis vinifera*" or "*Prunus dulcis*". For the second field; the search terms "soil organic carbon" and "soil organic matter" were used. Thereafter; we only included in the meta-analysis those studies conducted under Mediterranean climate. We obtained 213 results; resulting in 60 potential articles. This search was completed with other studies cited in Aguilera et al. (2013).

2.2. Definition of categories

The types of C input were those summarised in Table 1, namely: (i) None: no external organic C input was applied. The growth of a cover consisting of natural plant cover was prevented by frequent tillage and/or by pre-emergence herbicides. (ii) CC: a cover crop (seeded plant cover) or a cover of spontaneous resident vegetation (unseeded cover) in the inter-row area. Sheep or goat excretion resulting from grazing of these plant covers was included in this category since it is not an external organic input and the input of organic C by this route is typically very low. (iii) CR: crop residues, such pruning residues or olive leaves which were left on the top soil. (iv) OA: an external organic amendment was applied frequently. This external amendment typically consisted of farmyard manure, composted or un-composted olive mill pomace or sewage sludge. A wide range of doses and biochemical properties of these external organic amendments was found.

For the tillage types we used the following categories (Table 1). (i) T: frequent tillage. Tillage consisted of 3 or more annual passes. Very often this is combined with the use of herbicides. This tillage method was common in the conventional (CONV) management. (ii) RT: reduced tillage. This tillage method was common in the studies where wild resident vegetation covered the inter-row area of the farm. Reduced tillage was usually done during the spring to control the vegetation. (iii) NTH: no tillage, and unwanted plants were controlled using pre-emergence herbicides. As a consequence, the soil is permanently bare. As in the case of the T, NTH is common in the CONV management. (iv) NTM: no tillage and wild resident plants are eliminated by mowing, or using post-emergence herbicides in the spring. (v) NTG: no tillage where unwanted plants were controlled by animal grazing.

The comparisons were classified by management according to Table 1. The management is a result of the tillage and the type of organic C input. Conventional management (CONV) was used as a control group in the majority of the comparisons. CONV management typically includes the use of mineral fertilizers under the T tillage category. The rest of the management practices belong to the RMPs group. Some of these were the same as those proposed by Aguilera et al. (2013). (i) CC: a cover crop was implemented in the inter-row area or the orchard or vineyard. In most cases the soils were covered by a community of natural resident vegetation which was allowed to grow, typically between early autumn to middle spring. This plant community was controlled by mowing, grazing, or by applying post-emergence herbicides during the spring. Aboveground plant residues were left on the soil surface or incorporated by tillage. (ii) OA: organic amendments (manure, compost, agroindustry by-products or other residual organic inputs) were applied. Crop residues, such as pruning debris were included in this category. The growth of unwanted plants was prevented by tillage or application of pre-emergence herbicides. (iii) CMP: combined management practices. This is the most environmentally-friendly management category. It includes the

Table 1
Description of the three managements studied in the meta-analysis. The management type is the result of combining an organic carbon input and a tillage practice.

Management Type	Description	Observations	C Input Type	Description	Tillage Type	Description
CONV	Conventional management	Used as a control. Management included T or NTH with none C input.	None	No organic C input	T	– Tillage with herbicides – Frequent tillage without herbicides
CC	Cover Crops	Cover crop or natural plant cover, which were eliminated by a combination of NTM and NTG or with a RT	CC	– Cover crop – Natural cover of resident vegetation	RT	Reduced tillage. Usually once in spring and once in autumn
OA	Organic amendment	Annual organic amendment consisting in compost, manure, olive mill waste, sewage sludge or CR. Soil were T or NTH.	CR	Crop residues (e.g. pruning debris)	NTH	No tillage with pre-emergence herbicides
CMP	Combined management practices	CC + OA/CR + RT/NTM/NTG	OA	– Manure – Olive mill waste – Sewage sludge – Other	NTM	– No tillage mowing – No tillage with post-emergence herbicides in spring
					NTG	No tillage with grazing. Implies small amounts of manure

existence of plant cover (cover crop or resident vegetation cover) or an inert cover (crop residues), combined with an organic amendment. In some cases, plants were controlled by grazing.

The influence of four variables (Table 2) on the calculated effect sizes was assessed: management, woody crop species, time and climate. Management is a variable which includes: OA, CC, CC + OA (CMP) and none. In this analysis, three typical Mediterranean woody crop species were distinguished: olive orchards (*Olea europaea*), vineyards (*Vitis vinifera*), and almond orchards (*Prunus dulcis*). According to the duration of the study, the studies were classified into 3 categories: (i) Short-term: less than 6 years, (ii) Medium-term: 6–10 years, (iii) Long-term: more than 10 years. Finally, 6 different sub-climates of the Mediterranean climate according to Köppen-Geiger classification were also distinguished (Kottek et al., 2006): Csa, Csb, Cfa, Cfb, BWh and BSk (see Table 2 for the description of the different Mediterranean sub-climates).

Unfortunately, the influence of the different Mediterranean sub-climates and the duration of the study were only assessed for the CC management, since for OA and CMP managements, the high variability of the C inputs and the low number of studies with a duration longer than 5 years made the analysis impossible.

2.3. Data management

An effect is a statistical measure that portrays the degree to which a given event is present in a sample (Cohen, 1969). An effect size is a standard measure which can be calculated from any number of statistical outputs. We assessed 3 effect sizes (Table 3): (i) SOC response ratio, (ii) C sequestration rate, and (iii) efficiency of C sequestration. Data measured in most studies were SOC concentrations (g C kg^{-1} soil, or mg C g^{-1} soil). When data of the studies were presented only in a figure and not in numeric format, data were extracted from figures using WebPlotDigitizer software (http://arohatgi.info/WebPlotDigitizer) after figure digitalization. When soil organic matter concentration was determined instead of SOC, SOC was calculated using the Mann (1986) relationship (SOC = 0.58 × SOM).

SOC response ratio was calculated applying the Eq. (1):

$$\text{SOC response ratio (RR)} = \frac{SOC_{RMP}}{\text{SOC}_{\text{Control}}} \quad (1)$$

where SOC_{RMP} and $SOC_{Control}$ are the SOC concentrations (g C kg^{-1} soil) measured in the RMP management and in the control (CONV management) farms, respectively. Nevertheless, in order to normalize the sampling distribution, the natural logarithm of the RR was used (Hedges et al., 1999). Thus, the final equation was (Eq. (2)):

$$\ln(RR) = \ln SOC_{RMP} - \ln SOC_{Control} \quad (2)$$

We assumed a significant response ratio under a specific management when values were significantly different from 1 (e.g. $SOC_{RMP} > SOC_{Control}$).

To calculate soil C sequestration rate (t C ha^{-1} yr^{-1}) the change in the SOC stock (t C ha^{-1}) was calculated according to Eq. (3).

$$\text{Soil C sequestrationrate} = \frac{C_{t-}C_{t'}}{t} \quad (3)$$

where C_t and $C_{t'}$ represent SOC stocks (t C ha^{-1}) at the end and the beginning of the experiment, respectively, while t stands for the duration of the experiment (years). We assumed significant positive C sequestration rate under a specific RMP management relative to CONV management when values were significantly different from zero.

When data of SOC at the beginning of the experiment were not available, values of SOC stocks in the CONV treatment were selected, assuming similar initial C levels in RPM and CONV plots, since the plots used for the comparisons in the different studies had similar pedoclimatic conditions. Some studies provided the data of SOC stocks. However, most studies did not show values of SOC stocks, so these were calculated following the equation (Eq. (4)):

$$\text{SOC Stock } (\text{t C } ha^{-1}) = \sum_{i=1}^{j} \frac{d_i \rho_i SOC_i}{10} \quad (4)$$

where d_i, ρ_i and SOC_i are soil depth (metres), bulk density (t m^{-3}) and SOC concentration (g C kg^{-1} soil) for the different soil layers (from i to j soil layers), respectively. The SOC stock was the sum of the stocks for the k soil layers considered in each study. Since bulk density was not provided in many of the studies, values were estimated using the algorithm used by Aguilera et al. (2013), which was modified from Howard et al. (1995) but re-parametrized with data from Mediterranean soils (Eq. (5)):

$$\rho\ (\text{t } m^{-3}) = 1.84 - 0.443 \log 10(\text{SOC } (\text{g C } kg^{-1}\ soil)) \quad (5)$$

In the case of the studies providing enough information on the amount and characteristics of the organic inputs (both internal and external) – especially for OA and CR inputs – we also calculated the efficiency (E) of soil C sequestration following the equation

Table 2
The four variables assessed in the study (management, species, duration and sub-climate) and their different categories.

Variable	Categories
Management	OA (organic amendments: compost, manure, crop residues, sewage sludge, other) CC (cover crops/seeded cover, natural plant cover/unseeded cover) CC + OA = CMP (combined management practices) None
Tree species	Olive orchards Vineyards Almond orchards
Duration	Short term (<6 years) Medium-term (6–10 years) Long-term (>10 years)
Mediterranean Sub-climates according to Köppen-Geiger classification (main climate, precipitation, temperature)	Csa (Warm temperate, dry summer, hot summer) Csb (Warm temperate, dry summer, warm summer) Cfb (Warm temperate, fully humid, warm summer) Cfa (Warm temperate, fully humid, hot summer) BWh (Arid, desert, hot arid) BSk (Arid, steppe, cold arid)

Table 3
Effect size, description and equations used for their calculation.

Effect size	Description	Equation
SOC response ratio	Shows the variation in the SOC content in the RMP relative to the CONV management. If > 1 the RMP increases the SOC, if <1 it decreases it.	1,2
C sequestration rate	Shows the variation per unit of time (year) in the SOC stock in the whole profile in the RMP relative to the CONV management. If >0 the RMP increases the SOC stock, if <0 it decreases it.	3,4,5
C sequestration efficiency	Shows the percentage of the incoming organic C that is fixed into the soil after the implementation of the RMP.	6

RMP = Recommended management practice; CONV = Conventional management; SOC = Soil organic carbon; C = Carbon.

(Eq. (6)):

$$E = \frac{\text{C sequestration rate}}{\text{Annual organic C input}} \text{ x } 100 \quad (6)$$

2.4. Statistical analysis

We used a meta-analysis technique to assess the influence of RMPs on SOC using data from CONV management as the reference. A meta-analysis is a quantitative research synthesis which analyses the results of a set of analyses (Glass, 1976). The meta-analysis used a methodology similar to that used previously by Aguilera et al. (2013). For the meta-analysis, only independent studies were considered. We considered as independent studies those differing in management, duration, pedoclimatic or geomorphology conditions. For the non-independent studies, an average was calculated in order to avoid redundancy of the data and, thus, to transform them into independent values.

A "random-effects model" was used to carry out the meta-analysis. This type of model allows data from a wide range of scenarios to be compared (Borenstein et al., 2009), and assumes that the dispersion of data for a given category is not only due to a sampling error, but also due to other sources of variation which might have an effect on the mean effect size and the dispersion of the data (Borenstein et al., 2009). The dispersion of the data was relatively high in some cases and, therefore, it was difficult to detect significant differences.

The database contains 144 comparisons from 51 references. Nevertheless, not all the references contained all of the necessary data to calculate the effect sizes. Thus, we found 135 comparisons of SOC concentrations, and in 123 the C sequestration rate was shown or was calculated. These 123 comparisons represent a strong increase of available data compared to those found by Aguilera et al. (2013), who assessed the C sequestration rate in Mediterranean woody crops by using 10 comparisons. Finally, in 49 comparisons, the efficiency of C sequestration was calculated. In the case of the efficiency calculations, the majority of the data belonged to the studies which applied an organic amendment or crop residues. The studies which included cover crops did not usually show the amount of the inputs of organic C through plant residues.

Results of effect sizes were weighted in order to give more importance to larger studies (those with higher number of samples). Meta-analysis studies usually use the inverse of the variance of each study to weight the results. However, it was not possible in our case because this information was not provided for most of the studies. Thus, studies were weighted by sample size according to the methodology proposed by Adams (1997) (Eq. (7)):

$$w'_i = \frac{N_i^{RMP} N_i^{CONV}}{N_i^{RMP} + N_i^{CONV}} \quad (7)$$

Where w' refers to the specific weight of the comparison, and the N^{RMP} and N^{CONV} represent sample sizes in the recommended (RMP) and control (CONV) treatments, respectively.

As a result of a bootstrapping procedure (999 iterations) using MetaWin software (Rosemberg et al., 2000), 95% confidence intervals (CIs) were generated for each weighted mean effect size. Resampling techniques can be important for determining the significance of meta-analytic metrics since data often have small sample sizes and may violate some basic distributional assumptions. Bootstrapping chooses n studies from a simple size of n and then calculates the statistic, and this process is repeated many times to generate a distribution of possible values. The lowest and highest 2.5% values are chosen to represent the lower and upper 95% bootstrap confidence limits.

3. Results and discussion

3.1. General information

A total of 51 studies were selected resulting in 144 independent comparisons between the RMP and the CONV managements, i.e. about 3 comparisons per study. The number of studies performed in Spain was the highest (33 studies), followed by Italy (7), Greece (2), France (2), Portugal (2), South Africa (2), Syria (1), Turkey (1) and the United States (California) (1). According to the crop type, olive orchards were the most common woody crop (31 studies) studied, followed by vineyards (16) and almond orchards (5). One study included olive orchards and vineyards.

The number of studies devoted to olive groves and vineyards was somewhat related to their areas. Indeed, olive orchards in Spain and Italy cover 2.5 and 1.14 million hectares, respectively. However, this was not the case for almond orchards, at least in Spain and California, where there are about 700,000 ha and 331,000 ha planted, respectively. Therefore, the number of studies on almond orchards was underrepresented in comparison to those on olives and vineyards. This fact might be due to the lower economic importance of the almond products in comparison to olive oil and wine. Most of the studies were published during the last 10 years, peaking during 2012 and 2013 (Fig. 1).

The duration of the study in 64 out of 144 comparisons was lower than 6 years, whereas in 37 and 22 of them it was between 6 and 10 years and more than 10 years, respectively (some studies do not show data about the duration of the management). The relatively short time frame (typically lower than 4 years) of most of research programs at National and EU levels is likely the responsible for the relatively high proportion of studies which evaluate changes in SOC over the short term. This contrasts with the fact that changes in SOC typically occur at different rates after a change in management practices. Indeed, Poeplau and Don (2015) found that highest rates of SOC accumulation occur during the first few years, and usually decline afterwards until near zero changes when the steady state is reached. Thus the data on SOC accumulation provided in most of the articles of this study might

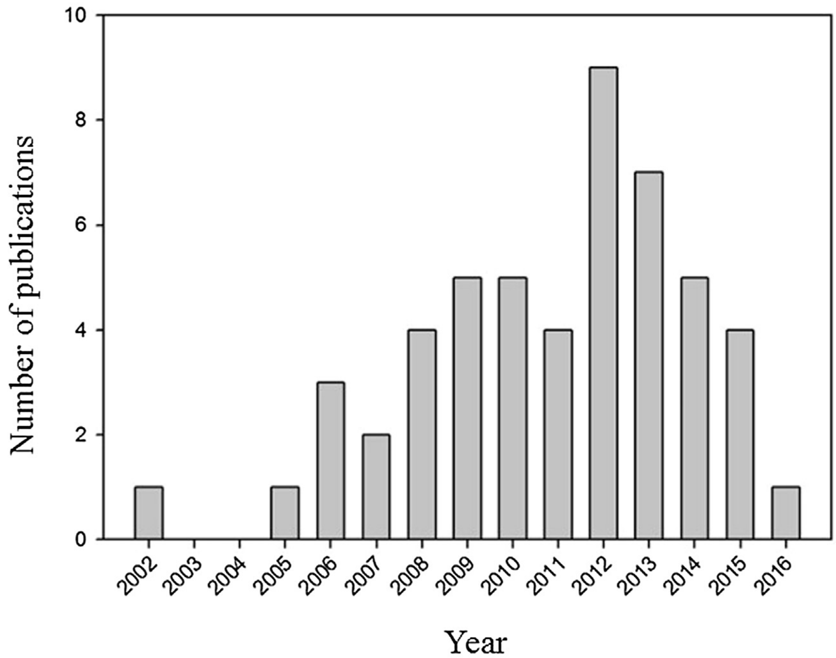

Fig. 1. Number of published articles used in the meta-analysis classified according to the year of publication.

be overestimated if interpolated over time. Clearly, long-term experiments would be highly valuable to fully understand soil C dynamics over long periods.

Only studies under a Mediterranean-type climate were selected. However, mean annual rainfall and temperature vary according to the geomorphological properties and other geographical features of the experimental sites. The great majority of the comparisons (95) were undertaken under warm temperate conditions with relatively hot and dry summers (Csa type climate), followed by BWh (14), BSk (11), Cfb (9), Csb (12) and Cfa (3). This was especially true for olive orchards. Nevertheless, studies on vineyards were also done in Csb, Cfb and BWh climate types, whereas for almonds, the studies were also performed under a BSk type climate.

3.2. Influence of management on the effect sizes of soil C sequestration

Response ratios of the three tested managements (CMP, CC and OA) ranged 1.35–1.45 and averaged 1.40, and were significantly different from 1.0. There were not large differences in the response ratios of the three managements (Fig. 2a). The mean lowest value (1.35) was observed in farms under CC, whereas intermediate values were obtained for the CMP (1.40) management, and the highest (1.45) for the farms that received organic amendments.

The similarity in the SOC response ratios among RMP managements contrasts with the relatively large differences in C sequestration rates. This might be related to the differences between bulk densities and soil depths considered among the studies, since the same response ratio does not mean same C sequestration rate when different depths and bulk densities are considered. Thus, the C sequestration rate would be more appropriate than response ratio when assessing the influence of management practices on the changes of SOC in studies which include data from different depths.

Fig. 2b shows that annual C sequestration rate averaged 4.07 t C ha^{-1} yr^{-1} under OA management. This figure was about 1.5 times higher than the rate found for CMP (2.62 t C ha^{-1} yr^{-1}) and four fold that under CC (1.03 t C ha^{-1} yr^{-1}), although these mean values were obtained with wide confidence intervals. For the whole set of studies and the three management types, minimum, mean and maximum annual C sequestration rates were −0.5, 3.8 and 6.6 t C ha^{-1} yr^{-1}. The fact that an accumulation of SOC was detected in the majority of the studies means that inputs of organic C and/or the slow-down of SOC losses under RMPs management compensate for SOC losses by organic matter decomposition and soil erosion. The average annual C sequestration rate for the whole set of studies was higher than that described for annual crops. For instance, Aguilera et al., 2013; in their meta-analysis involving Mediterranean crops, found a change of only about +8% in SOC content in the cereal rotations in the organic treatments compared to conventional management. The majority of cropping systems are dominated by annual plants that rely on cycles of tillage and planting of seed to ensure sufficient productivity. By comparison, fruit tree orchards, such as olives, almond and vineyards are capable of surviving many seasons requiring less soil disturbance. Perennial cropping systems have been recently proposed as systems that could protect soil C well, and since perennial plants often rely on more extensive root systems to ensure longevity, they likely produce more belowground biomass (Cox et al., 2006).

The highest SOC sequestration rate in the fruit tree orchards and vineyards was achieved for organic amendment management. For the whole set of studies under the OA management, the mean rate of organic C added was about 1.6 times higher than in CMP management, so the relatively high annual rate of C sequestration under the OA management compared to the other management practices is not surprising. The lower annual rate of organic carbon inputs in CMP compared to OA treated farms was likely due to the fact that farmer think that there is no need to add a high annual dose of organic matter when a cover crop is implemented in the inter-row area.

The fact that organic amendment additions represent direct inputs of organic C into the soil systems, and that these materials are often in forms that are much more recalcitrant than plant fresh residues should, in the absence of additional constraints, translate into moderate to high C sequestration rates. It is important to note that applications of manure are often assumed to increase C sequestration in soils at farm scale, but not at higher spatial scale (e.g. application of manure in one farm means an inefficient

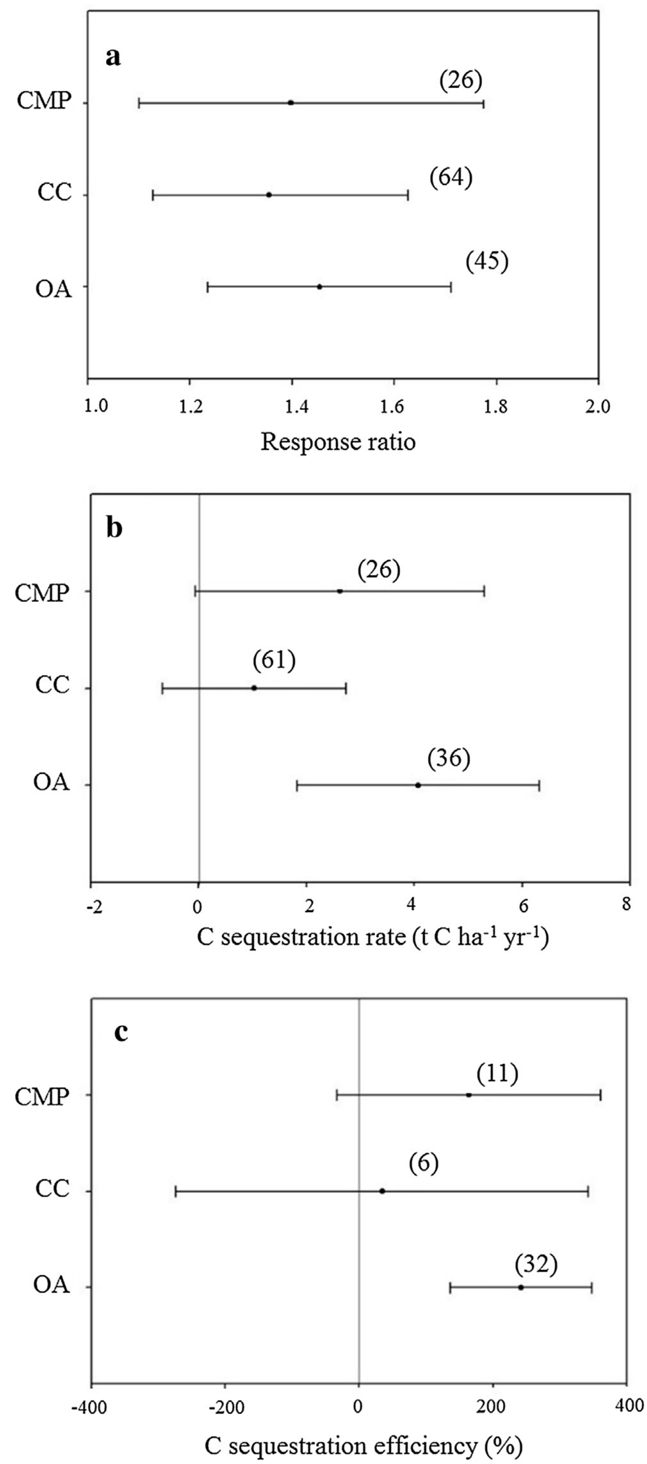

Fig. 2. Influence of the three managements (organic amendments, OA; cover crops, CC; and combined management practices, CMP) on natural logarithm of the response ratio (a), C sequestration rate (b) and C sequestration efficiency (c). The zero line represents the limit between a positive and negative response of each size effect. Numbers in brackets represents the number of comparisons used in each category. Points represents average values, whereas extremes corresponds to confidence intervals at 95%.

transport of organic C from other ecosystems to this farm), but manure is not likely to yield a net sink for C in soils (Smith, 2012), as would be required by the Kyoto protocol and also the Paris Agreement. Therefore, an ideal option would be apply organic C sources coming from the by-products of olive oil, wine and almond industries, thus avoiding CO_2 emissions from long-distance transportation, and from waste management.

The mean annual C sequestration rate reported here for CC was lower than the average of 1.59 t C ha^{-1} yr^{-1} found by the meta-analysis carried out by González-Sánchez et al. (2012) from 13 olive farms of Andalusia with CC. By using plant cover in the inter-row of tree orchards, an annual input of C is ensured, and this is true independently of the plant cover control technique. For instance, Castro et al. (2008) found a 3-year average annual aboveground biomass input of between 2.6 and 4.0 t ha^{-1} in an olive farm in Jaén (East Andalusia) with unseeded plant cover. The relatively high C sequestration rate under the CC treatment might be due not only to the annual C input of the plant residues, but also due to a decrease in C losses from soil erosion. In this line, Gómez et al. (2004) found a reduction in soil losses (and thus of organic matter and C) of about 70% in an olive farm after the implementation of unseeded plant cover.

In addition, the diversity of unseeded plant cover might have an important impact on soil C accrual by improving the ability of soil microbial communities to rapidly process plant residues and protect them into aggregates. The presence of many different annual plants in unseeded plant cover also introduces a greater diversity of C compounds into the soil, some of which may be more resistant to decomposition (Tiemann et al., 2015). While previous theories stated that microbial processing of residues in soils eventually produced similar C pools and compounds, a recent laboratory experiment found that the initial chemistry of the plant residues and the microbial community had a strong influence on which C compounds are present in the soil (Wickings et al., 2012). The presence of a diversity of plants, then, might ensure that a diversity of C compounds is present in the soil, improving soil C sequestration potential. Thus, strategies which increase productivity of non-commercial biomass without compromising the quantity and quality of the economic products, such as the inter-row seeded or unseeded cover in fruit tree orchards, is desirable to increase the amount of biomass C returned to the soils, which can affect the size, turnover, and vertical distribution of SOC (Franzluebbers et al., 1994). If suited to the climate and the technical and economic viability of the farming operation, then such cropping systems provide an opportunity to produce more biomass C than in a monoculture system, and to thus increase SOC sequestration. Lal (1997) reviewed the literature on this topic and concluded that the potential for sequestering C by the application of cover crops residues was about 0.1–0.3 t C ha^{-1}, values much lower than those reported in our study for fruit tree orchards. However, the degree of intensification (more tillage events) of soils in these crops systems reviewed by these authors was much higher, likely with more SOC losses.

C sequestration efficiency is commonly expressed by the relationship between annual C input and SOC accumulation rate, which is an indicator of soil C sequestration ability (Mclauchlan, 2006). Therefore, information about C sequestration efficiency is useful for seeking management strategies of enhancing the SOC stocks and soil fertility. On average, C sequestration efficiency was over 100% for OA (241%) and CMP (164%), whereas it was as low as 34% under CC management (Fig. 2c). Variability in soil C sequestration efficiency was ample, especially for CC and CMP managements, and no significant differences between groups were found. C sequestration efficiency is regulated by climate, the quantity and quality of added organic materials, soil organic C and inherent soil properties (Freibauer et al., 2004). These factors might explain the great variability observed in this study, which compiles many studies with wide pedoclimatic variability, and diverse quantities and qualities of the organic C amendments. High soil C efficiency in fruit tree orchards systems was expected, as these are usually cultivated on soils with low organic matter, and a negative linear relationship between C sequestration efficiency and initial SOC content has been reported, mainly because SOC tends to increase faster if initial SOC content is far from its saturation level. C sequestration efficiency of most of the studies used was lower than 50%. For instance, after 29 years, the C sequestration efficiency after application of pig and cattle manure and wheat straw ranged between 11 and 17% in a Vertisol cultivated by a soybean-wheat rotation (Hua et al., 2014). Triberti et al. (2008) found C sequestration efficiencies between 3.7 and 8.1% in a maize-wheat rotation after applying organic amendments. The unrealistically high C sequestration efficiency in the examined studies of our analysis could be due to four major reasons: (i) Uncertainties in the quantification of annual entry of some of the organic C inputs and lack of quantification for others. These uncertainties are quite common to many long-term field studies. For CC and CMP managements, only aboveground biomass of the unseeded or seeded plant cover were recorded or estimated; in some studies it was quantified on only one occasion. The C input *via* roots of the plant cover might represent a significant input of C which was not taken into account in the examined studies. Guzmán et al., 2014 found a root/shoot ratio of 0.8 (about 44% of the organic C in the biomass belongs to the belowground biomass) for cover crops, and also Ludwig et al. (2007) estimated an incoming organic C through the rhizodeposition process of 50% of the organic C content of the incoming biomass. (ii) Inaccuracies in the estimation of SOC stock (Aguilera et al., 2013). SOC stocks calculations require the measurement of soil bulk density, and in some of the studies soil bulk density was estimated but not experimentally calculated. Moreover, changes in bulk density lead to changes in sampled soil mass when a fixed sampling depth is used, possibly biasing the results. (iii) Positive feedback between the incoming organic C and the improvement of soil fertility features, which might reduce SOC oxidation and increase SOC protection mechanisms of the native SOC. (iv) RMPs (such as organic amendments, plant or pruning debris cover) tend to decrease soil loss, and therefore SOC, by erosion. For instance, in experimental olive plots with a relative low slope (about 4%), Gomez et al. (2011) found soil losses about 2.6 t ha^{-1} yr^{-1} under conventional tillage, whereas for those plots under vegetation cover this value was one order of magnitude lower (0.17 t ha^{-1} yr^{-1}), in a relatively rainy year (845 mm).

Furthermore, when organic materials, such as manure, compost and by-products of the olive oil and wine industries, are added to the soil, at least a share of their organic C is decomposed producing CO_2, while another part is sequestered in the soil. Increase in the SOC pool in the 0–0.3 m depth after long-term use of manure when compared with chemical fertilizers was 10 percent over 100 years in Denmark (Christensen, 1996), 22 percent over 90 years in Germany (Korschens and Muller, 1996), 100 percent over 144 years at Rothamsted, United Kingdom (Jenkinson, 1990) and 44 percent over 21 years in Sweden (Witter et al., 1993). Triberti et al. (2008) reported that 29 years after the start of a trial comparing different off-farm organic amendments, the cattle manure gave the quickest organic C stock build-up: 0.26 t organic C ha^{-1} yr^{-1}. In another study, about 25 and 36% of applied manure and compost C remained in the soil after 4 years of application, indicating greater C sequestration efficiency with composted than non-composted manure (Eghball, 2002). Annual off-farm organic amendments Zhang et al. (2010) encouraged significant SOC increase of about 7–45% after 25–28 years compared with the mineral fertilizer treatments, with a sequestration rate of about 0.70 to 0.88 t ha^{-1} yr^{-1}. Recently, Hua et al. (2014) found a linear relationship between off-farm organic C inputs (from 0.5 to 7.0 t ha^{-1} yr^{-1}) and SOC sequestration, although a linear relationship is not always observed (see for example Stewart et al., 2009 and Chung et al., 2009)

3.3. C *sequestration rate in the 3 types of fruit tree orchards and under RMPs management*

The effects of RMP management on C sequestration were only evaluated on olive orchards and vineyards, due to the lack of sufficient comparative data for almond orchards. The C sequestration rates in olive orchards were as follows: OA > CMP > CC (5.36, 3.33, and 1.10 t C ha^{-1} yr^{-1}, respectively) (Fig. 3a). The relatively large differences among management types, although with mean values with a wide dispersion which prevented statistical significance from being determined, were not found for vineyards, where C sequestration rates under the different management practices were relatively similar and not significantly different: CC > OA > CMP (0.78, 0.65 and 0.34 t C ha^{-1} yr^{-1}, respectively) (Fig. 3b).

In all cases, C sequestration rates were the highest for olive orchards, especially for OA and CMP managements. These differences were due to two main factors. Firstly, the mean annual rate of application of organic amendments to olive orchards was more than 25 times higher than that of vineyards. Secondly, the area covered by plant cover in olive orchards is much higher than in a vineyard, and thus aboveground and belowground biomass is expected to be much higher.

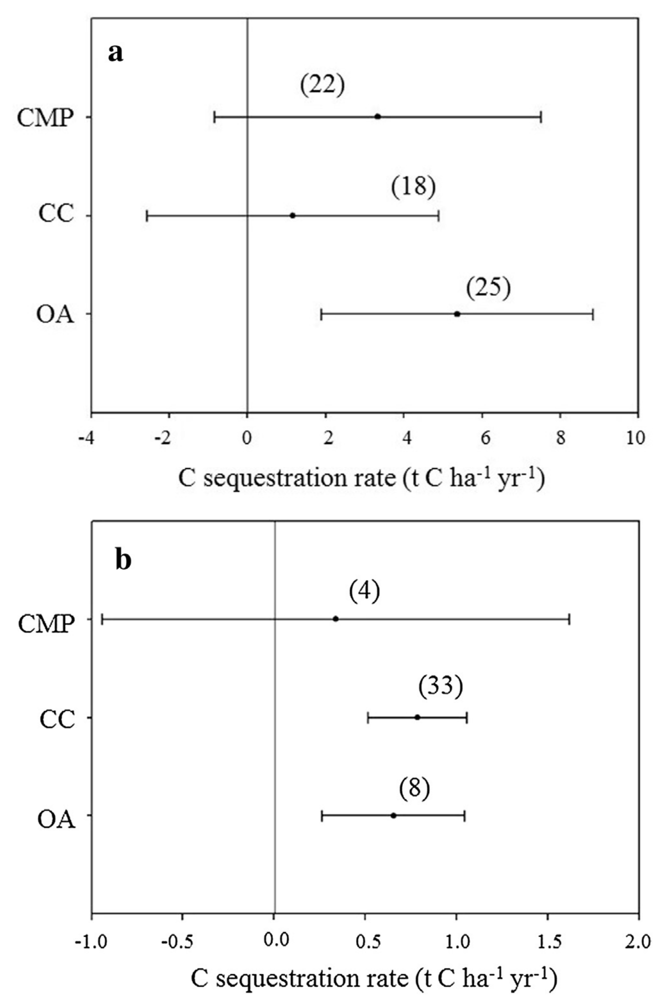

Fig. 3. Influence of the three managements (organic amendments, OA; cover crops, CC; and combined management practices, CMP) on C sequestration rate in olive orchards (a) and vineyards (b). The zero line represents the limit between a positive and negative response of the C sequestration rate. Numbers in brackets represents the number of comparisons used in each category. Points represents average values, whereas extremes corresponds to confidence intervals at 95%.

In the case of almond orchards, the C sequestration rate was 2.04 t C ha^{-1} yr^{-1} for CC management (n = 6) (figure not shown). For the rest of the management types it was not possible to assess the C sequestration rate due to the low number of available comparisons. This value is about 1.9 times that of the olive orchards and 2.6 times that of vineyards. Nevertheless, more studies should be carried out with cover crops in almond orchards to obtain consistent results.

Smith (2004) estimated with relatively high uncertainty the potential SOC sequestration for European croplands, mainly for herbaceous crops, according to different managements. For example, in the case of the organic farming the potentially SOC sequestration rate would be between 0 and 0.54 t C ha^{-1} yr^{-1}, for the use of animal manure was about 0.38 t C ha^{-1}, whereas with the use of cereal straw it was about 0.69 t C ha^{-1} yr^{-1}. Zero tillage potential SOC sequestration was about 0.38 t C ha^{-1} yr^{-1}, whereas for reduced tillage, this value was lower. Comparing these results for herbaceous crops with those obtained in our study, for olive orchards the C sequestration rate was about one order of magnitude higher after the use of organic amendments, whereas it was about 1.7 times for vineyards. In the case of SOC sequestration for CC management, the values estimated by Smith (2004) were in most cases lower than those obtained in this study for olive orchards, vineyards and almond orchards. Triberti et al. (2008) found in soils under a maize-wheat rainfed rotation C sequestration, rates between 0.16 and 0.26 t C ha^{-1} yr^{-1} by using residues, slurry and manure. Again, these values in herbaceous cropping systems are lower than those we found in woody cropping systems. The relatively high annual dose of organic matter application in treated woody crops, the implementation of cover crops, where residues are left annually on the soils, and the lower soil perturbations of woody crops, especially in olive orchards, compared to herbaceous crops, might explain the higher C sequestration rate.

3.4. Influence of duration of the experiment on C sequestration rate for CC management

The average of soil C sequestration rates for studies with duration of less than 6 years, between 6 and 10 years and higher than 10 years were significantly higher than zero. On average, soil C sequestration for the studies with a duration of less than 6 years was 1.22 t C ha^{-1} yr^{-1}, a figure which was 1.7 times higher than that observed in studies carried out during 6 to 10 years (0.72 t C ha^{-1} yr^{-1}) (Fig. 4).

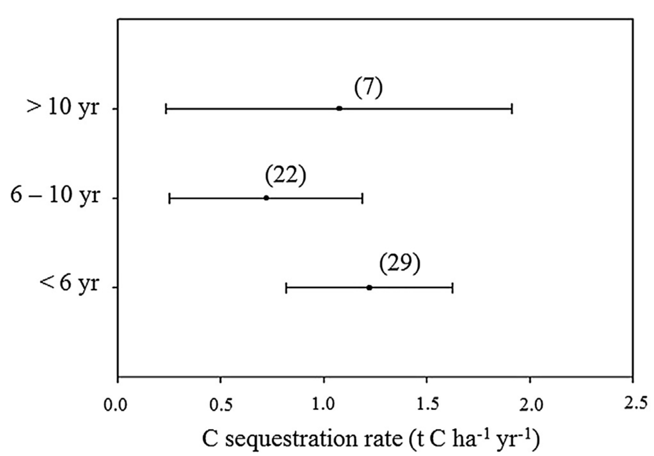

Fig. 4. Influence of the time on C sequestration rate in the cover crop management (CC) in the three crops. Numbers in brackets represents the number of comparisons used in each category. Points represents average values, whereas extremes corresponds to confidence intervals at 95%.

Higher C sequestration rates in studies with a duration of less than 6 years were not unexpected, since changes in SOC are projected to be faster just after a change in a management practice, and decline thereafter until a new equilibrium is reached some time later (Smith, 2005). For instance, West and Post (2002) found that the majority of SOC change in response to a change to no tillage occurred within the first 10–15 years following the implementation of this practice, and Rui and Zhang (2010) found that there was a negative correlation between soil C sequestration rate and duration of soil C sequestration. Finally, Poeplau and Don (2015) found an average C sequestration rate of 0.23 t C ha^{-1} yr^{-1} during the first 54 years after a change in the management, but an average of 0.11 t C ha^{-1} y^{-1}, thereafter reaching the new equilibrium (steady state) after 155 years following the adoption of the new management. Thus the soil C sequestration and soil C efficiency reported in this study should be treated with caution, as the experiment duration in about 44% of the studies of this meta-analysis was lower than 6 years.

Soil C sequestration rates for studies longer than 10 years, tended to be higher, although differences were not significant, than that of studies between 6 and 10 years of duration. However, caution should be applied as the number of studies of a duration of more than 10 years is scarce (n = 7) and with wide confidence intervals due to the high dispersion of the data.

3.5. Influence of mediterranean sub-climates on C sequestration rate for CC management

Soil C sequestration rate under CC management varied according to the sub-climates of the studies. Values averaged 1.18, 1.22 and 1.27 t C ha^{-1} yr^{-1} for Cfb, Csb and Csa sub-climates, respectively. Averages of soil C sequestration rates of studies under B-type climates (semiarid to arid) were 0.39 t C ha^{-1} yr^{-1} and 0.53 t C ha^{-1} yr^{-1} for BWh and Bsk, respectively (Fig. 5), but these were not significantly higher than zero. In general, it is acknowledged that the C sequestration potential of semiarid to arid soils is relatively low, because of water and edaphic limitations such as fertility, and chemical (i.e. sodicity and acidity) and physical constraints (Post et al., 1996). Soil C storage is controlled by a series of hierarchical processes, including C inputs and outputs. For example, the upper limit of C input to the soil is determined by net primary productivity of plants, which is in turn

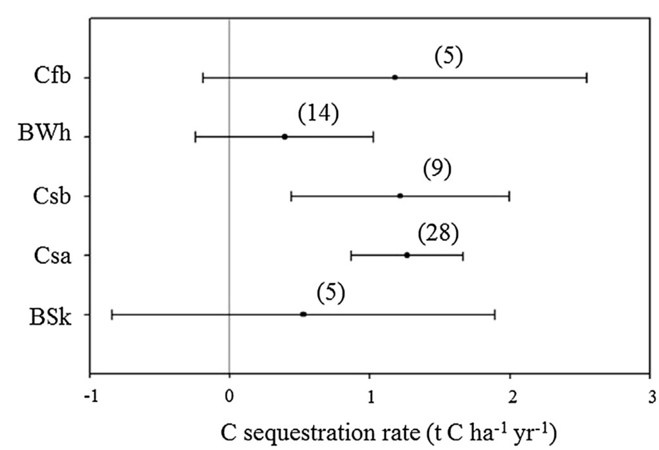

Fig. 5. Influence of the climate on C sequestration rate in the cover crop management (CC) in the three crops. The zero line represents the limit between a positive and negative response of the C sequestration rate. Note that Cfa sub-climate is not included in the analysis due to not to have enough number of comparisons. Points represents average values, whereas extremes corresponds to confidence intervals at 95%. Csa (Warm temperate, summer dry, hot summer), Csb (Warm temperate, summer dry, warm summer), Cfb (Warm temperate, fully humid, warm summer), BWh (Arid, desert, hot arid), BSk (Arid, steppe, cold arid).

constrained by solar radiation, climate, and limitations in soil water and nutrients. Thus, the lower soil C sequestration measured in olive and almond orchards and vineyards on semiarid to arid climates was likely due to the fact that crop productivity in these dry locations is low, and thus so is the annual rate of organic amendments. In addition, C inputs throughout the above and belowground biomass of the plant cover under these climates is expected to be low, and thus so is the soil C sequestration rate.

4. Conclusions

Specific recommended management practices (RPMs) increased C sequestration in Mediterranean olive and almond orchards and vineyards compared to conventionally-managed cropping systems. Nevertheless, soil C sequestration was highest when applying organic amendments due to the relatively high annual doses of organic material applied, especially in olive orchards (e.g. pruning debris, composted olive mill pomace). However, the plant cover management, used as green manure, amounted lower values of SOC sequestration rates, but the importance of this management is that it is relatively easy to be implemented by farmers, and with a relative low cost for farmers. Therefore, a combination of a plant cover in the inter-row of orchards with the application of external organic amendments (e.g. compost) or crop residues (e.g. pruning debris) would be a suitable management. Furthermore, the SOC sequestration would be higher during the first years after implementing the RMPs. Therefore, we recommend that future researches consider different time intervals for the estimation of soil C sequestration. Overall, this research shows that the relatively high sequestration rate combined with the relatively large spatial extent of these cropping systems areas allows the conclusion that the adoption of RMPs is a sustainable and efficient measure to mitigate climate change.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2016.10.024.

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