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FACULTAD DE BIOLOGÍA

Carbon Sequestration Mechanisms in Semiarid Soils according to Land Use and Management Practices

Mecanismos de Secuestro de Carbono en Suelos Semiáridos en función del Tipo de Uso y Prácticas de Manejo

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A mis padres,
porque su apoyo es una fuente inagotable

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Resumen

Capítulo 1. *Introducción*

El uso del suelo y las prácticas de manejo regulan su comportamiento como fuente o sumidero de gases de efecto invernadero

En la actualidad, a nivel global se está produciendo una retroalimentación negativa en el ciclo terrestre del carbono (C) con emisiones a la atmosfera de 3-6 Gt de CO_2 por año, debido a la mineralización del carbono orgánico del suelo (COS), con la consecuente aceleración del cambio climático.

De la pérdida de 136 ± 55 Pg del C del reservorio terrestre se estima que 78 ± 12 Pg es debido a la reducción del carbono orgánico del suelo (COS) (Lal et al., 2003). Esta parte del ciclo del C puede ser gestionada mejorando la estructura del suelo a través de usos de suelo y prácticas de manejo sostenibles, tales como la reforestación para la restauración de suelos degradados y prácticas agrícolas de laboreo de conservación. En la actualidad, no existe una relación, universalmente consensuada, de las mejores prácticas de manejo aplicables para proteger y aumentar el COS; por consiguiente, estas prácticas deben ser evaluadas y adaptadasa escala local o regional para cada sistema agrícola (IPCC, 2007).

Caracterización de la materia orgánica del suelo

La descripción clásica de la material orgánica del suelo se basa en la combinación de diferentes métodos químicos para la extracción diferencial y posterior identificación de los compuestos que la componen. Pero este enfoque ha contribuido poco a una comprensión funcional de la dinámica y el tiempo de permanencia del COS (Collins et al., 2000) y no se ajusta al concepto de estabilización y protección de la materia orgánica frente a la mineralización (von Lützow et al., 2006). Como alternativa, los investigadores han tendido a adoptar un modelo donde el COS se encuentra localizado en diferentes compartimentos, separados en función de su tiempo de permanencia en el suelo, denominados "*pools* funcionales de carbono orgánico" (Jones y Donelly, 2004). Sin embargo, en la actualidad, no hay demasiado acuerdo sobre la definición precisa de la mayoría de estos *pools*, los cuales pueden tener significados distintos según sean considerados por diferentes científicos (Jenkinson et al., 1992; Smith et al., 2002).

En este contexto, a nivel general existen tres *pools*, los cuales varían en su inherente tasa de descomposición y están representados por un conjunto de materiales orgánicos con tiempos de permanencia definidos (Krull et al., 2003): (a) *pool* activo o lábil, también conocido como "sensible" (Zimmermann et al., 2007) que consiste en la biomasa microbiana, restos de plantas y raíces con un tiempo de permanencia de 1-2 años, (b) *pool* intermedio o lento, con un tiempo de permanencia de 10-100 años que consiste en compuestos orgánicos recalcitrantes y materia orgánica físicamente protegida y (c) *pool* pasivo que está compuesto por materiales más antiguos protegidos físicamente y bioquímicamente, con un tiempo de permanencia aproximado de > 100 años (Figura 1.1).

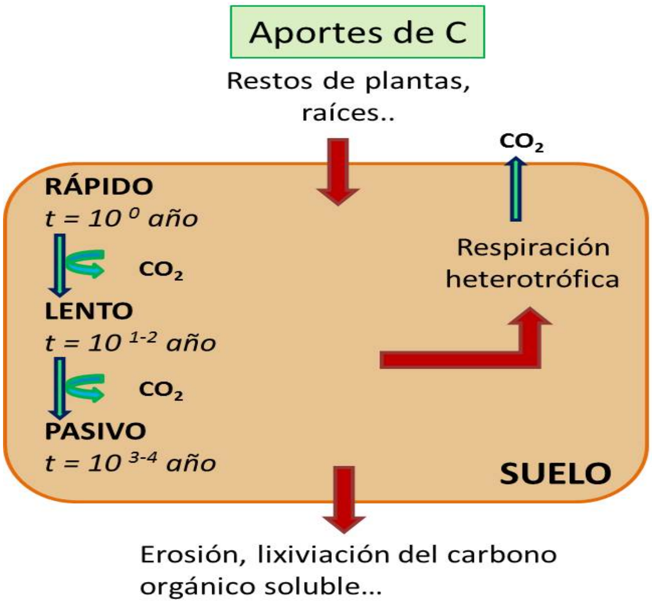


Figura 1.1. Balance simplificado del COS. Esquema adaptado de FAO, 2007.

Este modelo de los *pools* funcionales es el que se adopta en esta tesis para el estudio y descripción del carbono orgánico donde los *pools* lábil, intermedio y pasivo serán cuantificados para cada uso y práctica de manejo estudiada.

Estabilización del carbono orgánico del suelo

Al margen de algunas discrepancias, parece existir un amplio acuerdo, entre los científicos del suelo, en la existencia de tres principales mecanismos de estabilización:

(i) *"Estabilización bioquímica"*, también llamada *"preservación selectiva", "recalcitrancia molecular"* o *"alteración (bio) química".* Este tipo de estabilización se corresponde con la estabilización del COS debida a su propia composición química. Von Lützow et al. (2006) diferencian entre recalcitrancia primaria: los restos de plantas, rizodepósitos; y recalcitrancia secundaria: productos microbianos, recalcitrancia de polímeros húmicos, recalcitrancia debida a la condensación y reacciones de polimerización que crean nuevas y mayores moléculas; siendo la comunidad biológica un factor clave en estos procesos (Figura 1.2).

(ii) *"Protección física",* también llamada *"inaccesibilidad espacial"* o *"baja accesibilidad de la degradación biológica".* Este mecanismo indica la influencia positiva de la agregación en la acumulación del CO. La estabilización es causada por la oclusión del CO en los agregados (principalmente en los microagregados libres y los microagregados contenidos en el interior de los macroagregados), filosilicatos o macromoléculas orgánicas, las cuales protegen al CO de la descomposición por los organismos del suelo (Figura 1.2).

(iii) *"Estabilización química"* también llamada *"interacción con las partículas minerales"*. Esto es el resultado de las uniones químicas o físico-químicas entre la MOS y los minerales del suelo. Estas uniones incluyen ligandos intercambiables, puentes de uniones polivalentes, fuerzas de Van der Waals, uniones de hidrógeno y la formación de complejos de iones metálicos con sustancias orgánicas (Figura 1.2).

La contribución de los microorganismos, tanto a la formación y estabilización de los agregados del suelo, como a la degradación de la materia orgánica ha sido revisada de manera exhaustiva por diversos autores (Lynch y Bragg, 1985; Oades, 1993; Degens, 1997; Nicolás et al., 2014). Los hongos actúan dando lugar a la unión mecánica de las partículas del suelo mediante las hifas y exudando bioproductos que promueven la coalescencia de las partículas primarias (Helfrich et al., 2008; De Gryze et al., 2005). También las bacterias pueden tener profundas influencias en la agregación del suelo, especialmente a un nivel de microescala (Six et al., 2004). Aun así, la respuesta de las comunidades de microorganismos a los cambios de uso de suelo o prácticas de manejo no es muy conocida (Macdonald et al., 2009, Bastida et al., 2013).

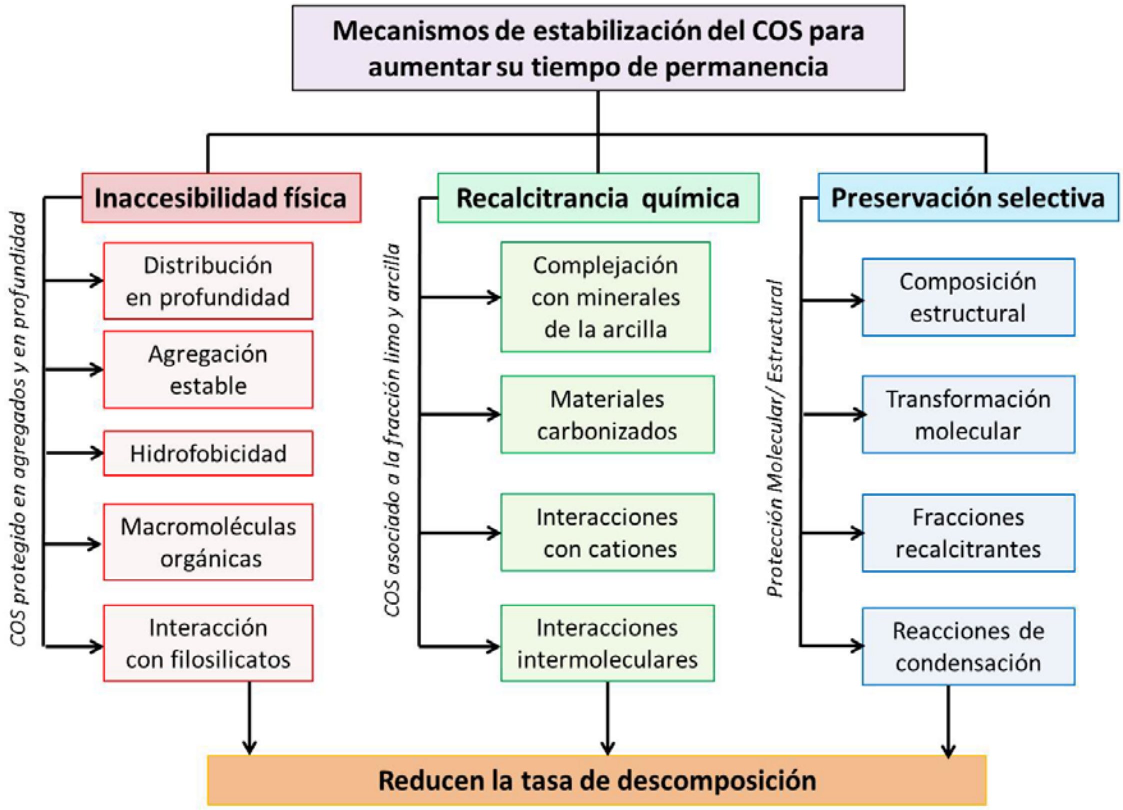


Figura 1.2. Mecanismos de estabilización de la materia orgánica (Adaptación de Lal et al., 2013).

Por lo tanto, todavía hay necesidad de un mayor conocimiento científico sobre los mecanismos de estabilización del COS y los diferentes factores (como por ejemplo: tipo de suelo, uso de suelo, litología, factores ambientales) que intervienen en esa estabilización en zonas semiáridas. En este estudio se ha profundizado en el conocimiento de los tres mecanismos de estabilización, descritos anteriormente, y se ha establecido la importancia relativa de cada uno de ellos, de acuerdo con los usos de suelo y prácticas de manejo. Este conocimiento permitirá determinar los usos adecuados y las mejores prácticas de manejo sostenible, para una mejora y aumento del secuestro del COS en áreas semiáridas.

Probablemente, con el objetivo de contribuir a la mitigación del cambio climático, la capacidad potencial de los suelos semiáridos para disminuir la concentración del CO_2 atmosférico, es cuantitativamente limitada. Sin embargo desde una perspectiva socioeconomic y ambiental, la implementación de los mejores usos y prácticas de manejo, es un aspect clave para la sostenibilidad y supervivencia en estas áreas, debido a:

1) las tendencisa actuales del cambio climático y global, *2)* la dependencia económica que tienen estas regiones del sector agroalimentario y *3)* la situación actual de pobreza que sufren las zonas áridas del Planeta.

Capítulo 2: ***Hipótesis de partida, objetivos y estructura de la tesis***

Los mecanismos de estabilización del COS y su relación con los diferentes factores abióticos y bióticos del suelo, en diferentes situaciones de uso y prácticas de manejo, son aún bastante desconocidos. A través del análisis comparativo de la distribución de agregados según su tamaño, de los *pools* funcionales de la materia orgánica y los mecanismos de estabilización del COS, entre los diferentes usos de suelo y las prácticas de manejo seleccionadas, esta tesis tiene como objetivo general llenar estos vacíos de conocimiento y proporcionar una base de datos y experiencia que permita determinar y recomendar los mejores usos y prácticas de manejo sostenibles, para la conservación del suelo y la mitigación del cambio climático en ambientes semiáridos. Para ello, se han sometido a estudio las siguientes hipótesis:

(i) Los factores bióticos y abióticos que controlan los contenidos de carbono orgánico en el suelo, pueden variar en función del tipo de uso y la profundidad en el perfil del suelo.

(ii) Los cambios de uso y las prácticas de manejo promueven cambios en la naturaleza de los aportes orgánicos, los cuales podrían afectar a la recalcitrancia y estabilización del COS, dando lugar a cambios en el tamaño y la composición de los *pools* funcionales de C.

(iii) Diferentes usos de suelo y prácticas de manejo inducen cambios en la distribución del tamaño de agregados y en su estabilidad, lo cual puede alterar la distribución del tamaño de poros en el suelo y la accesibilidad de los microrganismos a la mineralización de la materia orgánica, afectando, en última instancia, al secuestro de carbono. La protección del CO en microagregados puede proteger los aportes orgánicos contra la descomposición y facilitar su permanencia y estabilización en el suelo. Un incremento en el CO almacenado en microagregados ocluidos dentro de macroagregados podría ser un indicador sensible y fiable del potencial de secuestro de C en el suelo.

(iv) Para propiciar un buen nivel de agregación en el suelo, que favorezca la estabilización del carbono orgánico, sería necesario un aporte adecuado de inputs orgánicos, especialmente del pool más lábil. Por consiguiente, la efectividad de las prácticas, para aumentar el secuestro de C, dependerá de la cantidad y calidad de los aportes provinientes de la cobertura vegetal en cada uso o práctica de manejo.

(v) Los cambios en la estructura de las comunidades de microorganismos y en su actividad, inducidos por los cambios de uso y prácticas de manejo, pueden afectar al potencial del suelo para el secuestro de C.

De acuerdo con estas hipótesis y para lograr alcanzar el objetivo general descrito al inicio de este capítulo, se establecieron los siguientes objetivos parciales más específicos:

1. Evaluar los stocks de carbono en el perfil del suelo, según los usos, y determinar los factores que controlan sus variaciones, para aumentar nuestra capacidad de predecir la dinámica del carbono orgánico del suelo, con el cambio climático, en las áreas semiáridas.

2. Cuantificar y caracterizar los *pools* funcionales de materia orgánica (lábil, intermedio y pasivo), bajo diferentes usos y prácticas de manejo, con el fin de obtener una mayor comprensión del tiempo de permanencia del CO y los procesos que intervienen en su almacenamiento.

3. Evaluar la efectividad de la reforestación y de las prácticas agrícolas de manejo sostenibleen la capacidad de secuestro de C en el suelo.

4. Determinar los procesos y mecanismos implicados en la acumulación y estabilización del carbono orgánico en el suelo, en diferentes condiciones de uso y prácicas de manejo.

La Memoria de esta Tesis se ha estructurado en los siguientes capítulos:

- Capítulo 1: Introducción. Se presenta el estado actual del conocimiento sobre secuestro de carbono en zonas semiáridas, se identifican las lagunas en el conocimiento y se especifica el interés socioeconómico y ambiental que justifica este estudio.

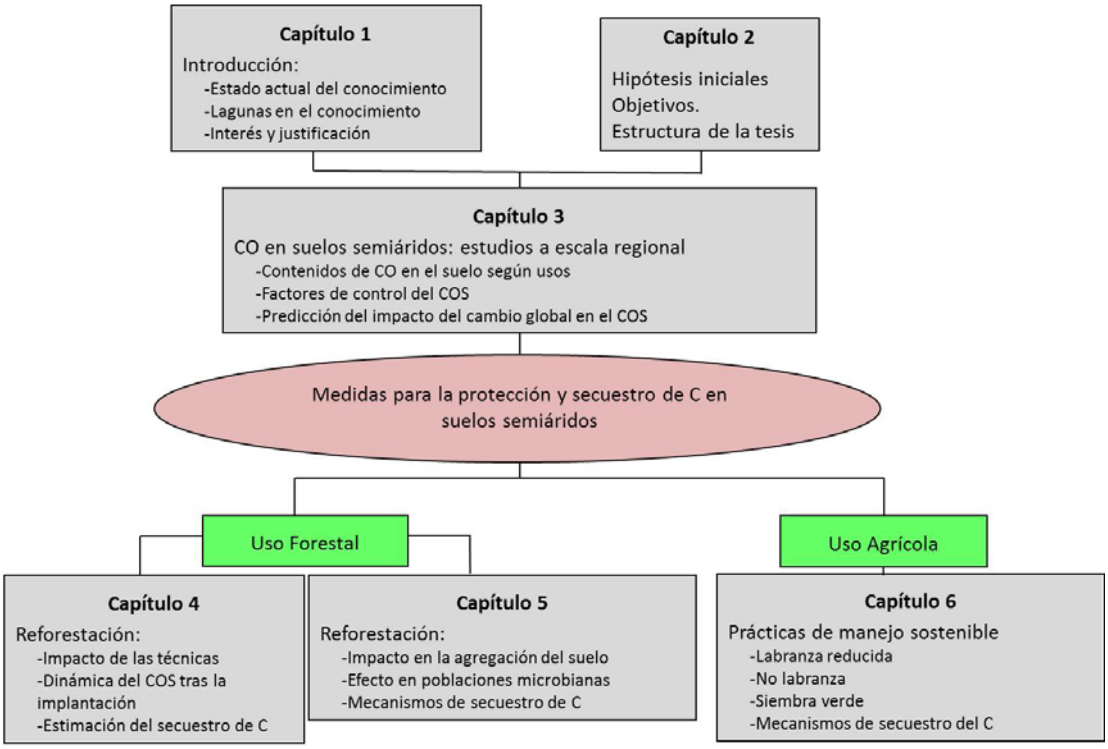


Figura 2.1. Estructura tesis.

- Capítulo 2: Se presentan las hipótesis de partida y los objetivos del estudio.

- Capítulo 3: A escala regional, se determina la distribución de los contenidos de CO en el perfil del suelo, en función del tipo de uso, y se analizan los factores que controlan las variaciones en estos contenidos. Se establecen correlaciones entre los factores de control y la concentración de CO, para mejorar las predicciones sobre el impacto del del cambio climático y uso del suelo sobre la materia orgánica de los suelos semiáridos. (Hipótesis i, Objetivo 1)

- Capítulo 4: Se estudia la dinámica de las propiedades del suelo y de los stocks de CO tras la reforestación de un matorral degradado, con dos técnicas de reforestación. (Hipótesis ii, objetivos 2 y 3)

- Capítulo 5: Se discuten los mecanismos de acumulación y estabilización del carbono en suelos forestales semiáridos. (Hipótesis iii, iv y v; objetivo 4)

- Capítulo 6. En un área experimental agrícola, se estudia el efecto de diferentes prácticas de manejo en la dinámica del COS y en los mecanismos que regulan su acumulación y estabilización. (Hipótesis ii, iii, iv, y v, objetivos 2, 3 y 4).

Capítulo 3. *Evaluación del COS y sus factores de control a escala regional: Influencia del uso de suelo y el cambio climático.*

El carbono orgánico del suelo (COS) es un componente importante del ciclo global del carbono, ya que representa el doble de la cantidad de carbono que se encuentra en la atmósfera y el 75% del reservorio total de carbono orgánico terrestre (Prentice, 2001). La sensibilidad de la descomposición del COS a los conductores del cambio global, de acuerdo con la profundidad del perfil, está recibiendo cada vez más atención debido a su importancia en el ciclo global del carbono y el potencial de retroalimentación con el cambio climático (Davidson y Janssens, 2006; von Lützow y Kögel-Knabner, 2009). Sin embargo, no hay demasiado acuerdo sobre los efectos del calentamiento global en los stocks de COS, creando una importante fuente de incertidumbre en las futuras predicciones sobre el cambio climático (Powlson, 2005; Schmidt et al., 2011). Una descripción clara de los cambios en la distribución del COS y de los factores que lo controlan puede ayudar a predecir las consecuencias del cambio climático.

Los objetivos específicos de este estudio son: *(1)* establecer la distribución vertical del COS en tres diferentes usos de suelo, contrastando diferentes litologías y tipos de suelo a través de toda la Provincia de Murcia, *(2)* determinar el factor principal que controla la variación de la concentración del COS a diferentes profundidades y en diferentes uso de suelo, y *(3)* incrementar nuestra capacidad para predecir el impacto de los principales conductores del cambio global sobre la concentración del COS en zonas semiáridas. Nuestra hipótesis se basa en que en las zonas semiáridas, los factores que controlan los niveles de COS cambian con la profundidad del suelo y que estos factores pueden ser dependientes de los cambios de uso de suelo.

El área de estudio fue la Provincia de Murcia (S.E de España) con un régimen climático Mediterráneo semiárido, con tendencia a árido. La base de datos usada (Proyecto LUCDEME) consiste en 312 perfiles de suelo con información sobre la concentración de COS (g kg^{-1}), tipo de suelo (Calcisoles, Regosoles, Fluvisoles, Cambisoles, Leptosoles, Kastanozems y Solonchaks), uso de suelo (forestal, matorral y cultivo), altitud, litología (sedimentos del Cuaternario, sedimentos aluviales, margas, y sedimentos metamórficos) textura y contenido de partículas gruesas (>2 mm). Los análisis estadísticos para estudiar las relaciones entre la concentración de COS y los factores de control en diferentes escenarios de uso de suelo se llevaron a cabo con profundidades fijas: 0-20, 20-40, 40-60, y 60-100 cm.

Los resultados obtenidos muestran que la concentración de COS en los 40 cm superficiales del suelo osciló entre 6.1 y 31.5 g kg^{-1}, con diferencias significativas según el uso de suelo, tipos de suelo y litología, mientras que por debajo de esta profundad no se observaron diferencias (concentración del carbono orgánico del suelo: 2.1-6.8 g kg^{-1}) (Tabla 3.1). El ANOVA mostró los cambios de uso como el factor de control más relevante en la concentración de COS en la capa de suelo de 0-40 cm de profundidad. Además, encontramos diferencias significativas en la importancia relativa de los factores ambientales y en los factores de la textura de acuerdo con el uso del suelo y la profundidad del suelo. También, se encontraron diferencias significativas en la importancia relativa de los factores ambientales y de la textura de acuerdo con el uso del suelo y de la profundidad del suelo. En el uso forestal, la precipitación media anual y la textura fueron los principales predictores del COS, mientras que en el uso agrícola y matorrales, los principales predictores fueron la temperatura media anual y la litología (Tabla 3.2).

Tabla 3.1. Media, desviación estándar (DS), coeficiente de variación (CV) del SOCc (g Kg^{-1}) agrupados por tipo de suelo, litología y tipos de uso de suelo en diferentes profundidades.

	Profundidad (cm)							
	0-20		20-40		40-60		60-100	
	Media ± DS	CV	Media ± DS	CV	Media±DS	CV	Media±DS	CV
Tipo de suelo								
Calcisol	12.2 ± 7.4a,b	60	7.0 ± 4.4ab	63	3.6 ± 2.2ab	61	2.2 ± 1.6a	72
Regosol	10.8 ± 9.1a	84	5.3 ± 4.4a	83	2.9 ± 2.0a	69	2.6 ± 1.7ab	65
Cambisol	23.5 ± 20.3bc	86	14.9 ± 10.3b	70	6.8 ± 4.4b	65	4.1 ± 3.7ab	90
Fluvisol	10.1 ± 6.6a,b	66	7.1 ± 4.5ab	63	4.6 ± 3.2ab	69	4.6 ± 3.8b	83
Kastanozem	31.5 ± 18.6c	58	18.3 ± 14.7b	80	3.7 ± 2.6ab	70	2.1 ± 2.4a	114
Leptosol	29.2 ± 18.0c	62	10.5 ± 6.3b	60	-		-	
Solonchak	11.3 ± 5.7a,b	50	8.5 ± 7.8 ab	92	3.8 ± 2.2ab	58	4.3 ± 3.4ab	79
Litología								
Sedimentos Cuaternarios	19.1 ± 16.5a	86	10.6 ± 9.5a	89	4.2 ± 4.2a	100	2.2 ± 2a	110
Sedimentos aluviales	9.8 ± 6.0b	61	6.7 ± 4.8a	72	4.5 ± 3.3a	73	2.5 ± 2a	80
Margas	9.3 ± 7.3b	78	6.1 ± 5.2b	85	3.0 ± 2.2b	73	4.1 ± 4a	97
Sedimentos metamórficos	12.3 ± 5.9a	50	6.6 ± 5.4a	81	4.3 ± 1.8a	41	4.7 ± 4a	85
Uso de suelo								
Forestal	30.4 ± 19.5c	64	13.4 ± 11.2c	83	5.8 ± 4.6a	79	4.4 ± 3.7a	84
Matorral	17.4 ± 13.9b	80	9.9 ± 9.1b	101	4.6 ± 4.4a	96	3.2 ± 3.2a	100
Agrícola	9.1 ± 4.9a	54	6.2 ± 4.0a	64	3.4 ± 2.3a	68	3.1 ± 2.8a	90

Letras diferentes indican diferencias significativas en cada profundidad entre tipos de suelo, litología y tipos de uso de suelo según el test de Tukey ($p<0.05$)

El COS total almacenado en 1 m de suelo en toda la Región fue aproximadamente de 79 Tg con una densidad media baja 7.18 kg C m^{-3}. La distribución vertical del COS es menos profunda en suelos forestales; y más profunda en las tierras de cultivo. Una reducción en la precipitación conduciría a la disminución del CO en bosques y matorrales, y un aumento de la temperatura media anual afectaría adversamente al COS en suelos de cultivo y matorral. Con el aumento de la profundidad, la importancia relativa de los factores climáticos disminuye y la textura cobra más importancia en el control del CO en todos los usos del suelo. En conclusión, con las tendencias actuales del cambio climático, nuestras predicciones indican que los impactos, en el COS, serán mayores en la superficie, por lo que las estrategias para el secuestro de C deben estar focalizadas al secuestro de C en el subsuelo, el cual en los suelos de uso forestal está en gran parte impedido, debido a las limitaciones en profundidad creadas por la roca madre. En estas condiciones se recomienda que las actuaciones dirigidas al secuestro de C en suelos, se realicen preferentemente en áreas agrícolas, de suelos más profundos, mediante de prácticas de manejo adecuadas.

Tabla 3.2. Coeficientes de correlación de Pearson entre SOCc y variables ambientales en diferentes profundidades en cada uso de suelo (p<0.05).

	Variables ambientales				
Contenido de COS según la profundidad (cm)	Arcilla	Limo fino	Altitud	Temperatura	Precipitación
Forestal					
0-20	ns	ns	ns	ns	0.53
20-40	**0.30**	ns	ns	ns	0.51
40-60	0.62	ns	ns	ns	0.54
60-100	ns	ns	ns	ns	0.62
Matorral					
0-20	ns	ns	0.53	-0.54	0.53
20-40	ns	ns	0.55	-0.58	0.51
40-60	ns	ns	0.43	-0.45	0.27
60-100	ns	0.57	ns	ns	ns
Agrícola					
0-20	ns	ns	0.22	-0.20	ns
20-40	ns	ns	0.21	-0.27	0.19
40-60	0.24	ns	ns	**-0.18**	**0.19**
60-100	ns	ns	0.26	-0.29	ns

En negrita p<0.1; ns: no correlaciones significativas

Capítulo 4. *Efecto de las técnicas de reforestación en el secuestro de C en el ecosistema*

En los últimos siglos, el suelo ha liberado grandes cantidades de CO_2 a la atmosfera como consecuencia de los cambios de uso (como por ejemplo la conversión de suelos forestales a un uso agrícola), la degradación del suelo y la desertificación (Lal, 2005; Jandl et al., 2007). Esto ha conducido a una disminución significativa de los diferentes *pools* de carbono orgánico en el suelo (COS) y un incremento de las concentraciones de gases de efecto invernadero en la atmósfera. A su vez, el cambio climático supone una potencial amenazada para el COS en los ecosistemas semiáridos. Los enfoques tradicionales en la restauración de los ecosistemas han considerado la reforestación como una herramienta importante para la rehabilitación de la capacidad de los ecosistemas para producir bienes y servicios, además de aumenta el secuestro del C. (Nosetto et al., 2006; Cao et al., 2010). Sin embargo, varios autores han reportado estudios contradictorios en cuanto al potencial de la reforestación para el secuestro de C (Wiesmeier et al., 2009; Fernández-Ondoño et al., 2010).

En este capítulo, partimos de la hipótesis que la captura de carbono tras la repoblación forestal en zonas semiáridas, puede ser aumentada mediante técnicas adecuadas, encaminadas a mejorar las condiciones del suelo y la disponibilidad de recursos para la planta. En un experimento a largo plazo (20 años), se analizó el efecto de dos técnicas de reforestación. Las técnicas de reforestación fueron: implantación de *Pinus halepensis* en, *(1)* aterrazado mecánico (T) y, *(2)* aterrazado mecánico con adición de enmienda orgánica (AT). Una zona de matorral típico Mediterráneo (S), adyacente a estas terrazas reforestadas, fue considerada como control. Veinte años después de la reforestación se midieron los cambios en: (i) las propiedades físicas, químicas y biológicas del suelo, (ii) el stock de C en el ecosistema y (iii) los tres *pools* funcionales de CO (lábil o activo, intermedio y pasivo).

Los resultados muestran que los diferentes tratamientos de reforestación tienen un impacto diferente en las propiedades del suelo. Comparado con la zona adyacente de matorral (S), el tratamiento AT condujo a una ganancia de C en el ecosistema de 1.3 kg C m^{-2}, mientras que con el tratamiento T hubo una disminución del 0.60 kg C m^{-2} a través de 20 años (Tabla 4.4). Este descenso fue debido al impacto de los trabajos previos en la construcción de las terrazas. La capacidad potencial de secuestro de C en los ecosistemas reforestados fue 160 y 65 g C m^{-2} $year^{-1}$ en AT y T, respectivamente. Centrándonos en el secuestro de C en el suelo mineral, la tasa de secuestro media anual fue de 28 g C m^{-2} y^{-1} en AT y de 17 g C m^{-2} y^{-1} en T.

En lo que respecta al stock de CO en la fracción lábil (CO-POM), fue significativamente mayor en AT, seguida de T y menor en S, en la capa superficial del suelo (0-5 cm). En los 5-20 cm, no se encontraron diferencias significativas en CO-POM entre tratamientos, pero la en los 20-25 cm, el OC-POM fue más bajo en S que en AT y T (Figura 4.5). El stock de CO en el *pool* intermedio (CO-S+A), en superficie (0-5 cm) fue mayor en AT que en S y T, sin diferencias entre S y T. En los 5-20 cm fue significativamente más bajo en T, pero sin diferencias entre AT y S, mientras que en la capa más profunda (20-25 cm) no se observaron diferencias entre tratamientos. El patrón de distribución de stock de CO en el *pool* estable (CO-S+C) fue diferente de las anteriores fracciones (Figura 4.6). A lo largo del perfil del suelo, el CO-S+C fue significativamente mayor en S que en AT y T, sin diferencias entre los tratamientos reforestados. Estos resultados muestran que el potencial de secuestro de C en los ecosistemas reforestados semiáridos, puede ser incrementado por el uso de técnicas adecuadas que propicien un mayor desarrollo de la vegetación. Al mismo tiempo, deben evitarse las tecnologías que impliquen grandes perturbaciones en el suelo. Veinte años después de la reforestación con *Pinus halepensis*, la capacidad potencial de secuestro de C de los ecosistemas reforestados está lejos de ser saturada y pueden continuar secuestrando C a medida que alcanzan la madurez.

Tabla 4.4. Distribución de los stocks de carbono orgánico (kg m^{-2}) (valores medios ± errores estándar) entre diferentes componentes del ecosistema en el tratamiento control (S), aterrazado mecánico (T) y aterrazado mecánico combinado con enmienda orgánica (AT).

Componentes del ecosistema	**Tratamientos**		
	S	**T**	**AT**
Suelo Mineral (0-25 cm)	3.6 ± 0.1b	2.9 ± 0.1a	3.5 ± 0.1b
Pinos	0*	0.4 ± 0.2a	1.6 ± 0.9b
Matorral (sotobosque)	0.4 ± 0.06b	0.05 ± 0.01a	0.05 ± 0.01a
Hojarasca	n	0.05 ± 0.01	0.2 ± 0.04
TOTAL	4.0	3.4	5.3

Letras diferentes en cada fila indica diferencias significativas entre tratamientos (Test de Tukey, p <0.05) n: despreciable. * No había pinos en el tratamiento S.

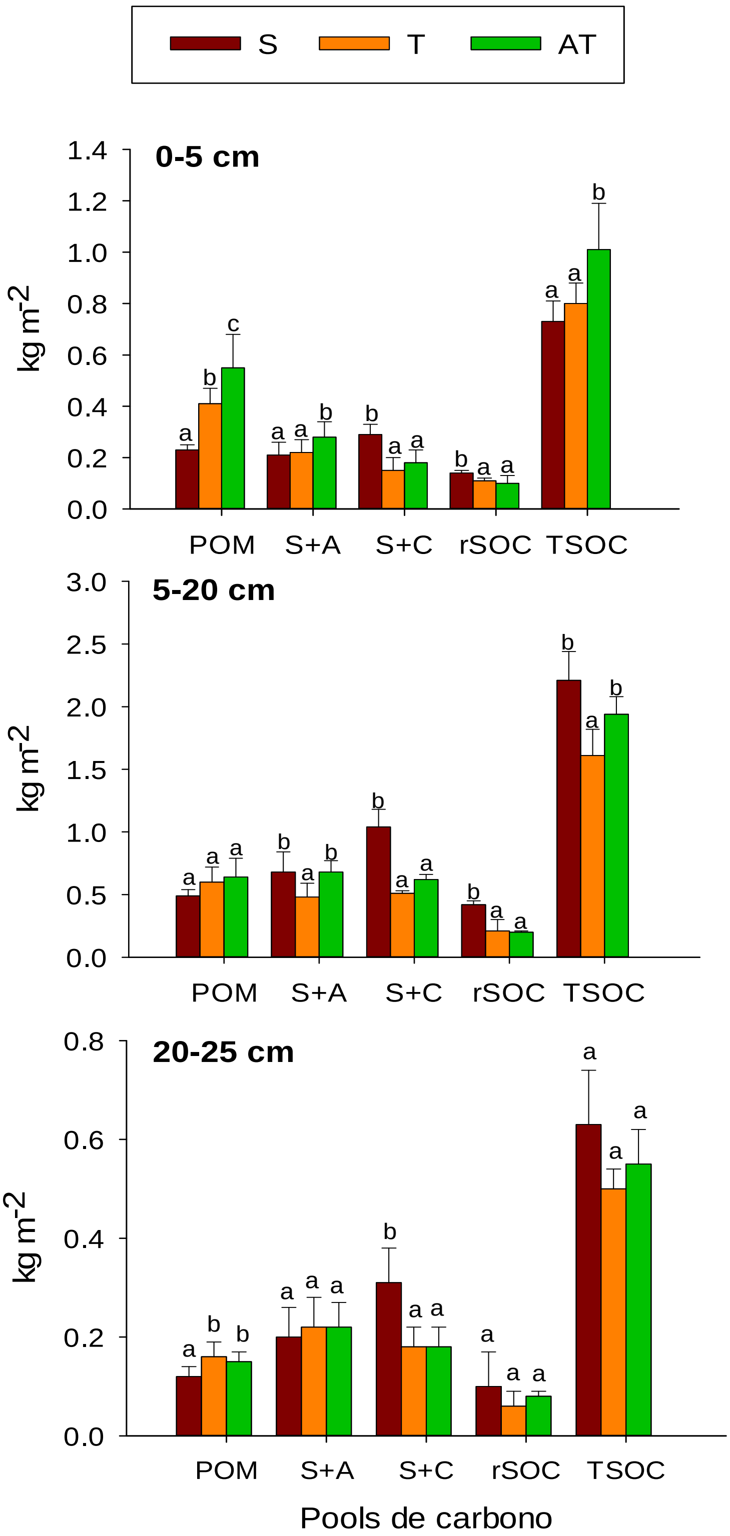


Figura 4.6. Stock de carbono orgánico (media ± error estándar) en la fracción de materia orgánica particulada (POM), arena y agregados estables (S+A), limo y arcilla (S+C), carbono orgánico resistente a la oxidación) y COS total (kg m^{-2}) en 0-5, 5-20 y 20-25 cm de profundidad en el tratamiento control (S), aterrazado mecánico (T) y aterrazado mecánico combinado con enmienda en el suelo (AT). Letras diferentes en las barras indican diferencias significativas entre tratamientos (Test de Tukey, $p < 0.05$) para cada pool.

Capítulo 5. *Mecanismos de estabilización del carbono orgánico en suelos forestales semiáridos*

El potencial de secuestro de C, en los ecosistemas semiáridos, puede ser aumentado mediante técnicas adecuadas de reforestación y otras conversiones del uso de suelo (De Gryze et al., 2004). Sin embargo, se necesita un mayor conocimiento sobre los mecanismos y factores que controlan la acumulación y estabilización del COS en las reforestaciones, para la optimización de estas técnicas.

La hipótesis de partida, específica para este estudio, fué: el cambio en la cobertura vegetal después de la reforestación, aumenta los aportes de restos vegetales frescos y promueve cambios en la comunidad microbiana, que pueden conducir a un aumento en la agregación y protección física del COS.

Se experimentaron dos técnicas de reforestación con *Pinus halepensis*: aterrazado mecánico (T) y aterrazado mecánico con adición de enmienda orgánica en el suelo (AT), comparadas con una zona adyacente de matorral mediterráneo (S). Los objetivos específicos fueron analizar: (*1)* cambios en la agregación del suelo, (*2)* remodelización de la estructura de la comunidad de bacterias y de hongos y, (*3)* procesos implicados en la protección y estabilización del COS.

Los resultados obtenidos muestran un aumento significativo en ambos *pools* (lábil: OCs; y pasivo: OCsw) en los 0-5 cm del suelo, y una disminución significativa del *pool* pasivo en las capas más profundas, en el tratamiento AT comparado con el control S. Sin embargo, en el tratamiento T se observa un descenso del contenido de OCsw respecto al tratamiento S a lo largo de todo el perfil del suelo, mientras que no se observaron cambios en OCs entre T y S (Tabla 5.1).

Además, en superficie (0-5 cm) se encontraron diferencias significativas en la composición química del *pool* funcional OCs entre los tratamientos reforestados y el matorral: mayores porcentajes en los compuestos de O-alkyl-C en AT: 51.1 ± 1.1 % y T: 49.2 ± 0.7 % que el matorral 45.1 ± 0.25 %) y menores porcentajes de compuestos de Aryl-C en AT: 30.3 ± 0.6 % y T: 31.7 ± 0.8 %) respecto a S (33.2 ± 0.05 %).

Tabla 5.1 Contenido de CO en el total del suelo (g kg^{-1}): *pool* sensible (OCs) y *pool* lento (OCsw) en AT (reforestación con enmienda orgánica), T (reforestación) y S (matorral) a 0-5, 5-20 y 20-25 cm de profundidad.

Profundidad	***Pool* sensible (OCs)**		
(cm)	**S**	**T**	**AT**
0-5	4.02 ± 0.10aC	5.90 ± 0.78aB	11.65 ± 1.20bB
5-20	2.59 ± 0.21aB	3.10 ± 0.26aA	3.46 ± 0.34aA
20-25	1.74 ± 0.24aA	2.45 ± 0.22aA	1.72 ± 0.31aA
Interacción: *Tratamiento x profundidad*	**	**	**
Profundidad	***Pool* lento (OCsw)**		
(cm)	**S**	**T**	**AT**
0-5	8.78 ± 0.45AbB	5.87 ± 0.26aA	10.92 ± 0.59cB
5-20	9.40 ± 0.73cB	5.09 ± 0.17aA	7.02 ± 0.45bA
20-25	7.28 ± 0.46bA	5.61 ± 0.52aA	5.62 ± 0.35aA
Interacción: *Tratamiento x profundidad*	**		**
***Significación P<0.01*			

Valores medios ± error estándar. Diferentes letras minúsculas en filas indican diferencias significativas entre tratamiento para cada profundidad y para cada *pool* funcional. Diferentes letras mayúsculas en columnas indican diferencias significativas entre profundidades dentro de cada tratamiento para cada *pool* funcional (Test de Tukey, p <0.05).

En cuanto a las diferencias en la estructura de la comunidad de microorganismos, en el caso de las bacterias no se encontraron diferencias significativas entre tratamientos. Sin embargo, la estructura de la comunidad de hongos mostró diferencias significativas entre tratamientos. Además, la riqueza de especies de hongos fue mayor en AT comprada con S, mientras que T no mostró diferencias con AT y S. También, la distancia filogenética entre especies de hongos fue mayor y marginalmente significativa en AT comparada con S, mientras que T no mostró diferencias con ninguno de los dos tratamientos. Los hongos de la división *Basidomicotas* (subdivisión *Agaricomicotinas*) fueron más abundantes en AT que en S, mientras que su abundancia relativa en T no difirió significativamente de S o AT. La abundancia relativa de los hongos *Chytridiomicotas* fue significativamente mayor en S que en los tratamientos reforestados.

Por otro lado, la distribución del tamaño de agregados estuvo correlacionada con la estructura de la comunidad de hongos, pero no con la de bacterias. La misma tendencia se observó en la correlación entre la respiración basal y la estructura de la comunidad de hongos. Asímismo, el análisis de componentes principales que se realizó mediante un análisis no ponderado de matrices de distancia UniFrac (Lozupone et al., 2006), mostró que la estructura de la comunidad, tanto de bacterias como de hongos, en las zonas reforestadas (AT y T) eran diferentes a la zona de matorral.

En lo que respecta a la distribución de agregados y carbono orgánico asociado a los agregados, al mismo tiempo que aumentó el porcentaje de macroagregados (> 250 µm) en AT, también lo hizo el porcentaje de microagregados ocluidos dentro de macroagregados y la concentración de CO dentro de ellos en AT con respecto a los otros dos tratamientos (T y S). Además el contenido de CO en los microagregados ocluidos fue mayor que el de los microagregados libres. Todos estos resultados sugieren un orden jerárquico de agregación del suelo en AT, donde los macroagregados actúan como núcleo de formación de microagregados que quedan protegidos en su interior (Oades, 1984).

La fuerte correlación entre la respiración basal medida en los macroagregados (RB-M) y el porcentaje de macroagregados (> 250 µm), en todos los tratamientos, sugiere que la actividad de los microorganismos fue un importante factor en la formación de nuevos macroagregados. Similares resultados sobre la influencia de la actividad biológica en la agregación del suelo, han presentado otros autores (Lynch y Braggs, 1985; Siddiky et al., 2012; Daynes et al., 2013). La alta significación de las correlaciones positivas en AT y T entre RB-M y el porcentaje de microagregados ocluidos en macroagregados, y con el contenido de CO de esos microagregados ocluidos en macroagregados, sugieren la presencia de un proceso activo de formación de microagregados dentro de agregados de mayor tamaño, siendo este proceso más activo en AT. Todo esto conduce a un aumento de la protección del CO asociado con estos microagregados y de su tiempo de permanencia en el suelo. En definitiva, aumenta la capacidad de secuestro de C en los ecosistemas reforestados. Por el contrario, en la zona de matorral no se encontró ninguna correlación entre la RB y los microagregados dentro de macroagregados.

En conclusión, en AT, el aumento del *pool* de C lábil (caracterizado por un mayor contenido relativo en O-alkyl-C) induce un aumento de la actividad microbiana, acompañada de cambios significativos en las comunidades de hongos. Estos cambios, promueven y activan los procesos de formación de nuevos macroagregados que actúan como núcleo de formación de microagregados ocluidos dentro de ellos y que están enriquecidos en carbono orgánico, principalmente en la capa de 0-5 cm de profundidad.

Tabla 5.7. Coeficientes de correlación de Pearson de los *pool* sensible (OCs), *pool* lento (OCsw) y respiración basal con el porcentaje de agregados y el CO asociado a ellos.

	Tratamientos		
Correlaciones:	**S**	**T**	**AT**
OCs:			
M (%)	0.816**	0.684**	0.828**
m (%)	ns	-0.714**	-0.836**
Mm (%)	ns	0.683**	0.860**
OC-M (g kg^{-1})	0.780**	0.659**	0.931**
OC-m (g kg^{-1})	ns	ns	ns
OC-Mm (g kg^{-1})	ns	ns	0.864**
BR-M (mg CO_2-C kg^{-1} d^{-1})	0.877**	0.784**	0.925**
OCsw:			
M (%)	0.567*	ns	0.786**
m (%)	ns	ns	-0.867**
Mm (%)	ns	ns	0.752**
OC-M (g kg^{-1})	0.552*	ns	0.743**
OC-m (g kg^{-1})	ns	ns	ns
OC-Mm (g kg^{-1})	ns	ns	0.746**
BR-M(mg CO_2-C kg^{-1} d^{-1})	ns	ns	0.896**
RB-M (mg CO_2-C kg^{-1} d^{-1})			
M (%)	0.798**	0.817**	0.933**
m (%)	ns	-0.922**	-0.918**
Mm (%)	-0.838**	0.855**	0.911**
OC-M (g kg^{-1})	0.719**	0.985**	0.917**
OC-m (g kg^{-1})	ns	ns	-0.481*
OC-Mm (g kg^{-1})	ns	0.823**	0.819**

*Significanción P <0.05;
**Significación P < 0.01;
ns, not significativo.
M: % de macroaggregates (> 250 µm); m: % de microagregados (250-63 µm), Mm: % de microagregados ocluidos en macroagregados; OC-M: contenido de CO en macroagregados; OC-m: contenido de CO en microagregados; OC-Mm: contenido de CO en microagregados ocluidos en macroagregados; RB-M: respiración basal de los macroagregados.

Capítulo 6. *Mecanismos de estabilización del COS en áreas agrícolas: efecto de las prácticas de manejo*

La cantidad y naturaleza de los restos vegetales y el grado de descomposición de la MOS son factores claves en la formación y estabilización de los agregados que promueven la protección y estabilización del CO y, por tanto, el secuestro de C (Haynes y Beare, 1996). Sin embargo, los mecanismos de las interacciones entre la estructura del suelo, prácticas de manejo y la dinámica del COS no son aún bien conocidos (Blanco-Canqui y Lal, 2004).

En en el marco general de la hipótesis (ii), en este capítulo pretendemos probar que el COS asociado a los agregados, aumenta con la adición de abono verde y con el cese del laboreo, y la protección físico-química del CO, en dichos agregados, es la responsable de la acumulación del CO.

El área experimental se estableció en un cultivo ecológico de almendro en secano, donde la práctica habitual de manejo durante 14 años fue el laboreo reducido (RT). Las prácticas de manejo estudiadas fueron: labranza reducida combinada con la adición de un abono verde (mezcla de *Vicia sativa* L. y *Avena sativa* L.) (RTG) y no laboreo (NT). Para el estudio analítico, se aislaron diferentes tamaños de agregados y pools de materia orgánica, contenida en estos agregados, a tres profundidades del suelo (0-5, 5-15, 15-30 cm), (figura 6.2).

Los resultados obtenidos muestran una mayor concentración del CO en RTG (13.74 ± 0.63 g kg^{-1}) respecto a RT (11.76 ± 0.50 g kg^{-1}) y NT (10.15 ± 0.37 g kg^{-1}) en la capa superficial del suelo (0-5 cm), mientras que no se observan diferencias por debajo de los 5 cm de profundidad. La misma tendencia se observó para el carbono de la biomasa microbiana.

En RT el porcentaje de macroagregados grandes (> 2000 μm, LM) y macroagregados pequeños (250-2000 μm, SM), fue reducido por el laboreo, mientras que en RTG la reducción fue solo en el porcentaje de LM comparado con NT. La incorporación del abono verde resultó en una acumulación de CO en todos los agregados del suelo con un incremento del 186% del CO asociado a los LM, 58% en SM y 45% en microagregados, relativo a RT en la superficie (0-5 cm).

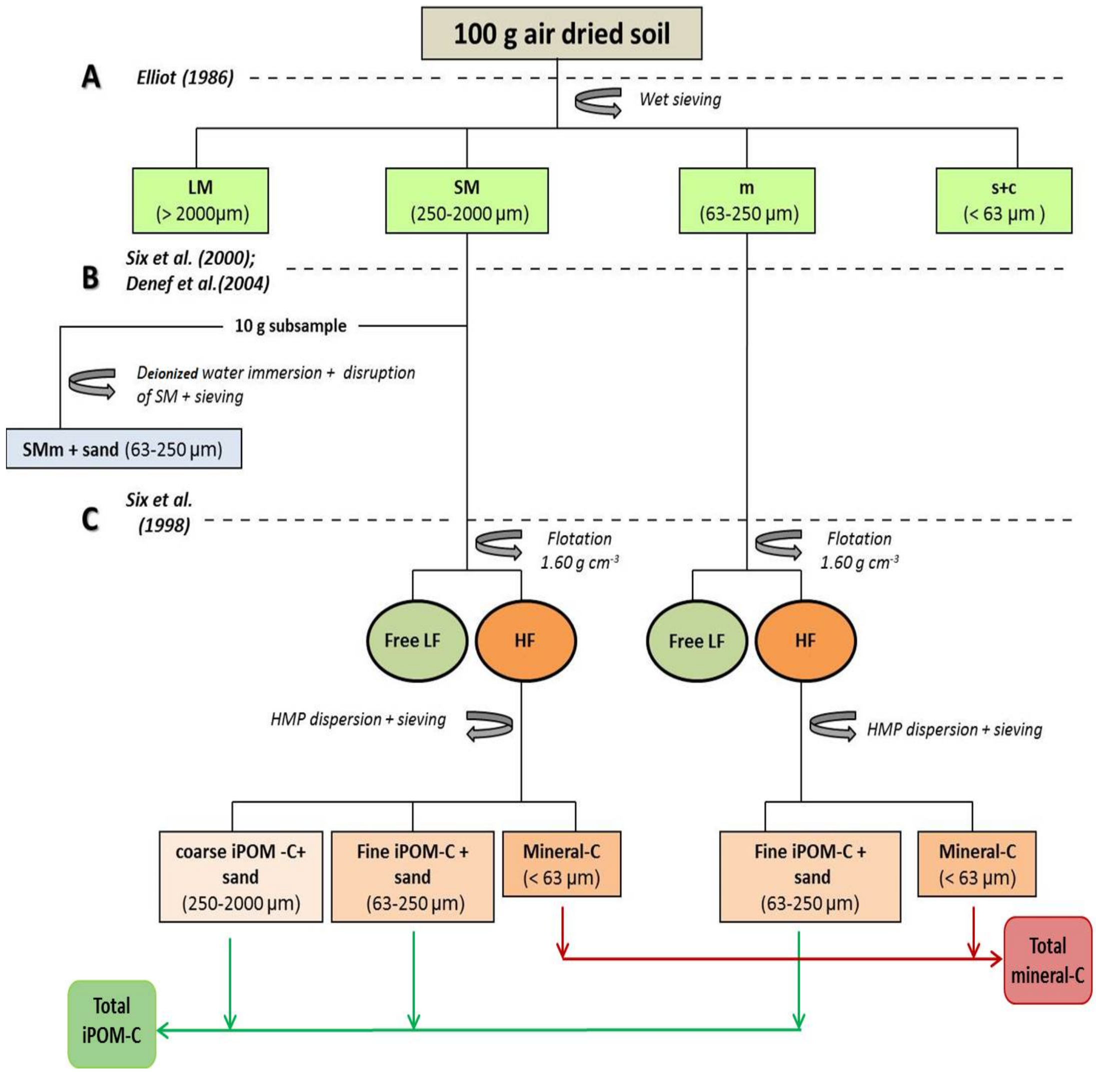


Figura 6.2. Esquema de los métodos de fraccionamiento usados: **(A)** Tamizado en húmedo (Elliot et al., 1986): macroagregados grandes (LM: >2000 µm), macroagregados pequeños (SM: 250-2000 µm), microagregados (m: 63-250 µm), y partículas de limo más arcilla (s+c: <63 µm); **(B)** Separación de microagregados ocluidos en macroagregados (Denef et al., 2004 y Six et al., 2000b): microagregados dentro de macroagregados más arena (SMm: 63-250 µm); y **(C)** Fraccionamiento por densidad (Six et al., 1998): fracción ligera libre (free LF, densidad < 1.60 g cm^{-3}), fracción pesada (HF, densidad > 1.60 g cm^{-3}): materia orgánica particulada intra-agregada en la fracción gruesa (coarse iPOM-C: 250-2000 µm), y en la fracción fina (fine iPOM-C: 63-250 µm), carbono orgánico asociado a la fracción mineral ocluida en los agregados (Mineral-C: <63 µm).

Así mismo, en RTG con el abono verde aumentó el porcentaje de microagregados dentro de macroagregados pequeños (SMm) y su contenido en CO (CO-SMm), con respeto a RT. Mientras que en NT aumentó el porcentaje pero no el CO asociado a los SMm (Tabla 6.2).

Tabla 6.2. Porcentaje en peso y concentración de CO (g kg^{-1} suelo) de SMm (microagregados ocluidos en macroagregados pequeños) a 0-5, 5-15, y 15-30 cm de profundidad, para los tratamientos de: laboreo reducido (RT); laboreo reducido con abono verde (RTG), no laboreo (NT).

	Prácticas de manejo del suelo		
Profundidad (cm)	RT	RTG	NT
SMm (%)			
0-5	4.96 ± 0.23aA	11.00 ± 0.41cA	6.55 ± 0.19bA
5-15	7.98 ± 0.21bB	11.38 ± 0.40cA	6.46 ± 0.34aA
15-30	8.45 ± 0.50aB	11.96 ± 0.29bA	8.60 ± 0.71aB
CO –SMm (g kg^{-1})			
0-5	0.76 ± 0.02aA	2.13 ± 0.27bA	0.75 ± 0.10aA
5-15	1.12 ± 0.04bB	2.07 ± 0.13cA	0.70 ± 0.13aA
15-30	1.19 ± 0.01abB	1.88 ± 0.30bA	0.93 ± 0.09aA

Valores medios ± errores estándar. Diferentes letras minúsculas en filas indican diferencias significativas entre tratamiento dentro cada profundidad. Diferentes letras mayúsculas en columnas indican diferencias significativas entre profundidades dentro de cada tratamiento (Test de Tukey, $p < 0.05$).

El CO de la fracción libre (free LF-C) representó entre un 3% y un 11% del CO total del suelo, según el tratamiento y la profundidad (Tabla 6.3). En superficie, el free LF-C fue 1.5 veces mayor en RTG comparado con RT y NT. Sin embargo por debajo de los 5 cm no se observaron diferencias entre RT y RTG. El CO total de la fracción de materia orgánica particulada intra-agregada (total iPOM-C) fue relativamente mayor en RTG y NT (20%) que en RT (12%) en todo el perfil del suelo (Tabla 6.3). Por otro lado, el CO asociado con la fracción mineral (total mineral-C) disminuyó en el siguiente orden: RTG ≥ RT ≥ NT en la capa superficial del suelo, pero por debajo de los 5 cm no se han encontrado diferencias entre los tratamientos.

No se observaron diferencias, en los 0-5 cm del suelo, en la fracción gruesa de materia orgánica particulada intra-agregada (coarse iPOM-C) entre los tratamientos, pero sí en la fracción fina (fine iPOM-C) ocluida en los macroagregados pequeños (SM): RTG y NT aumentaron un 63 y un 118% respectivamente, comparados con RT (Figura 6.5). Sin embargo, en profundidad (5-15 cm), el tratamiento NT mostró un aumento del 310 % en la fracción coarse iPOM-C comparado con el tratamiento RT. Por otro lado, la fracción fine iPOM-C ocluida dentro de los microagregados, fue la fracción que más CO ganó con el abono verde (138%) respecto a RT, en la profundidad de 0-5 cm. Mientras que en NT esta ganancia fue del 57.5% respecto RT en la profundidad de 5-15cm. Por último, el CO asociado a la fracción mineral (mineral-C) ocluida en los SM, fue el principal responsable de las ganancias de CO en el total mineral-C en RTG comparado con RT. (Figura 6.5).

Tabla 6.3. Concentración de CO (g C kg^{-1} suelo) de fracción ligera libre (free LF-C), total de la fracción de material orgánica particulada intra-agregada (iPOM-C contenido en macroagregados pequeños + en microagregados) y total de la fracción mineral (mineral-C contenido en macroagregados pequeños + en microagregados) a 0-5, 5-15, y 15-30 cm de profundidad, para los tratamientos de: laboreo reducido (RT); laboreo reducido con abono verde (RTG), no laboreo (NT).

	Soil management practices		
Depth (cm)	**RT**	**RTG**	**NT**
Free LF-C			
0-5	0.45 ± 0.02aA	0.71 ± 0.04bA	0.46 ± 0.01aA
5-15	1.21 ± 0.11bB	1.21 ± 0.12bB	0.53 ± 0.05aA
15-30	0.58 ± 0.07bA	0.58 ± 0.02bA	0.37 ± 0.05aA
Total iPOM-C			
0-5	1.36 ± 0.08aA	2.91 ± 0.11cC	1.81 ± 0.07bA
5-15	1.55 ± 0.07aA	2.20 ± 0.10aB	2.40 ± 0.72aA
15-30	1.52 ± 0.08aA	1.70 ± 0.13aA	1.64 ± 0.10aA
Total mineral-C			
0-5	2.70 ± 0.13abA	3.20 ± 0.14bA	2.31 ± 0.14aA
5-15	3.07 ± 0.44aA	3.80 ± 0.23aB	3.34 ± 0.19aB
15-30	2.38 ± 0.14aA	2.94 ± 0.27aA	2.66 ± 0.10aA

Valores medios ± errores estándar. Diferentes letras minúsculas en filas indican diferencias significativas entre tratamientos dentro cada profundidad. Diferentes letras mayúsculas en columnas indican diferencias significativas entre profundidades dentro de cada tratamiento (Test de Tukey, $p < 0.05$).

En resumen, a pesar del poco tiempo que lleva este experimento, se observaron diferencias en todos los pools de C debido a las prácticas agrícolas de manejo sostenible (RTG y NT), principalmente en el horizonte superficial del suelo. Además, los resultados muestran que las fracciones de carbono: (i) asociado a la fracción mineral, y (ii) materia orgánica particulada intra-agregada, son las principales responsables de la acumulación del CO en todo el perfil del suelo. Ambas fracciones aumentan con la incorporación del abono verde o con el cese del laboreo, en las prácticas habituales de cultivo, proporcionando mayor sostenibilidad a los agroecosistemas de cultivos leñosos de secano.

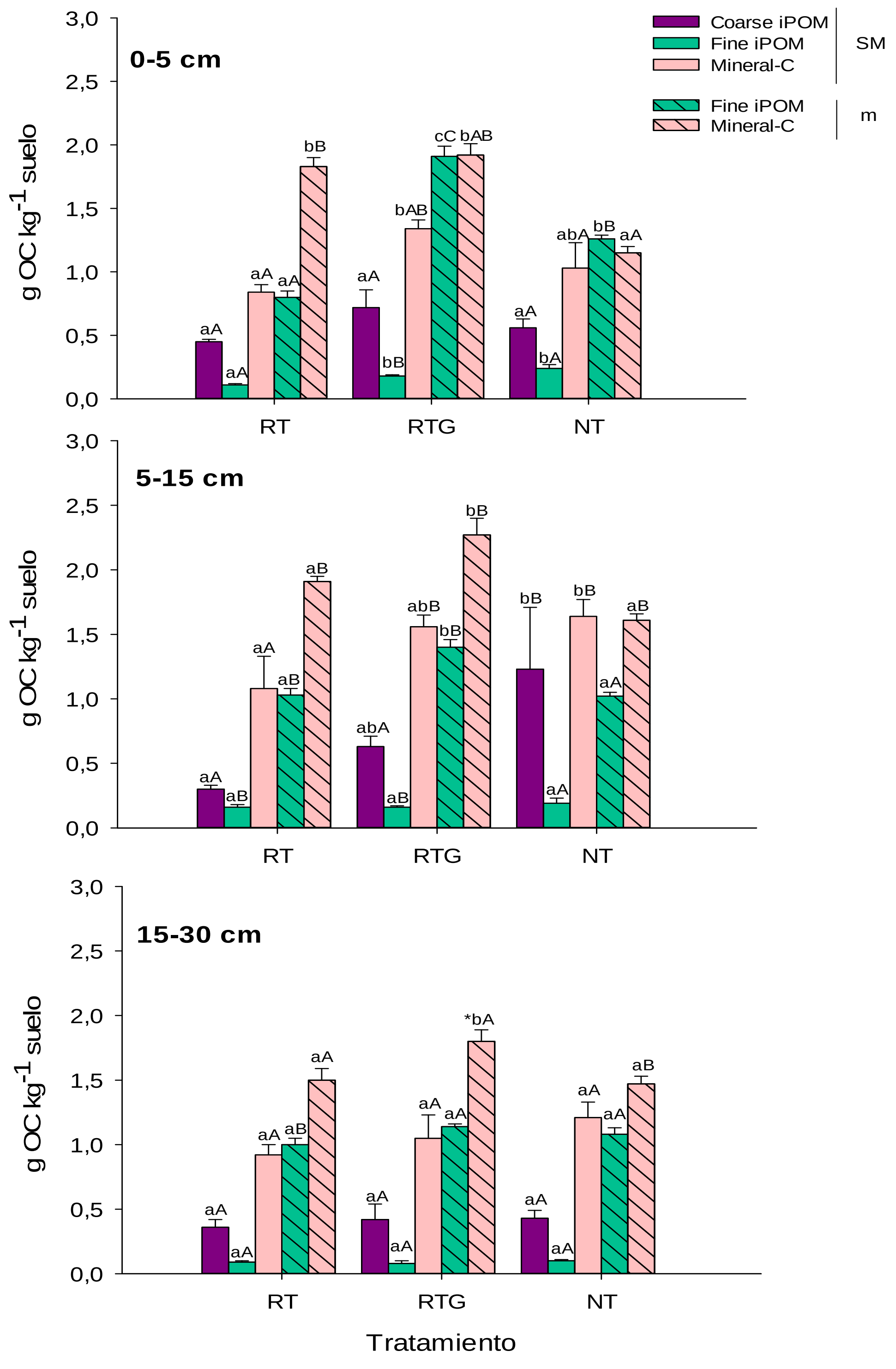


Figura 6.5. Distribución del contenido de CO (g kg^{-1} suelo) en los sub*pools* de macroagregados pequeños (SM) y microagregados (m): materia orgánica particulada intra-agregada (iPOM-C): coarse iPOM-C (250-2000 µm), fine iPOM-C (63-250 µm); y C asociado a la fracción mineral (mineral-C) en las profundidades de 0-5, 5-15 y de 15-30 cm para los tratamientos de: laboreo reducido (RT); laboreo reducido con abono verde (RTG) y no laboreo (NT). Letras minúsculas diferentes sobre las barras del gráfico indican diferencias significativas entre tratamientos dentro de cada profundidad (Tukey, $p < 0.05$; * $p < 0.06$). Diferentes letras mayúsculas sobre la barras del gráfico indican diferencias entre profundidades para cada tratamiento (Test de Tukey, $p < 0.05$).

Los mayores aumentos en el total de CO del suelo y CO asociado a los agregados se han observado en el tratamiento RTG, sugiriendo que la combinación de abono verde y laboreo reducido es una buena opción para el secuestro de COS pues: i) laboreo verde representa continuos inputs de MO que, a su vez, activan: a) la protección física del CO a través de la formación de nuevos agregados y b) la protección físico-química asociado a las partículas minerales del suelo; y ii) es necesario un mínimo de laboreo porque favorece la incorporación de los restos vegetales a las capas más profundas del suelo, promoviendo a su vez la formación de nuevos agregados.

Conclusiones generales

1. El tipo de uso de suelo se reveló como el factor con mayor peso en el control de la concentración de carbono orgánico en el suelo, en los ecosistemas semiáridos estudiados. La conversion de zonas de bosque en tierras de cultivo implica una considerable reducción (alrededor del 70% en los 0-20 cm) en el contenido en COS, por lo que debe estar rigurosamente restringido.

2. Los factores que controlan los cambios en el contenido de carbono orgánico del suelo, varían en función del tipo de uso y la profundidad del suelo. En relación al uso, mientras que en los ecosistemas forestales los principales factores fueron la precipitación media anual y la textura, en los ecosistemas de matorral y agrícolas los principales factores de control fueron la temperatura y la litología. Respecto a la profundidad, al aumentar la profundidad disminuye la importancia relativa de los factores climáticos (precipitación y temperatura) y la textura se convierte en el factor con más peso, en todos los usos de suelo. Estos resultados sugieren que, en futuros escenarios de cambio climático, los cambios de temperatura tendrán mayor impacto sobre los suelos agrícolas, mientras que los cambios de precipitación afectarán, en mayor medida a los suelos forestales. Nuestros resultados predicen una disminución de los contenidos de COS, en un escenario de cambio climático con un aumento de la temperatura y una disminución de la precipitación, como se espera en las áreas semiáridas. En todos los usos, los suelos con texturas finas serán más resistentes a los cambios que los de textura gruesa.

3. El análisis de los factores ambientales, muestra que el impacto del cambio climático será mayor en la superficie del suelo que en profundidad, por lo que las

actuaciones para el secuestro de carbono deberían focalizarse al secuestro en el subsuelo. En este sentido, las actuaciones tradicionales de reforestación en áreas de montaña, están potencialmente limitadas por la presencia de la roca madre, a escasa profundidad. La distribución de los stocks de carbono en el perfil del suelo (capitulo 3), sugiere que las actuaciones en áreas agrícolas (incentivar prácticas de manejo adecuadas a estos objetivos), son potencialmente mas efectivas y, probablemente, más rentables económica y socialmente, ya que además de reducir el CO_2 atmosférico, aumentan la productividad del suelo y el rendimiento agrícola.

4. El potencial de secuestro de C, en las áreas reforestadas semiáridas, depende, en gran medida, de las técnicas usadas para la reforestación. Los stocks de C, en los ecosistemas reforestados, son directamente proporcionales a la cantidad de biomasa producida, la cual, a su vez, viene determinada por la productividad del suelo. Por tanto, se han de utilizar métodos que mejoren la productividad del suelo. El uso de enmiendas orgánicas del suelo, previo a la implantación, fue muy efectivo en términos de secuestro de C. Se recomienda la supresión de prácticas que impliquen una gran perturbación de los horizontes del suelo, ya que, además de reducir su productividad, suponen la emisión de altas cantidades de CO_2 a la atmósfera.

5. La estabilización química del CO, a través de la formación de complejos con las partículas de limo y arcilla, y la protección física en microagregados formados dentro de macroagragados, fueron los principales mecanismos de secuestro de C en los suelos estudiados, tanto en las áreas forestales como en las agrícolas.

6. La estabilización química fue promovida por la composición mineral de la matriz del suelo, principalmente la alta concentración de cationes multivalentes, mayoritariamente Ca^{2+}, y la presencia de superficies minerales con gran capacidad de absorver compuestos orgánicos, como los interestratificados ilita-montmorillonita que tienen un área superficial específica muy alta.

7. La protección física estuvo impulsada por los cambios, cualitativos y cuantitaivos en los aportes vegetales al suelo. Tanto en el área forestal, especialmente en el tratamiento con enmienda orgánica, como en el cultivo con la aplicación de la siembra verde, se produce un aumento en el pool lábil de CO en el suelo. Este aumento promueve

la formación de macroagragados, en una doble vía: a) actuando directamente como agente de enlace entre las partículas del suelo y, b) de forma indirecta, activando la actividad microbiológica, especialmente de los hongos, que “empaquetan” las partículas con sus hifas. La constitución de estos nuevos macroagregados propicia la formación de microagregados, que ocluyen materia orgánica en su interior y la hacen inaccesible a los microrganismos.

8. Los cambios en la cobertura vegetal inducen variaciones en la estructura de la comunidad de hongos del suelo que, en el caso de la reforestación con Pinus halepensis, favorecen la formación de macroagregados, al aumentar la proporción de especies formadoras de hifas.

9. Las fuertes correlaciones entre respiración basal y porcentaje de microagragados dentro de macroagregados, positiva en los suelos reforestados y negativa en el matorral degradado, sugieren que: a) la formación de microagragados, enriquecidos en CO, dentro de macroagregados es un mecanismo de autodefensa del suelo, para la protección del CO frente al aunnnmento de la actividad microbiológica y, 2) esta correlaciones podrían servir como indicador de procesos de agradación, en el caso de correlación positiva, o degradación, correlación negativa, del suelo.

Summary

Chapter 1. *General introduction*

Soil use and management practices control soil behavior as a sink or source of greenhouse gases

At present, in the terrestrial carbon (C) cycle a negative feedback is occurring, with emission to the atmosphere of 3-6 Gt CO_2 per year through soil organic carbon (SOC) mineralization, with the subsequent acceleration of climatic change. Of the historic loss from the terrestrial C pool of 136 ± 55 Pg, 78 ± 12 Pg is estimated to have arisen from depletion of SOC (Lal et al., 2003). This part of the C cycle could be managed by improving soil structure through adequate and sustainable land uses, such as restoration of marginal land and sustainable land management practices (SLM) like conservation tillage. Therefore, there are many factors and processes, linked to land use and management, which determine the direction and rate of change in SOC content when vegetation and soil management practices are changed.

Soil organic matter characterization

Classical descriptions of SOM have normally combined chemical extractions with the identification of specific chemical compounds, but this approach has contributed little to a functional understanding of the dynamic and the turnover time of SOC (Collins et al., 2000) and does not conform to the concept of SOM stabilization as the protection from mineralization (von Lützow et al., 2006). As an alternative, researchers have tended to adopt a model where organic carbon is located in more or less discrete functional "*pools*" in the soil (Jones and Donnelly, 2004). In a general context, three pools, which vary in their inherent rate of decomposition and are represented by a suite of organic materials with defined turnover times (Krull et al., 2003), may be differentiated: (a) the active or labile pool also named "sensitive" (Zimmermann et al., 2007), consisting of microbial biomass and plant detritus with a turnover time of about 1-2 years, (b) the intermediate or slow pool with a residence time of about 10-100 years, consisting of recalcitrant organic compounds and physically protected organic matter, and (c) the passive pool, consisting of very old material that is physically or biochemically protected, with a turnover time of about 100 to > 1000 years (Figure 1.1).

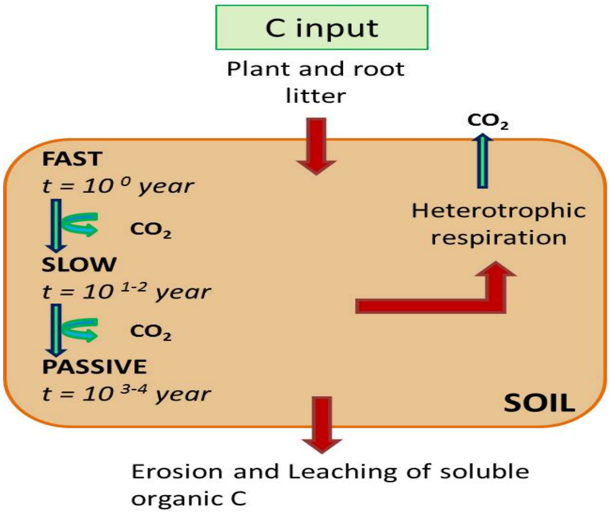


Figure 1.1. Scheme of the soil organic carbon balance, adapted from FAO (2007).

Stabilization of soil organic carbon

At present, there is broad agreement with three main mechanisms of SOC stabilization:

(i) *"Biochemical stabilization"*, also called *"selective preservation"*, *"molecular recalcitrance"*, or *"(bio) chemical alteration"*. This is understood as the stabilization of SOC due to its own chemical composition (molecular recalcitrance). Von Lützow et al. (2006) differentiated primary recalcitrance, as the recalcitrance of plant litter and rhizodeposits, and secondary recalcitrance, including microbial products, recalcitrance of humic polymers, and recalcitrance due to production of charcoal. Jastrow et al. (2007) also considered recalcitrance induced by condensation and polymerization reactions that create new, larger molecules, the soil biotic community being a key factor in these processes (Figure 1.2).

(ii) *"Physical protection"*, also called *"spatial inaccessibility"* or *"low accessibility for biological degradation"*. This mechanism indicates the positive influence of aggregation on the accumulation of OC. The stabilization is caused by the occlusion of OC in aggregates (mainly in free microaggregates and microaggregates within macroaggregates), phyllosilicates, or organic macromolecules, which protect OC from the microbes and enzymes controlling food web interactions and - consequently - microbial turnover (Figure 1.2).

(iii) *"Chemical stabilization"*, also called *"interactions with mineral particles".* This is the result of chemical or physical-chemical binding of SOM by soil minerals. It includes ligand exchange, polyvalent cation bridges, Van der Waals forces, H-bondings, and complexation of metal ions with organic substances (Figure 1.2).

The contribution of microbial activity to aggregate formation, stabilization, and eventually degradation has been extensively reviewed before (Lynch and Bragg, 1985; Oades, 1993; Degens, 1997; Nicolás et al., 2014). However, the response of soil microbial communities to changes of land use or management practices is not well understood (Macdonald et al., 2009, Bastida et al., 2013).

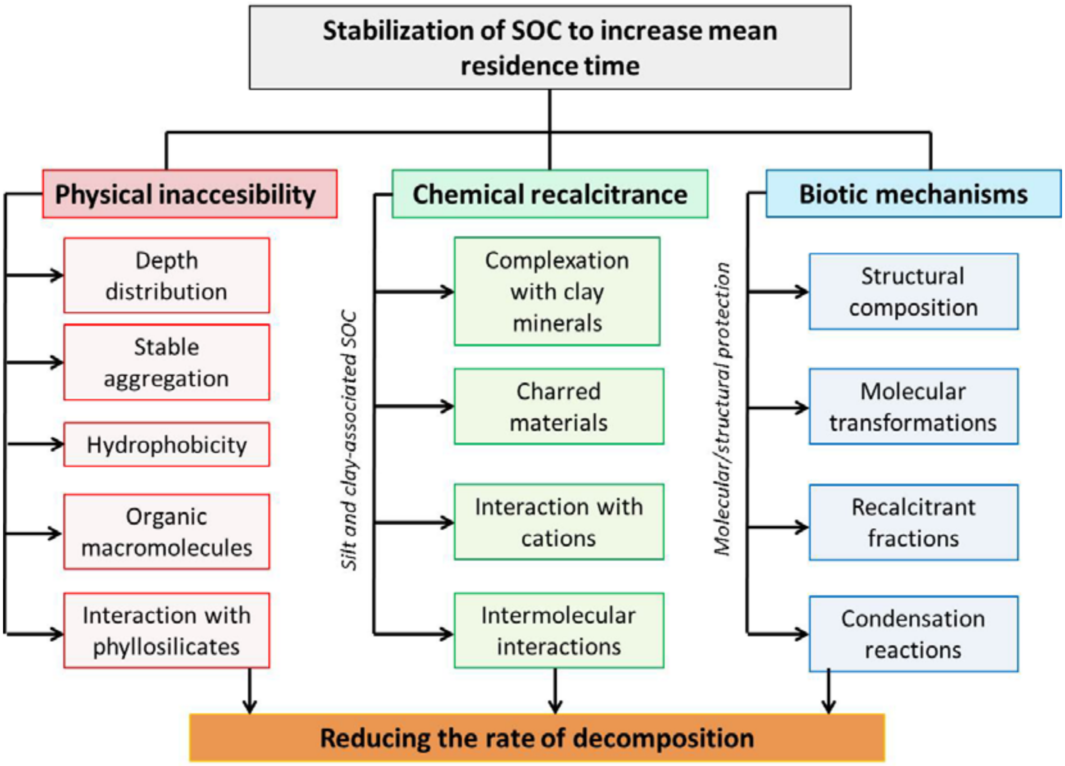


Figure 1.2. Mechanisms of stabilization of soil organic matter (Adapted from Lal et al., 2013).

Therefore, there is still a lack of scientific literature about SOC stabilization mechanisms and the different factors (for example, soil type, land use, lithology, and environmental factors) involved in this stabilization in semiarid areas. Hence, the above three mechanisms will be studied in this thesis and the relative implication of each one, according to land use and management practices, will be discussed. The new knowledge acquired in this thesis could be very useful for the implementation of the best land use and management practices in semiarid areas. Quantitatively, with the aim of climate change mitigation, the potential capacity of these soils may be low, but from a socioeconomic and environmental point of view, the implementation of the best uses and management practices could be key to human survival in these areas because of: a) the trends of global change, b) the economic dependence on the agricultural sector, and c) the current poverty in the dry areas, at the global level.

Chapter 2: ***Initial hypothesis, objectives, and structure of the thesis***

The mechanisms of stabilization of the SOC and its relationship with different abiotic and biotic factors of the soil, in different situations of use and management practices, are still quite unknown. Through the comparative analysis of the distribution of aggregates according to their size, the functional pools of organic matter and the mechanisms of stabilization of the SOC for different land uses and selected management practices, the general objective of this thesis is to fill these gaps in knowledge and provide a valid set of data to be able to recommend the best uses and sustainable management practices for soil conservation and mitigation of climate change in semiarid environments. To this end, the following hypotheses have been subjected to study:

(i) The biotic and abiotic factors controlling the content of SOC may vary according to the type of use and the depth in the soil profile.

(ii) Changes of use and management practices promote changes in the nature of the organic inputs into the soil, which could affect the recalcitrance and stabilization of SOC - resulting in changes in the size and composition of the functional C pools.

(iii) Different land uses and management practices induce changes in the size distribution of aggregates and their stability, which can alter the distribution of pore sizes in the soil and the accessibility of the microorganisms to the mineralization of the organic matter, ultimately affecting the carbon sequestration. The protection of OC in free microaggregates and microaggregates occluded within macroaggregates can protect organic inputs against decomposition and facilitate their permanence and stabilization in the soil. An increase in the OC stored in microaggregates occluded within macroaggregates could be a sensitive and reliable indicator of the soil C sequestration potential.

(iv) A good level of aggregation in the soil, which favours stabilization of OC, would require a proper supply of organic inputs, especially of the more labile pool. Therefore, the effectiveness of the practices intended to increase C sequestration will depend on the quantity and quality of the contributions arising from the type of coverage in each use or management practice.

(v) Changes in the structure of the communities of microorganisms and their activity, induced by changes of use and management practices, can affect the potential for C sequestration of the soil.

In accordance with these hypotheses, and to achieve the overall objective described at the beginning of this chapter, the following more specific, partial targets were established:

1. To assess the stocks of carbon in the soil profile, according to its uses, and determine the factors controlling its variations - to increase our ability to predict the dynamics of the SOC with climate change in semiarid areas.

2. To quantify and characterize the functional organic matter pools (labile, intermediate, and passive) under different uses and management practices, to obtain a better understanding of the residence time of the OC and the processes involved in its storage.

3. To assess the effectiveness of reforestation and agricultural practices of sustainable management, regarding the capacity of the soil for C sequestration.

4. To determine the processes and mechanisms involved in the build-up and stabilization of the SOC, under different conditions of use and management practices.

This thesis has been arranged in the following chapters:

- Chapter 1: Introduction. This describes the current state of knowledge on carbon sequestration in semiarid areas, identifies gaps in this knowledge, and specifies the socio-economic and environmental interest that justifies this study.

- Chapter 2: The initial hypothesis and the objectives of the study are stated.

- Chapter 3: This describes the estimation, at the regional scale, of the distribution of OC contents in the soil profile, depending on the type of use, and discusses the factors that control the variations in these contents. Correlations between these factors and the concentration of OC are established, to improve predictions of the impact of climate change and land use on the organic matter of semiarid soils. (Hypothesis i; Objective 1).

- Chapter 4: Explores the dynamics of soil properties and OC stocks after the reforestation of a degraded shrubland, with two reforestation techniques. (Hypothesis ii; Objectives 2 and 3).

- Chapter 5: The mechanisms of the accumulation and stabilization of carbon in semiarid forest soils are discussed. (Hypotheses iii, iv, and v; Objective 4).

- Chapter 6: In the agricultural experimental area, the impact of the management practices on carbon sequestration and agroecosystem sustainability are studied. (Hypotheses ii, iii, iv, and v; Objectives 2, 3, and 4).

Chapter 3. *SOC Quantification at regional scale: Impact of land use and climate change*

Purpose: The sensitivity of soil organic carbon to global change drivers, according to the depth profile is receiving increasing attention because of its importance in the global C cycle and its potential feedback to climate change. A better knowledge of the vertical distribution of SOC and its controlling factors – the aim of this study - will help scientists predict the consequences of global change.

Materials and methods: The study area was the Murcia Province (S.E. Spain) under semiarid Mediterranean conditions. The database used consists of 312 soil profiles collected in a systematic grid, each 12 km^2 covering a total area of 11004 km^2. Statistical analysis to study the relationships between SOC concentration and control factors in different soil use scenarios was conducted at fixed depths of 0-20 cm, 20-40 cm, 40-60 cm and 60-100 cm.

Results and discussion: SOC concentration in the top 40 cm ranked between 6.1 and 31.5 g kg^{-1}, with significant differences according to land use, soil type and lithology, while below this depth no differences were observed (SOC concentration 2.1-6.8 g kg^{-1}). The ANOVA showed that land use was the most important factor controlling SOC concentration in 0-40 cm depth. Significant differences were found in the relative importance of environmental and textural factors according to land use and soil depth. In forestland precipitation and texture were the main predictors of SOC, while in cropland and shrubland the main predictors were mean annual temperature and lithology. Total SOC stored in the top 1m in the region was about 79 Tg with a low mean density of 7.18 kg Cm^{-3}. The vertical distribution of SOC was shallower in forestland and deeper in cropland. A reduction in rainfall would lead to SOC decrease in forestland and shrubland, and an increase of mean annual temperature would adversely affect SOC in croplands and shrubland. With increasing depth, the relative importance of climatic factors decreases and texture becomes more important in controlling SOC in all land uses.

Conclusions: Due to climate change impacts will be much greater in surface SOC; the strategies for C sequestration should be focused on subsoil sequestration, which was hindered in forestland due to bedrock limitations to soil depth. In these conditions, sequestration in cropland through appropriate management practices is recommended.

Chapter 4. *Effect of afforestation techniques on C sequestration*

Purpose: Climate change is a potential threat to soil organic carbon (SOC) in semiarid ecosystems. Several studies advocated afforestation as an important way to achieve soil C accumulation, but few deal with the mechanisms of C stabilization. The knowledge of these mechanisms is a key aspect in the preservation of SOC in the face of climate change.

Material and methods: In a long-term experiment in southeast Spain, we analyzed the effect on C sequestration and stabilization mechanisms of two *Pinus halepensis* afforestation treatments: (a) terracing (T) and (b) terracing with soil amendment (AT). Twenty years after installing the pine plantations, changes were measured in: a) chemical, physical, and biological soil properties, b) ecosystem C stocks, and c) three functional SOC pools: particulate organic matter (POM), sand and stable aggregates (S+A), and silt plus clay (S+C).

Results and discussion: The results showed that the afforestation treatment had a distinct impact on soil properties. Compared with the adjacent native shrubland, the AT treatment led to improved soil fertility, while the T treatment had a negative impact on soil properties. In turn, AT led to a C gain in the ecosystem of 1.3 kg C m^{-2}, while with T there was a decline of 0.60 kg C m^{-2} over 20 years. This decline was due to the impact of the terracing work. The potential ecosystem C sequestration capacity of the afforestation treatments was 160 and 65 g C m^{-2} y^{-1} in AT and T, respectively. Focusing on sequestration in the mineral soil, the average annual sequestration rate was 28 g C m^{-2} y^{-1} in AT and 17 g C m^{-2} y^{-1} in T. In relation to the functional SOC pools, the C sequestered showed the following distribution: 30% POM, 46% (S+A), and 24% (S+C).

***Conclusions*:** The C sequestration, through afforestation of semiarid areas, can be increased by using suitable afforestation techniques. Site preparation involving large soil disturbance is not recommended. Twenty years after planting, the potential capacity for C sequestration of the afforested ecosystems is far from being saturated and they will continue sequestering C as they reach maturity.

Chapter 5. *Stabilization mechanisms of SOC under semiarid forest soil use*

Purpose*:* Changes in plant cover, after afforestation, induce variations in litter inputs and soil microbial community structure and activity, which may promote the accrual and physical-chemical protection of soil organic carbon (SOC) within soil aggregates. In a long-term experiment (20 years) we study the effects, on soil aggregation and SOC stabilization, of two afforestation techniques.

Material and methods: The two afforestation methods tested were: a) amended terraces with organic refuse (AT), and b) terraces without organic amendment (T). We used the adjacent shrubland (S) as control. Twenty years after stand establishment, aggregate distribution including microaggregates within larger aggregates, functional organic carbon pools, basal respiration and microbial community structure were measured for all the soils.

Results and discussion: The main changes occurred in the top layer (0-5 cm) were: i) both, sensible and passive organic carbon (OC) pools increased in AT compared to S and T, ii) the percentage of microaggregates within macroaggregates (mM) and OC concentration in these mM was higher in AT than in S and T, iii) basal respiration was also higher in AT, and iv) significant changes in fungal community structure, but not in bacterial community, were observed in the afforested soils (AT and T) as regard to shrubland soil.

Conclusions: These results suggest that the increase in OC pools linked to the changes in microbial activity and fungal community structure, after afforestation with AT treatment, promoted the formation of macroaggregates which acted as the nucleus for the formation of OC-enriched microaggregates inside. According with our results, the correlations between microbial activity and percentage of mM could be an indicator for degradation or rehabilitation trend in soil processes.

Chapter 6. *Stabilization mechanisms of SOC in agricultural areas: Effect of management practices*

Purpose: In Mediterranean, semiarid agroecosystems, generally poor in soil organic matter (SOM), sustainable land management (SLM) practices are necessary in order to increase the SOM level. This helps to maintain soil fertility, improving carbon sequestration and mitigating CO_2 emissions to the atmosphere. In a medieum-term experiment (4 years) on rain-fed almond orchard under reduced tillage (RT) as the habitual management practice during 14 years, we studied the effect on soil aggregation and OC accrual and stabilization of sustainable management practices.

Material and methods: Two agricultural management practices were tested: i) reduced tillage with a mix of *Vicia sativa L*. and *Avena sativa L*. as green manure (RTG), and ii) no-tillage (NT). Four aggregate size classes (large- and small-macroaggregates, microaggregates, and the silt+clay), likewise the microaggregates occluded into small-macroaggregates (SMm), were differentiated through wet sieving. The free light fraction (free LF-C), intra-aggregate particulate OM (iPOM-C), and mineral fraction (mineral-C) were differentiated using a density fractionation method.

Results and discussion: Our data showed that the combination of reduced tillage plus green manure (RTG) was the most-efficient SLM practice for SOC sequestration. Furthermore, green manure offseted the effect of tillage on aggregate rupture. The continuous input from green manure and its incorporation by reduced tillage into deeper layers promoted new aggregate formation and activated the subsequent physical-chemical protection of OC in the iPOM-C and mineral-C pools. Both pools together represented more than 42% of the total OC accumulated in the bulk soil.

Conclusions: The total SOC increased by about 14% in the surface layer when compared to RT without OM addition. No tillage favored OC accumulation in the fine iPOM-C occluded within small-macroaggregates and microaggregates at the surface layer and in the coarse iPOM-C at 5-15 cm soil depth, but four years of cessation of tillage was not enough to increase the total OC in the bulk soil.

Chapter 1. *General Introduction*

Soil use and management practices control soil behavior as a sink or source of greenhouse gases

At present, in the terrestrial carbon (C) cycle a negative feedback is occurring, with emission to the atmosphere of 3-6 Gt CO_2 per year through soil organic carbon (SOC) mineralization, with the subsequent acceleration of climatic change. Of the historic loss from the terrestrial C pool of 136 ± 55 Pg, 78 ± 12 Pg is estimated to have arisen from depletion of SOC (Lal et al., 2003). This part of the C cycle could be managed by improving soil structure through adequate and sustainable land uses, such as restoration of marginal land and sustainable land management practices (SLM) like conservation tillage. At the same time, a stable soil structure stores and protects soil organic matter (SOM) from rapid decomposition (Blanco-Canqui and Lal, 2004), leading to enhanced nutrient recycling, water availability and biodiversity while reducing water and wind erosion and improving surface and ground water quality (Bronick and Lal, 2005).

Therefore, there are many factors and processes, linked to land use and management, which determine the direction and rate of change in SOC content when vegetation and soil management practices are changed. So, the loss of SOC by conversion of natural vegetation to cultivated use is well known (Post and Kwon, 2000). Various land uses result in very rapid declines in SOC (Schlesinger, 1985; Post and Mann, 1990; Davidson and Ackerman, 1993). In contrast, the conversion of cropland into areas with perennial vegetation leads to an increase in SOC (Geesing et al., 2000; Nagarajan and Sundaramoorthy, 2000). This type of conversion has been traditionally used in ecosystems restoration projects, such as afforestation, which may be an important tool to increase C sequestration (Nosetto et al., 2006; Shwendenmann et al., 2006; Wiesmeier et al., 2009; Cao et al., 2010; Langanière et al., 2010; O' Brien et al., 2013), even in semiarid areas (Fernández-Ondoño et al., 2010; Garcia-Franco et al., 2014).

Likewise, agricultural practices with sustainable management - such as conservation tillage , incorporation of leguminous residues (Lal et al., 1997; Del Grosso et al., 2002), organic amendments, soil fertility management, and nutrient cycling (Bronick and Lal, 2005) - play a major role in greenhouse gas emissions and offer many opportunities for mitigation (Lal, 2004a). The potential of agriculture to mitigate climatic change arises from soil C sequestration, which has strong synergies with sustainable, conservation, and/or organic agriculture practices. In addition, the use of SLM practices is being widely advocated by development organizations (World Bank and FAO) and bilateral programs (USAID) (Schwilch et al., 2011). The environmental implications of SLM practices have been reviewed for the USA (Soil and Water Conservation Society, 1995; Uri et al., 1998; Uri, 2001) and for Canada (McLaughlin and Mineau, 1995). However, there is no universally applicable list of best management practices to enhance SOC; practices need to be evaluated for individual agricultural systems and settings (IPCC, 2007). As the same time, in Europe, and in particular in semiarid areas under rainfed crops, SLM is a relatively new concept that, if broadly implemented, may have substantial environmental benefits (Almagro et al., 2013). However, several recent studies have demonstrated that increased application of SLM, such as no tillage or minimum tillage, can play a great role in the mitigation of CO_2 gas emission to the atmosphere in semiarid Mediterranean areas (Álvaro-Fuentes et al., 2009, 2012a; Plaza-Bonilla et al., 2013, 2014; López-Garrido et al., 2014). For the establishment of sustainable management of the carbon budget, while maintaining or even improving major soil functions, understanding of the mechanisms of SOC stabilization and the factors regulating them is needed (Kyoto Protocol on Climate Change, 1997).

Soil organic matter characterization

Classical descriptions of SOM have normally combined chemical extractions with the identification of specific chemical compounds, but this approach has contributed little to a functional understanding of the dynamic and the turnover time of SOC (Collins et al., 2000) and does not conform to the concept of SOM stabilization as the protection from mineralization (von Lützow et al., 2006). As an alternative, researchers have tended to adopt a model where organic carbon is located in more or less discrete functional “pools” in the soil (Jones and Donnelly, 2004). However, there is at present little agreement on the precise definition of most of these pools and they can mean different things to different researchers (Jenkinson et al., 1992; Smith et al., 2002). In a general context, three pools, which vary in their inherent rate of decomposition and are represented by a suite of organic materials with defined turnover times (Krull et al., 2003), may be differentiated: (a) the active or labile pool also named "sensitive" (Zimmermann et al., 2007), consisting of microbial biomass and plant detritus with a turnover time of about 1-

2 years, (b) the intermediate or slow pool with a residence time of about 10-100 years, consisting of recalcitrant organic compounds and physically protected organic matter, and (c) the passive pool, consisting of very old material that is physically or biochemically protected, with a turnover time of about 100 to > 1000 years (Figure 1.1).

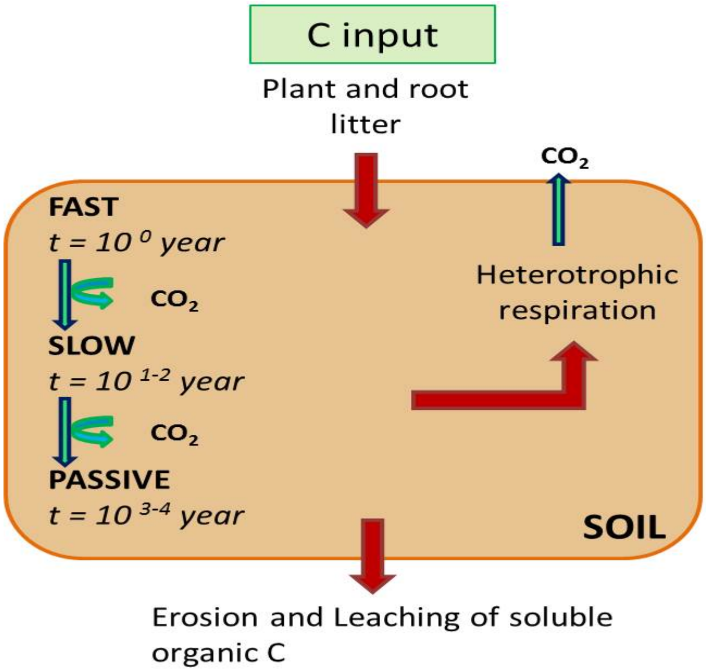


Figure 1.1. Scheme of the soil organic carbon balance, adapted from FAO (2007).

Little is known of the distribution of SOC in the different pools or of the dynamics of these pools with changes in land use and management practices in semiarid environments, although several authors reported that labile SOC fractions were the most sensitive and consistent indicators for assessment of the impact of different soil management regimes on soil quality and of the enhancement of SOC and biological status by long-term conservation tillage (Álvaro-Fuentes et al., 2008; Melero et al., 2009; Plaza-Bonilla et al., 2014). The above model will be adopted in this thesis, where labile, intermediate, and passive pools will be quantified for every use and agricultural management practice.

Stabilization of soil organic carbon

In relation to SOC stabilization mechanisms, there is no general agreement. Sollins et al. (1996) identified three major mechanisms of SOC protection from decomposition:

the molecular character of the SOC (recalcitrance), low accessibility for biological degradation, and interactions with mineral particles.

Baldock et al. (2004) suggested a scheme based solely on biological stabilization. Krull et al. (2003) reviewed the mechanisms and processes of SOM stabilization and concluded that, in active surface soils, adsorption and aggregation can retard degradation processes but "*molecular recalcitrance*" appears to be the only mechanism by which SOM can be stabilized for longer periods of time. Mayer (2004) suggested that all hypotheses fit into two categories regarding the stabilization of SOM, namely "*organic recalcitrance*" and "*biotic exclusion*".

Jastrow et al. (2007) focused the stabilization on the mechanisms controlling soil C turnover. Since turnover is primarily a biological process, they first considered the overall role of the soil biotic community and then reviewed how (bio) chemical alteration and physicochemical protection mechanisms control the stabilization of SOC. A more detailed review of SOM stabilization mechanisms in soils of the temperate zone can be found in von Lützow et al. (2006). However, at present, it seems that there is broad agreement with three main mechanisms of SOC stabilization:

(i) *"Biochemical stabilization"*, also called *"selective preservation", "molecular recalcitrance"*, or *"(bio) chemical alteration".* This is understood as the stabilization of SOC due to its own chemical composition (molecular recalcitrance). Von Lützow et al. (2006) differentiated primary recalcitrance, as the recalcitrance of plant litter and rhizodeposits, and secondary recalcitrance, including microbial products, recalcitrance of humic polymers, and recalcitrance due to production of charcoal. Jastrow et al. (2007) also considered recalcitrance induced by condensation and polymerization reactions that create new, larger molecules, the soil biotic community being a key factor in these processes (Figure 1.2).

(ii) *"Physical protection"*, also called *"spatial inaccessibility"* or *"low accessibility for biological degradation"*. This mechanism indicates the positive influence of aggregation on the accumulation of OC. The stabilization is caused by the occlusion of OC in aggregates (mainly in free microaggregates and microaggregates within macroaggregates), phyllosilicates, or organic macromolecules, which protect OC from the microbes and enzymes controlling food web interactions and - consequently - microbial turnover (Figure 1.2).

(iii) *"Chemical stabilization"*, also called *"interactions with mineral particles".* This is the result of chemical or physical-chemical binding of SOM by soil minerals. It includes ligand exchange, polyvalent cation bridges, Van der Waals forces, H-bondings, and complexation of metal ions with organic substances (Figure 1.2).

The contribution of microbial activity to aggregate formation, stabilization, and eventually degradation has been extensively reviewed before (Lynch and Bragg, 1985; Oades, 1993; Degens, 1997; Nicolás et al., 2014). Fungal activity results in the mechanical union of soil particles by fungal hyphae and the exudation of metabolites that promote the coalescence of primary particles (Helfrich et al., 2008; De Gryze et al., 2005). But, bacteria also can have profound influences on aggregation, especially at the microscale (Six et al., 2004). However, the response of soil microbial communities to changes of land use or management practices is not well understood (Macdonald et al., 2009, Bastida et al., 2013).

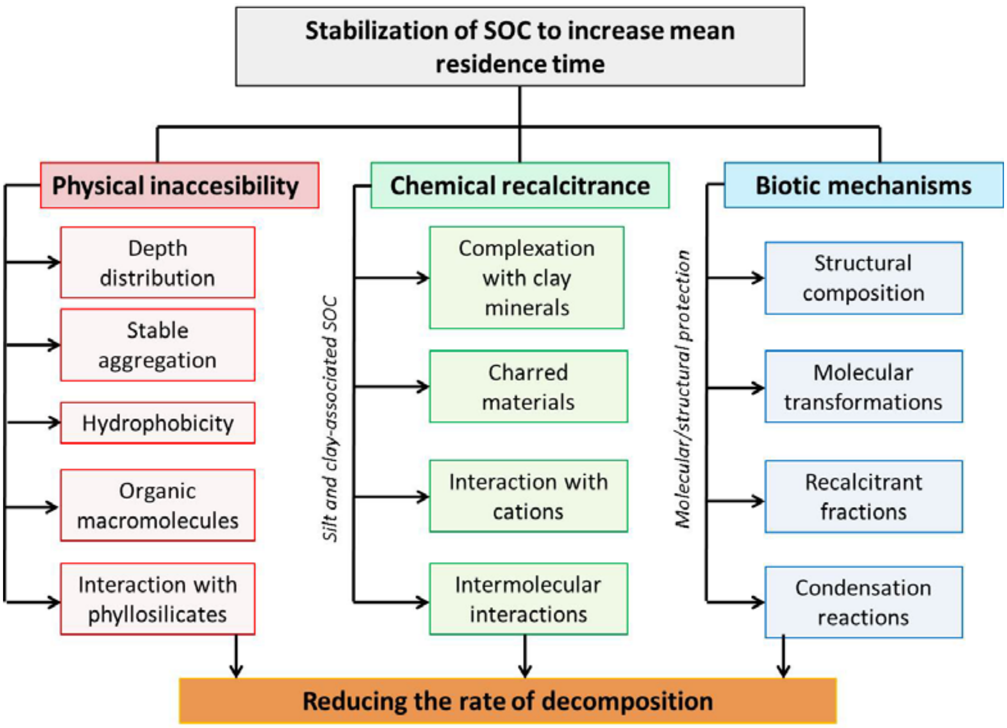


Figure 1.2. Mechanisms of stabilization of soil organic matter (Adapted from Lal et al., 2013).

Therefore, there is still a lack of scientific literature about SOC stabilization mechanisms and the different factors (for example, soil type, land use, lithology, and environmental factors) involved in this stabilization in semiarid areas. Hence, the above three mechanisms will be studied in this thesis and the relative implication of each one, according to land use and management practices, will be discussed. The new knowledge acquired in this thesis could be very useful for the implementation of the best land use and management practices in semiarid areas. Quantitatively, with the aim of climate change mitigation, the potential capacity of these soils may be low, but from a socioeconomic and environmental point of view, the implementation of the best uses and management practices could be key to human survival in these areas because of: a) the trends of global change, b) the economic dependence on the agricultural sector, and c) the current poverty in the dry areas, at the global level.

Chapter 2. *Hypothesis, objectives and structure of the thesis*

The mechanisms of stabilization of the SOC and its relationship with different abiotic and biotic factors of the soil, in different situations of use and management practices, are still quite unknown. Through the comparative analysis of the distribution of aggregates according to their size, the functional pools of organic matter and the mechanisms of stabilization of the SOC for different land uses and selected management practices, the general objective of this thesis is to fill these gaps in knowledge and provide a valid set of data to be able to recommend the best uses and sustainable management practices for soil conservation and mitigation of climate change in semiarid environments. To this end, the following hypotheses have been subjected to study:

(i) The biotic and abiotic factors controlling the content of SOC may vary according to the type of use and the depth in the soil profile.

(ii) Changes of use and management practices promote changes in the nature of the organic inputs into the soil, which could affect the recalcitrance and stabilization of SOC - resulting in changes in the size and composition of the functional C pools.

(iii) Different land uses and management practices induce changes in the size distribution of aggregates and their stability, which can alter the distribution of pore sizes in the soil and the accessibility of the microorganisms to the mineralization of the organic matter, ultimately affecting the carbon sequestration. The protection of OC in free microaggregates and microaggregates occluded within macroaggregates can protect organic inputs against decomposition and facilitate their permanence and stabilization in the soil. An increase in the OC stored in microaggregates occluded within macroaggregates could be a sensitive and reliable indicator of the soil C sequestration potential.

(iv) A good level of aggregation in the soil, which favours stabilization of OC, would require a proper supply of organic inputs, especially of the more labile pool. Therefore, the effectiveness of the practices intended to increase C sequestration will depend on the quantity and quality of the contributions arising from the type of coverage in each use or management practice.

(v) Changes in the structure of the communities of microorganisms and their activity, induced by changes of use and management practices, can affect the potential for C sequestration of the soil.

In accordance with these hypotheses, and to achieve the overall objective described at the beginning of this chapter, the following more specific, partial targets were established:

1. To assess the stocks of carbon in the soil profile, according to its uses, and determine the factors controlling its variations - to increase our ability to predict the dynamics of the SOC with climate change in semiarid areas.

2. To quantify and characterize the functional organic matter pools (labile, intermediate, and passive) under different uses and management practices, to obtain a better understanding of the residence time of the OC and the processes involved in its storage.

3. To assess the effectiveness of reforestation and agricultural practices of sustainable management, regarding the capacity of the soil for C sequestration.

4. To determine the processes and mechanisms involved in the build-up and stabilization of the SOC, under different conditions of use and management practices.

This thesis has been arranged in the following chapters:

- Chapter 1: Introduction. This describes the current state of knowledge on carbon sequestration in semiarid areas, identifies gaps in this knowledge, and specifies the socio-economic and environmental interest that justifies this study.

- Chapter 2: The initial hypothesis and the objectives of the study are stated.

- Chapter 3: This describes the estimation, at the regional scale, of the distribution of OC contents in the soil profile, depending on the type of use, and discusses the factors that control the variations in these contents.

Correlations between these factors and the concentration of OC are established, to improve predictions of the impact of climate change and land use on the organic matter of semiarid soils. (Hypothesis i; Objective 1).

- Chapter 4: Explores the dynamics of soil properties and OC stocks after the reforestation of a degraded shrubland, with two reforestation techniques. (Hypothesis ii; Objectives 2 and 3).

- Chapter 5: The mechanisms of the accumulation and stabilization of carbon in semiarid forest soils are discussed. (Hypotheses iii, iv, and v; Objective 4).

Chapter 6: In the agricultural experimental area, the impact of the management practices on carbon sequestration and agroecosystem sustainability are studied. (Hypotheses ii, iii, iv, and v; Objectives 2, 3, and 4).

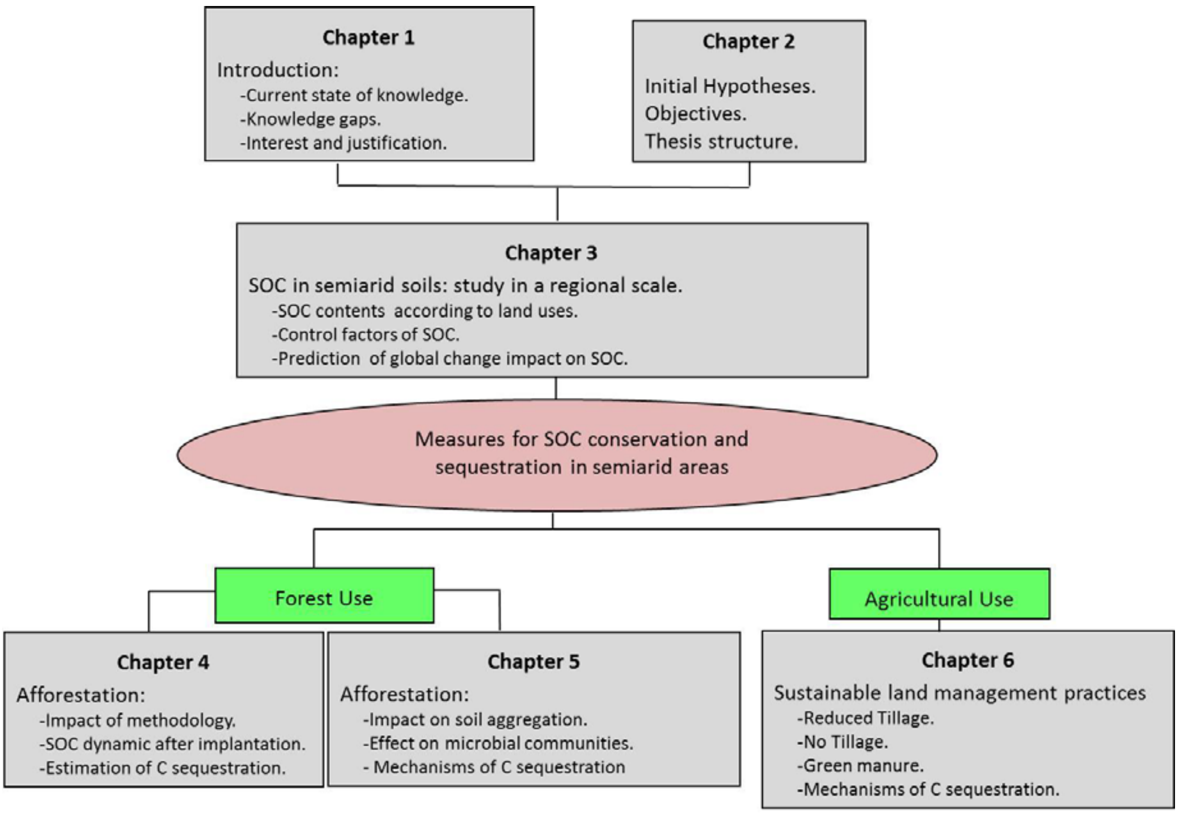


Figure 2.1. Thesis structure.

Chapter 3. ***SOC quantification at regional scale: Impact of land use and climate change***

3.1. Introduction

Soil organic carbon is an important component of the global carbon cycle since it represents twice the amount of carbon found in the atmosphere and about 75% of the total terrestrial organic carbon pool (Prentice, 2001). The sensitivity of decomposition of soil organic carbon (SOC) to global change drivers is receiving increasing attention, because of its importance in the global C cycle and potential feedback to climate change (Davidson and Janssens, 2006; von Lützow and Kögel-Knabner, 2009). However, there is little agreement concerning the effects of global warming on SOC stocks - a major source of uncertainty in future climate change predictions (Powlson, 2005; Schmidt et al., 2011).

The storage of organic carbon in the soil depends on the balance between gains and losses of C. Biotic characteristics such as biomass production and microbial abundance, environmental variables (e.g. mean annual precipitation and temperature), soil characteristics including texture and lithology and anthropogenic controls, like land use and management, influence the processes of SOC storage or losses. A clear description of the distribution and changes of SOC and its factors of control will help predict the consequences of climate change.

The strong relationship between environmental conditions and the accrual of organic carbon in the soil has been widely proven (Jenny, 1941). However, there are important gaps in the knowledge of the role played by each one of the environmental factors (Jobbágy and Jackson, 2000; Garten and Hanson, 2006). There is a need for a better understanding of the link between SOC and environment. In addition, most of the studies related with the controlling factors of SOC have been restricted to the surface soil horizon and only a few include the total soil profile. However, more than 50% of the total SOC is stored below 20 cm depth (Batjes, 1996). Jobbágy and Jackson (2000) reported that in temperate grasslands, 59% of SOC is located between 20 and 100 cm depth. Consequently, minor shifts in these subsoil stocks of organic C will have considerable impact on the entire C balance (Don et al., 2007).

Carbon turnover models tend to assume that the mechanisms controlling C dynamics are the same in both topsoil and subsoil (Jenkinson and Coleman, 2008). However, there is sufficient evidence to suggest that the factors controlling C dynamics in topsoil and subsoil may be different and, yet, this possibility has not really been considered or investigated (Salomé et al., 2010). In this regard, some research has shown that subsurface SOC is significantly more sensitive to increases in temperature or nutrient availability than surface SOC (Fierer et al., 2003), while for others upper SOC is more sensitive to climate and texture than deeper SOC (Liu et al., 2011; Jobbágy and Jackson, 2000).

On the other hand, soil C sequestration has been consistently defined as an important component of a comprehensive greenhouse management strategy for climatic change mitigation (Pacala and Socolow, 2004). However, the potential of soil to sequester C and thus to mitigate climate change remains controversial (Smith, 2005). Several studies have proven that it is feasible to restore the organic carbon lost in degraded soils. For example, 20 years after of a single addition of organic compost, Albaladejo et al. (2008) reported an increase in SOC stock of 9.5 Mg ha^{-1} in the upper 20 cm of a semiarid degraded soil included in the area covered by this study. To increase our knowledge of the soil´s potential for climate change mitigation it is the utmost importance to investigate the impact of the key factors controlling the levels of organic matter in soils, in order to identify optimal strategies for land management (Chaplot et al., 2010).

Despite several studies on global SOC inventories, quantitative data on the relationship between SOC concentration and the controlling factors do not exist at a large scale (Lal, 2004b). The paucity of data on soil carbon distribution in the profile in different landscapes has been identified as one of the major knowledge gaps in soil science (Lal et al., 1998). This is especially significant in the very fragile ecosystems of semiarid regions (Hoffmann et al., 2012).

The aims of this study were: *(1)* to establish the vertical distribution of SOC in three different land uses with contrasting lithology and soil type throughout the Murcia Province, *(2)* to determine the main factors controlling variation in SOC concentration at different depths and for different land uses, and *(3)* to increase our ability to predict the impact of global change drivers on SOC concentration in semiarid areas.

3.2. Materials and methods

3.2.1. Study area

The study area was located in Murcia Province, southeast Spain (38° 45´ - 37° 23´ North and 0° 41' - 2° 21' West). The climate is semiarid Mediterranean, where maximum temperatures coincide with minimum rainfall levels. The mean annual temperature is 17°C, the potential evapotranspiration about 900 mm, and mean annual rainfall is 330 mm. The lithological substrate is composed mainly of limestone materials. Climax vegetation of the area has been mainly attributed either to the mesomediterranean (*Quercus rotundifolia, Quercus coccifera* and *Pinus halepensis* as dominant species) or thermomediterranean thermotypes of the Murcia-Almeriense Province (*Pistacea lentiscus* and *Rhamnus lycioides* as dominant species).

3.2.2. Data source

The present study was based on the data from LUCDEME (Combating Desertification in the Mediterranean Area) project. This project started in Spain in 1981 on the initiative of UN Conference on Desertification of Nairobi in 1977, and is in force at present. 312 soil profiles were dug between 1986 and 1998 at the nodes of a 12x12 km^2 grid of an 11004 km^2 area of the Murcia Province of Spain (Figure 2.1). The profile were described thoroughly and sampled at fixed depth intervals from 0-20, 20-40, 40-60 to 60-100 cm, resulting in 913 samples. This database seeks to characterize the diversity of soils and land uses and environments in the study area.

Each profile provided information on soil organic carbon concentration (SOCc, grams per kilogram), land-use type, soil type, altitude, lithology, soil texture and coarse particle content (>2 mm). For this study, the land uses were grouped into three classes: forestland, shrubland and cropland. An area was considered forestland when trees were present and no distinctions were made as regards the density. Shrubland use includes typical Mediterranean matorral (*Rosmarinus officinalis, Thymus sp., Stipa tenacissima*) and abandoned croplands recovered by matorral, with a vegetal cover ranging between 50% and 70%. Most of the cropland is dedicated to irrigated crops (cereals, fruit trees and citrus) and a small proportion is not irrigated (cereals and almonds). Of all the soil profiles analysed, 46.7% represented croplands, 39% forestlands and 14.3% shrublands.

The samples were representative of the most characteristic soil and lithology types in the region. The soil types were: 39.1% of the profiles were Calcisols, 20.2% Regosols, 14.5% Fluvisols and 26.2% Cambisols, Leptosols, Kastanozems and Solonchaks. The distribution by lithology was: 60% of the profiles were developed from Quaternary sediments, 15% from Alluvial sediments, 16% from Marls and 9% from Metamorphic sediments. As regards the relation between soil type and land use, Calcisols, Cambisols and Regosols were not associated with any particular use and can be found under all the land uses; Kastannozems and Leptosols only appeared in forestland and shurbland; Fluvisols was predominantly associated with cropland and Solonchacks with shrubland.

Mean annual precipitation and mean annual temperature (*T*) at the sampling sites was estimated from available monthly climate datasets (73 stations) for the period 1966-2006. Based on the high negative correlation between altitude and temperature (r = -0.89), values of *T* were corrected for each sampling point through Kriging with External Drift with altitude as drift variable using *Rs* gstat library.

The altitude of the sampling points ranged between 0 and 1700 m, with an average value for forestlands that was double than of shrublands and croplands. The mean temperature *(T)* of the sampling points ranged between 11°C and 18°C being higher in shrublands and croplands (16.4) than in forestlands (15.3) reflecting the higher altitude in the last. The average precipitation of the sampling points varied between 200 mm and 550 mm, the highest mean values being for forestlands. Temperatures higher than 17°C were observed, mostly in areas located at altitudes lower than 700 m and temperatures lower than 14°C applied mainly to in areas located at altitudes greater than 700 m altitude.

3.2.3. Soil organic carbon density and stocks estimation

The soil organic carbon density (SOCD kg m^{-2}) in each layer interval was determined by the product of SOC concentration, thickness of the layer interval and the bulk density in each layer of the soil profile. SOCD was also corrected for the coarse particle content of each soil layer.

$$\textbf{SOCD} = \sum_{n}^{i=1} SOCci \; x \; BDi \; x \; thi \; x \; (1 - \frac{CPi}{100}) \qquad (3.1)$$

where *i* represents each sampled depth of the soil profile, *SOCc* is the organic carbon concentration (percent) of the fine earth (< 2 mm), *BD* is the soil bulk density depth (grams per cubic centimetre), *thi* is the thickness (meter) of each sampled depth, and *CP* is the coarse-particle content (volume percentage of the fraction > 2mm).

Given that the LUCDEME data base does not include soil bulk density data, this parameter was estimated from a pedotransfer function (PTF). To select the PTF that best fits in the study area, different PTFs were tested, using 118 analytical data from the other soil studies in the region. Two of the tested PTFs were internationally referenced (Tomasella and Hodnett, 1998 and Bernoux et al., 1998), and the other, Barahona and Santos Francés (1981) was developed from data of soil profiles representative of Southeastern Spain, where our study area is located. The tests showed the following Pearson correlation coefficient: Barahona and Santos Francés (r=0.673, p=0.0001); Tomasella and Hodnett, 1998 (r=0.309, p=0.001); and Bernoux et al. 1998 (r=0.007, ns). From these results the following equation of Barahona and Santos Francés was selected:

$$\textbf{BD (g cm}^{-3}\textbf{) =} 1.5456 + (\%sand \times 0.0015) - (\%clay \times 0.0022) - (\%OC \times 0.1219) \qquad (3.2)$$

The authors point to an r^2 of 0.45 for this equation, which allows the bulk density to be determined with a standard error of 0.136 and a 95% confidence interval ± 0.267. The equation was developed from soils of S.E. Spain, as indicated above.

SOC stocks according to soil type were obtained from the product of the SOCD of each layer and the corresponding area covering each soil type. The area of each soil type was taken from the Soil Map 1:100,000 of the LUCDEME Project (Faz Cano, 2003). The total SOC stock in the first meter of the soil profile, for the study area, was obtained by summing the stocks for each soil type.

SOC stocks according to land use were obtained by the product of the SOCD of each layer and the total area covering each land use. In forestland and shrubland the stocks for layers below 40 cm were corrected by the percentage of Leptosols (soils with bedrock limitation to soil depth). The percentage of Leptosols, determined from the LUCDEME Soil Map (Faz Cano, 2003) were 70% in forestland and 30% in shrubland.

3.2.4. Statistical analysis

Prior to analysis, data were examined for normality by the Kolmogorov–Smirnov test and homogeneity of variances by the Levené test. Data that were not distributed normally were ln- transformed (precipitation, temperature and SOC concentration).

The differences in SOC concentration between land uses, soil types and lithologies, were examined by ANOVA using Tukey´s test for multiple pairwise comparisons. The ANOVAs were conducted separately for different soil depths (0-20, 20-40, 40-60, 60-100 cm). The relationships between SOC concentration and quantitative control factors (precipitation, temperature, altitude, clay and clay plus fine silt) were examined through correlation analysis. In addition, stepwise multiple regressions analysis with backward and forward selection techniques was used to identify the main predictor variables (Ramsey and Schafer, 1997). The entered variables were temperature, precipitation, clay, fine silt and the categorical variable lithology as dummy variable, indicating for each observation the presence (1) or absence (0) of a given parent material. We used multiple linear regressions to test whether changes in the main predictor variables had a positive, negative or neutral effect on SOC at each layer of the soil profile. All data were analyzed using SPSS 13.0 software (SPSS Inc., Chicago. Illinois). Statistical significance was set at $p < 0.05$.

3.3. Results

3.3.1. Distribution of organic carbon concentration in the soil profiles

3.3.1.1. SOC concentration according to soil type and lithology

Significant differences in SOCc were found between the soil types of the uppermost layers (0-20 cm) (Table 3.1). Kastanozems (KS) and Leptosols (LP) 31.5±18.6 and 29.2±18.0 g kg^{-1}, respectively, showed the highest SOC concentrations followed by Cambisols (CM) 23.5±20.3 g kg^{-1}. Lower SOC concentrations occurred in Calcisols (CL), Solonchaks (SC), Regosols (RG) and Fluvisols (FL): 12.2±7.4, 11.3±5.7, 10.8±9.1 and 10.1±6.6 g kg^{-1}, respectively. Between 20 and 40 cm, KS, LP and CM were grouped together, and below this depth, less pronounced differences were obtained between soil types. FL showed the highest average SOCc in the deepest layer (60-100 cm), although, given the high value of the standard deviation, no significant differences with CL, SC and RG were observed. The coefficients of variation for SOCc, according to soil type, were very high (50-114%). Likewise, Batjes (1996) reported an average coefficient of variation of 79% for SOCc in profiles grouped according to soil taxonomy orders. This high variability implies that other factors, in addition to soil type, are important for SOCc, and that the grouping of SOCc data into large aggregated units may mask the influence of other control factors (Jobbágy and Jackson, 2000).

For all soil types, SOCc decreased significantly with depth. This vertical distribution of SOCc was fitted to a power function with R^2 values ranging between 0.19 and 0.46, depending on soil type, with $p<0.001$ (Figure 3.1). Different patterns in the vertical SOCc distribution were observed among the soil types, the slope of the curve being higher in KS and CM than in the rest of the soils. The specific soil formation processes of each soil type were reflected in these SOCc distributions through the soil profiles, due to the genetic nature of soil classification system used. The most contrasting behaviour was observed between FL and KS. FL displayed the lowest SOCc at the surface and the highest in depth, while KS showed the highest SOCc at surface and the lowest at 100 cm. This can be due to differences in the soil formation processes. KS was developed from limestone under a dense vegetation cover typical of Mediterranean forest and shrubland. Due to the shallow root distribution of this vegetation type the organic inputs into the soil occur mainly in the surface horizons decreasing sharply with depth. However, FL was formed from alluvial stratified sediments and is the result of different buried soils. The stratification in FL soils is evident from the irregular decrease in the SOC content with depth (FAO, 2006). Similar vertical distribution patterns of SOC to this study were found for CM, RG, and FL in a study of the major soil groups of Brazil (Batjes, 2005). The soils developed from quaternary sediments showed the highest SOCc in the top 20 cm, although no significant differences were found with the metamorphic sediments because of the high standard deviation of the former (Table 3.1).

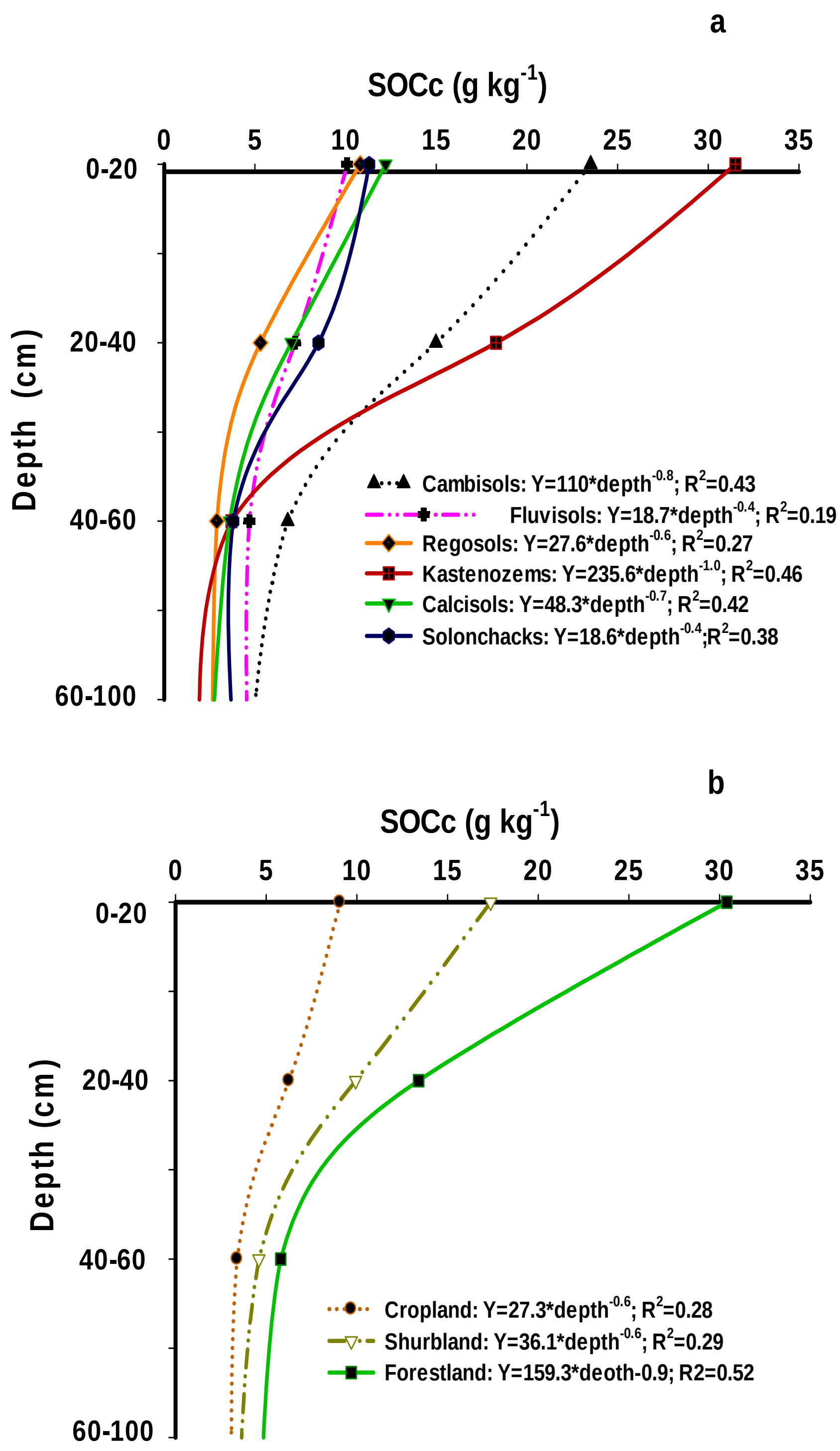


Figure 3.1. Vertical distribution of SOC concentration (g kg^{-1}) in the soil profile of different soil types (a) and land uses (b). Note that the lines represent the connection between plots of the data, not the regression lines.

Table 3.1. Mean, standard deviation (SD), and coefficient of variation (CV) of SOCc (g Kg^{-1}) grouped by soil type, lithology and land use type at different depths.

	Depth (cm)							
	0-20		20-40		40-60		60-100	
	Mean ± SD	CV	Mean ± SD	CV	Mean ± SD	CV	Mean ± SD	CV
Soil type								
Calcisol	12.2 ± 7.4a,b	60	7.0 ± 4.4ab	63	3.6 ± 2.2ab	61	2.2 ± 1.6a	72
Regosol	10.8 ± 9.1a	84	5.3 ± 4.4a	83	2.9 ± 2.0a	69	2.6 ± 1.7ab	65
Cambisol	23.5 ± 20.3bc	86	14.9 ± 10.3b	70	6.8 ± 4.4b	65	4.1 ± 3.7ab	90
Fluvisol	10.1 ± 6.6a,b	66	7.1 ± 4.5ab	63	4.6 ± 3.2ab	69	4.6 ± 3.8b	83
Kastanozem	31.5 ± 18.6c	58	18.3 ± 14.7b	80	3.7 ± 2.6ab	70	2.1 ± 2.4a	114
Leptosol	29.2 ± 18.0c	62	10.5 ± 6.3b	60	-		-	
Solonchak	11.3 ± 5.7a,b	50	8.5 ± 7.8 ab	92	3.8 ± 2.2ab	58	4.3 ± 3.4ab	79
Lithology								
Quaternary sediments	19.1 ± 16.5a	86	10.6 ± 9.5a	89	4.2 ± 4.2a	100	2.2 ± 2a	110
Alluvial sediments	9.8 ± 6.0b	61	6.7 ± 4.8a	72	4.5 ± 3.3a	73	2.5 ± 2a	80
Marls	9.3 ± 7.3b	78	6.1 ± 5.2b	85	3.0 ± 2.2b	73	4.1 ± 4a	97
Metamorphic sediments	12.3 ± 5.9a	50	6.6 ± 5.4a	81	4.3 ± 1.8a	41	4.7 ± 4a	85
Land use								
Forestland	30.4 ± 19.5c	64	13.4 ± 11.2c	83	5.8 ± 4.6a	79	4.4 ± 3.7a	84
Shrubland	17.4 ± 13.9b	80	9.9 ± 9.1b	101	4.6 ± 4.4a	96	3.2 ± 3.2a	100
Cropland	9.1 ± 4.9a	54	6.2 ± 4.0a	64	3.4 ± 2.3a	68	3.1 ± 2.8a	90

Different letters means significant differences in each depth between soil types, lithology and land use type at $p<0.05$ according to Tukey´s test.

The soils from marls and alluvial sediments displayed the lowest SOCc at this depth. However, in the 60-100 cm depth, the soils from alluvial sediments showed the highest SOCc. The higher values in the subsoil (60-100 cm) for alluvial sediments must be a consequence of the stratification, as indicated above for Fluvisols.

3.3.1.2. SOC concentration according to land use type

Significant differences in SOCc were found in the top 40 cm of the soil according to land use (Table 3.1). Forest soils showed significantly higher values than shrublands and croplands in the upper (0-20 and 20-40 cm) layers. SOCc in forestlands was more than three times higher in the top 20 cm and more the double in the 20-40 cm layer of the soil than in croplands. With regards to differences between shrubland and cropland use, the results indicated that the SOCc was significantly higher in the top 40 cm of the shrubland soils. These results showed that the conversion of forestland or shrubland to cultivation implies a reduction in SOCc. This reduction was higher in the upper soil horizons and decreases in intensity with soil depth. Below 40 cm depth, no differences were found in SOC concentration in relation to land use.

The SOC concentration coefficient of variation (CV) was always higher than 50% for all land use types and depths, ranging between 54% and 80% near surface and between 84% and 100% at 60-100cm depth, although, in general, cropland showed lower CV than the other land uses at all depths (see Table 3.1). As indicated above, this high variability suggests that, being land use a very important factor, there are other factors not tested in this study, (such as the percentage of vegetation cover, microclimate conditions, or agricultural practices), which are also important for the SOCc.

For all the land uses the SOCc decreased significantly with depth. The vertical distribution of SOCc was fitted to a power function with R^2 values between 0.28 and 0.52 depending on land uses and p<0.001 (see Figure. 3.2b). The relative distribution of SOCc with depth was shallower in forestland than in the other uses. This would reflect the different allocation of roots through the soil profile according to vegetation types (Jobbágy and Jackson 2000) or, in our case, land use.

3.3.2. Correlations between SOC concentration and environmental and textural variables

The ANOVA clearly showed that land use is a key factor in the SOC concentration of these semiarid soils. So, for a better understanding of the influence of the environmental and textural variables as control factors of SOCc, correlation analyses were performed separately for each land use (Table 3.2).

Different relationships were observed according to land use. In forestland, precipitation showed a strong correlation with SOCc at all depths (*r* values between 0.51 and 0.62 at $p< 0.001$) and clay was positively correlated ($r=0.30$, $p<0.10$) at 20-40 cm and ($r=0.62$, $p<0.01$) at 40-60 cm depth. However, temperature and altitude were no correlated at any depth with SOCc in this land use (see Table 3.2). In shrubland, the environmental variables altitude and precipitation were correlated positively and temperature negatively with SOC between 0-60 cm, while below 60 cm depth only fine silt showed a significant correlation with SOCc ($r=0.57$, $p<0.01$).

Table 3.2. Pearson correlations coefficients between SOCc and environmental variables at different depths in each land use at $p<0.05$.

	Environmental variables				
SOC content by depth (cm)	**Clay**	**Fine silt**	**Altitude**	**Temperature**	**Precipitation**
Forestland					
0-20	ns	Ns	ns	Ns	0.53
20-40	**0.30**	Ns	ns	Ns	0.51
40-60	0.62	Ns	ns	Ns	0.54
60-100	ns	Ns	ns	Ns	0.62
Shrubland					
0-20	ns	Ns	0.53	-0.54	0.53
20-40	ns	Ns	0.55	-0.58	0.51
40-60	ns	Ns	0.43	-0.45	0.27
60-100	ns	0.57	ns	Ns	ns
Cropland					
0-20	ns	Ns	0.22	-0.20	ns
20-40	ns	Ns	0.21	-0.27	0.19
40-60	0.24	Ns	ns	**-0.18**	**0.19**
60-100	ns	Ns	0.26	-0.29	ns

in bold p<0.1; ns: not significant correlation.

In cropland areas, the correlations coefficients between SOCc and control variables were, generally, lower than those in non-cultivated areas. Temperature was negatively correlated at all depths and altitude was positively correlated at nearly all depths. SOC was positively correlated with precipitation at 20-40 cm and 40-60 cm depths (r=0.19, p<0.05) and with the clay content at 40-60 cm depth (r=0.24, p<0.05). Overall, for all land uses, the textural factors only showed some correlation in the subsoil layers (below 20 cm).

3.3.3. Regression models of SOC concentration

With the stepwise multiple regression analysis, we examined the relationships between SOCc and the following predictor variables: lithology, precipitation, temperature, clay, and fine silt. This analysis was accomplished for forestland, shrubland and cropland separately at four different depth layers. Significant differences were found in the relative importance of the predictor variables according to soil use and depth (Table 3.3).

In forestland, precipitation and texture (clay) were the main predictor variables explaining the SOCc variability at the different depths. Precipitation explained 15% and 12% of the SOCc variability at 0-20 and 20-40 cm depth, respectively. Clay explained 40% of SOCc variability between 40 and 60 cm depth and precipitation plus clay explained 45% of the SOCc variability below 60 cm. In this land use type the model does not selected the temperature and lithology.

In shrubland, 36% of SOCc variability was explained by temperature and lithology in the 0-20 cm layer and 39% in the 20-40 cm interval. Temperature was the main predictor variable at 40-60 cm and lithology plus fine silt explained 33% of the variability.

In cropland soils the R^2 values were, in general, lower than in the other land uses and decreased with depth. The most significant factors controlling SOCc in cropland were lithology (marls), texture (fine silt or clay), and temperature at 0-40 cm depth, marls plus clay at 40-60 cm depth and quaternary sediments below 60 cm depth.

Table 3.3. Stepwise multivariate regression model of SOCc against predictor variables at different depths and land uses.

Land use	Depth increments (cm)	Term	Unstandard coefficient	Standard coefficient	R^2 adj.	*P*-value
Forestland	0-20	Intercept	-3.96		0.15	0.008
		lnP	0.85	0.37		
	20-40	Intercept	-5.76		0.12	0.027
		lnP	0.99	0.38		
	40-60	Intercept	-2.48		0.40	0.000
		Clay	0.06	0.53		
	60-100	Intercept	-16.10		0.45	0.003
		lnP	2.67	0.63		
		Clay	0.06	0.54		
Shrubland	0-20	Intercept	10.26		0.36	0.000
		lnT	-2.94	-0.46		
		L_1	0.75	0.44		
		L_2	0.51	0.18		
	20-40	Intercept	11.77		0.39	0.000
		lnT	-3.48	-0.41		
		L_2	-0.58	-0.24		
		L_3	-0.89	-0.35		
		L_4	-0.74	-0.30		
	40-60	Intercept	9.18		0.10	0.008
		lnT	-2.80	-0.34		
	60-100	Intercept	-0.54		0.33	0.000
		L_1	-0.64	-0.35		
		Fine silt	0.02	0.41		
Cropland	0-20	Intercept	6.33		0.21	0.000
		L_3	-0.61	-0.41		
		Fine silt	0.012	0.21		
		lnT	-1.59	-0.23		
	20-40	Intercept	7.04		0.16	0.000
		L_3	-0.76	-0.34		
		Cl	0.016	0.21		
		lnT	-2.05	-0.19		
	40-60	Intercept	0.44		0.18	0.000
		L_3	-0.91	-0.39		
		Clay	0.025	0.34		
	60-100	Intercept	1.24		0.11	0.002
		L_1	-0.66	-0.35		

T, mean annual temperature; *P*, mean annual precipitation; *L*, lithology type: L_1, quaternary sediments; L_2, alluvial sediments; L_3, marls; L_4, metamorphic sediments.

3.3.4. Distribution of organic carbon stocks in the soil profiles

The total SOC stored in the top 1 m of the soil in the Murcia Province was about 79 Tg (Teragram), with a mean density of 7.18 kgC m^{-3} (Table 3.4). The vertical distribution was shallow, with more than 70% stored in the top 40 cm of the soil.

The soils types with highest SOCD in the top 1 m were CM and KS, followed by FL and CL (see Table 4). In the case of LP it must be taken into account that soil depth is limited by taxonomic characteristics (FAO, 2006).

The vertical distribution of SOCD was deepest in FL and shallowest in KS, which is consistent with their vegetal cover and soil formation processes as discussed above. Considering the area covered by every soil type in the Murcia Province, only the CL stored half of the total SOC (Figure 3.2a).

According to land use type, higher SOCD was found in forestland and shrubland (8.4 and 8.1 kg C m^{-3}, respectively) than in cropland (6.3 kg C m^{-3}) (see Table 3.4). However, considering the total area covered by each use, the greater amount of SOC was stored in cropland (48.9%), followed by forestland (31.5%) and shrubland (19.6%), (Figure 3.2b). The vertical distribution showed marked differences among the uses, being shallowest in forestland and deepest in cropland, which is in agreement with the results reported by other researchers (Yang et al., 2007). The top 40 cm contained 87% of the SOC stored in forestland, while in cropland the SOC in this layer accounted for the 63.5% (see Table 3.4). Of the total SOC stored in the study area below 40 cm depth, 63% occurred in cropland and only 15% was found in forestland. Subsoil forestland SOC (below 40 cm depth) contributed only 4% to the total SOC stored in the Murcia Province, while this contribution from cropland was 18%. This was due to the bedrock limitation to soil depth and the greater stoniness of forestland.

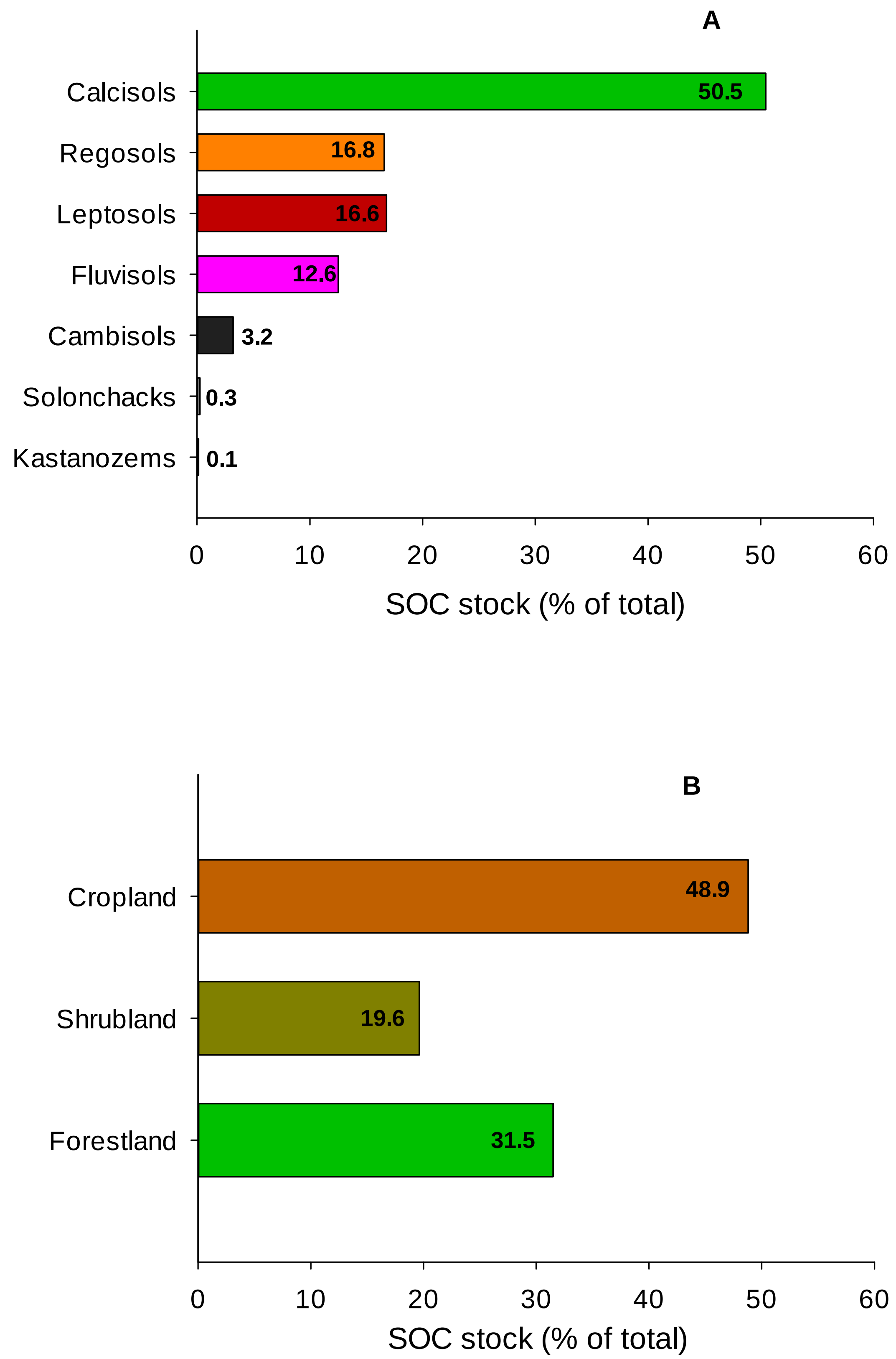


Figure 3.2. Percentage of SOC stored at 0-100 cm soil layer under different soil types **(a)** and land uses **(b).**

Table 3.4. Mean and standard deviation of SOC density (SOCD) and total SOC stock in each soil and land use type.

		SOCD (kg m^{-3})					**Total SOC storage (Tg)**				
	Land area (10^6 m^2)	Depth (cm)					Depth (cm)				
Soil type		0-20	20-40	40-60	60-100	0-100	0-20	20-40	40-60	60-100	0-100
Calcisols	5521	3.0 ± 1.2a,b	1.8 ± 0.9a,b	0.9 ± 0.6a	1.3 ± 0.4a,b	7.0	17.0	10.0	5.0	7.2	39.2
Regosols	2100	2.6 ± 1.5a	1.5 ± 1.0a	0.8 ± 0.5a	1.4 ± 0.3a,b	6.3	5.4	3.2	1.7	3.0	13.3
Leptosols	1986	4.1 ± 1.2c	2.6 ± 1.1a,b,c	-	-	6.7	8.1	5.2	-	-	13.3
Fluvisols	1144	2.7 ± 1.4a	1.9 ± 1.1a,b	1.5 ± 0.8a,b	2.7 ± 0.8b	8.8	3.1	2.2	1.7	3.1	10.1
Cambisols	214	3.9 ± 1.7b,c	3.1 ± 1.6 b,c	1.9 ± 1.1b	2.3 ± 0.8 a,b	11.2	0.9	0.7	0.4	0.5	2.5
Solonchaks	29	2.7 ± 1.2a	1.9 ±0.8 a,b	0.9 ± 0.4a	1.4 ± 0.6 a,b	6.9	0.08	0.05	0.03	0.04	0.2
Kastanozems	10	4.8 ± 1.5c	3.3 ± 1.7 c	1.2 ± 0.7a,b	1.1 ± 0.6 a,b	10.4	0.05	0.04	0.01	0.01	0.1
Total	11004						34.63	21.39	8.84	13.85	78.7
Land Use											
Forestland	2963	4.6 ± 1.5	2.7 ± 1.6	0.5 ± 0.1	0.6 ± 0.1	8.4	13.6	8.0	1.5	1.8	24.9
Shrubland	1898	3.3 ± 1.6	2.1 ± 1.6	1.2 ± 1.0	1.5 ± 1.4	8.1	6.3	4.0	2.3	2.9	15.5
Cropland	6143	2.3 ± 1.1	1.7 ± 0.9	0.9 ± 0.6	1.4 ± 1.1	6.3	14.1	10.4	5.5	8.6	38.6
Total	11004						34.0	22.4	9.3	13.3	79.0

Different letters within columns means significant differences between soil types at each depth at *p*<0.05 according to Tukey's test.

3.4. Discussion

3.4.1. SOC concentration

The results of this study suggest that land use type will be of great importance in the impact of global change on SOCc in semiarid areas. Although several studies have proved that changes in land uses involve changes in SOC content (Post and Kwon, 2000), considerable uncertainties remain as to the magnitude of these changes in different regions (Schimel et al., 2001).

In our study area, the average SOCc (see Table 3.1) near the surface (0-20 cm) was more than three times higher in forestland (30.4 g kg^{-1}) than in cropland (9.1 g kg^{-1}), so a very important reduction of SOCc, in the top 20 cm of the soil may be expected following a change from forestland to cropland, if actions are not taken to protect the SOC accumulated under forestland. This SOCc reduction implies an average emission to the atmosphere of 1 t of carbon (3.66 t of CO_2) per 47 t of surface forestland soil converted to cropland. Other authors found land cover to be a key predictor variable of SOCc and stock in arid and semiarid areas (Lo Seen et al., 2010, Wiesmeier et al., 2011; Hoffmann et al., 2012). Boix Fayos et al. (2009) found similar losses of SOC in the first 20 cm of soil when forest was converted to cropland in an area located at the north of the Murcia Province.

The conversion from forest to cultivated areas reduce the surface SOCc mainly through reducing biomass inputs into the soil, increasing soil erosion rates and accelerating the decomposition of soil organic matter. Some examples of these effects in Murcia Province have been quantified in others studies (Martínez-Mena et al., 2002; Almagro et al., 2010). In addition, there are evidences that the magnitude of this loss of SOC through cultivation in semiarid areas could be greater than in more humid areas (Celik, 2005; Martínez-Mena et al., 2008). This impact decreased with depth. So, in the 20-40 cm interval the loss in SOCc was 53% and no differences in SOCc were observed between land uses below 60 cm depth (see Table 3.1). Therefore, little changes may be expected in deeper SOCc following the conversion of forestland into cropland.

The SOC according to parent material showed lower average concentration in soils developed from marls than in the rest of the soils, although no significant differences could be established, in all cases, due to the high standard deviation (see Table 3.1). The chemical, physical and mineralogical properties of the parent material play an important role in the composition and amount of organic matter stored in the soil. In environmental conditions similar to those of this study, Turrion et al. (2009) reported that bedrock type was the principal factor affecting soil development and the fertility of forest soil in central western Spain. Also, Aranda et al. (2011), found that the evolution of soil organic matter to more stable and evolved forms of humus was less pronounced in soils from marls than in soils derived from colluvial limestones, which might lead to a faster turnover rate and lower SOC content in marly soils.

On the other hand, the high susceptibility to soil erosion could reduce SOC accumulation through the removal of surface litter particles attached to SOC (Lal, 2004b), a fact corroborated by Liu et al. (2011), in semiarid areas of the Loess Plateau, China. In arid and semiarid Mediterranean areas, since soils derived from marls are very susceptible to erosion (Imeson et al., 1998), we suggest that the lowest content of SOC in marly soils is mainly attributable to the removal of surface particles by water erosion, which is accelerated by the very poor vegetal cover due to the low soil fertility.

3.4.2. Factors controlling SOC concentration variability

The results of the stepwise multivariate regression between SOCc and environmental and textural control factors (see Table 3.3), for the different depth intervals, showed that the relative importance of each factor changed with depth and with land use type. Similar results were reported by Jobbágy and Jackson (2000). This suggests that the control factors or their relative importance and the mechanisms involved in the stabilization and dynamics of SOC may be different at the surface and in subsurface soil (Salomé et al., 2010; Rumpel and Kögel-Knabner, 2011), and also may change depending on land use.

Better relationships between the controlling factors and SOCc were obtained in the deeper layers than in the surface layers in forestland. In this use, the main factor controlling SOC was precipitation up to 40 cm depth, clay content at 40-60 cm, and precipitation plus clay below 60 cm depth. In shrubland and cropland, temperature and lithology were the main control factors explaining the variability of SOCc through the soil profile. In general, in all the soil uses the relative importance of climatic factors (precipitation and temperature) decreased with depth, while the importance of textural factors (clay and fine silt) and lithology increased.

As regards predictions of the impact of climatic change according to land use, our results point to decreasing SOCc as precipitation becomes less, which was evident at all depths in forestland. This can be attributed to the key role of precipitation in biomass productivity, which is the determinant of litter input (Chaplot et al., 2010). However, the relative importance of precipitation decreases with depth and the relative weight of texture increases. This may be due to the higher stabilization of organic matter through organo-mineral associations (Torn et al., 1997; Six et al., 2002a) in deeper layers than at the surface.

In shrublands and croplands, a reduction of SOCc with increasing temperature was evident at all depths. In shrubland, precipitation showed a positive correlation with SOC concentration in the Pearson correlation coefficient (see Table 3.2) but no in the stepwise regression (see Table 3.3), while no impact of precipitation was observed in cropland. This result might be due, in the case of cultivated areas, to the irrigation carried out in most of the areas considered in this study.

In cropland, a very low percentage of variation of SOCc can be explained by the variables considered. The weak relationships obtained might be due to the wide range of crops and management techniques covered by this land use.

In relation with the predictions of the impact of global warming on SOCc, our results also suggest differences according to land use and depth. In cropland and shrubland, losses of SOCc can be predicted to occur with increases in temperature (see Tables 3.2 and 3.3). Similar results were reported by other authors (Leifeld et al., 2005; Garten and Hanson, 2006; Yang et al., 2007). These results might be attributable to the greater increase in decomposition rate compared with the increase in photosynthesis due to temperature increase, which is particularly favoured when soils are intensively managed (Balkovic et al,. 2011), as occur in cropland. This effect of temperature on the mechanisms controlling the C dynamics was different with depth, being more evident at the upper soil layers (see Table 3.3). Likewise, Jobbágy and Jackson (2000) found a decreasing correlation between SOCc and temperature as depth increased and suggested that temperature may have a proportionally higher effect on the decomposition of shallow SOC than on deep SOC. The different temperature sensitivities between surface and deep SOCc found in this study may be attributable to changes in the kinetic properties of soil organic compounds due to the greater physical and chemical protection in depth (Davidson and Janssen, 2006). The higher correlation between SOCc and the clay content found in deep layers support this affirmation.

The feedbacks between SOC and climate are not fully understood (Schmidt et al., 2011). In contrast with croplands and shrublands, the lack of correlation between *T* and SOCc, at any depth, in forestland suggests that no effect on SOCc in forestland is to be expected in short-term, with the current trend in global warming. This may be due to the increase in net ecosystem productivity (NEP) with increased atmospheric CO_2 and global warming (Cao and Ian Woodward 1998; Melillo et al., 2011), which could compensate the warming-induced SOC losses through the gain of vegetal input in the soil. In any case, these results should be considered with care because: (a) the increase in NEP will decline as the CO_2 fertilization effect becomes saturated (Cao and Ian Woodward, 1998), so, the long-term prediction could be different; and (b) the sensitivity of SOCc to changes in *T* is less in non-perturbed soils (soils at their native temperature) than in perturbed soils (Agren and Bossata, 2002). This could mean that the effect of temperature in forestland could be masked by other factors.

3.4.3. SOC stocks

Considering the entire soil profile (0-100 cm), the average SOC stock in the Murcia Province was 71.8 Mg C ha^{-1}, being 42% lower than the worldwide average (Batjes, 1996) and, in the 0-30 cm soil profile, it was 39.8% lower than the European average (Smith et al., 1997). The mean SOCD in the study area is within the range reported by Wu et al. (2003) for soils from China (8.01 kg C m^{-2}), due to the extended arid and semiarid regions, and slightly higher than those reported by Batjes (1996) for Xerosols (4.2-6.2 kg C m^{-2}). As regards to land use, in our study average SOCD in forestland and shrubland were 84 and 81 Mg C ha^{-1}, respectively, which were smaller than the presented by Lettens et al. (2005) for coniferous forest (155 Mg C ha^{-1}) and grassland (130 Mg C ha^{-1}) of Belgium. Likewise, the SOC stock in cropland was lower than in other European countries. The average SOCD in the 0-100 cm of the cropland soils found in our study was 63 Mg C ha^{-1}, lower than the presented by Krogh et al. (2003) for arable soils in Denmark (132-150 kg C m^{-2}), or by Batjes (2002) for eastern Europe (158 Mg C ha^{-1}). The mean SOC content in the top 30 cm of the European agricultural area is 53 Mg C ha^{-1} (Smith et al., 1997) which is 62% higher than in the cropland area of the Murcia Province. These results corroborate that SOCD is positively correlated with precipitation (Post et al., 1982), so that when the potential evapotranspiration is higher than annual precipitation, it leads to a deficit in organic carbon in dry soils.

Given that our results, in agreement with other studies (Batjes, 1996; Rumpel and Kögel-Knabner, 2011), predict that the impact of climate change on SOC content will be larger in surface soils than in depth, we suggest that the strategies for C sequestration in semiarid areas should be focused on subsoil C sequestration. Both, the reforestation of degraded forestland and the adoption of recommended management practices (RMPs) in cropland and grazing land, have been advocated as effective options for C sequestration in soils (Smith, 2004), and both lead to additional environmental and socio-economic benefits (UNEP, 2012). Taking into account the number of factors and complexity involved in this issue, it seems reasonable to think that the best options may change according to the specific conditions of each area.

The vertical distribution of SOC stocks showed that subsoil forestland (below 40 cm depth) contributed only 4.4% to the total SOC stored in the study area, while cropland contributed 17.8% and shrubland 6.6% of total SOC. This was due to the fact that forestlands in arid and semiarid areas are usually confined to mountainous areas, in which the soil capacity for C sequestration is restricted to shallow soil profiles due to bedrock limitation to soil depth. In these situations, the option of C sequestration and improvement in soil productivity through implementation of RMPs in cropland and shrubland soils might be economically and environmentally more profitable than reforestation, unless reforestation is carried out in deep soils of marginal areas. As reported Álvaro-Fuentes et al. (2012a), under climate change conditions, Spanish agricultural soils could act as potential atmospheric C sinks. Note that, although SOCD was higher in forestland than in cropland and shrubland, the potential capacity for additional C sequestration is higher in cropland and shrubland soils due to their deeper soil profiles and lower C saturation.

This suggestion could be extrapolable to large areas in arid and semiarid conditions since Leptosols, the most extensive reference soil group on Earth, are particularly abundant in mountains in the extensive desert regions of North Africa and Southwest Asia (Spaargaren, 2008). An increase of 1 t of soil carbon pool of degraded cropland soils may increase crop yield by 20 to 40 kg ha^{-1} for wheat (Lal, 2004b). Restoration of degraded cropland soils is, therefore, a key aspect for a sustainable development and to meet basic needs of the present and future population (Lal, 2010).

3.5. Conclusions

The average density of SOC in these semiarid areas was low (7.18 kg C m^{-3}) in the 0-100 cm layer, being 70.12 % located in the top 40 cm. Land use was the main factor controlling SOC concentration in these semiarid areas. The conversion of forestland to cropland involves a substantial decrease in SOC concentration and it should be strictly restricted in these areas.

The analysis of environmental control factors suggested a negative effect on the SOC concentration in a climatic change scenario with increased temperature and a decrease in rainfall, as expected in semiarid areas. The results showed that this impact would be much greater in surface than in the subsurface SOC. Therefore, the strategies for soil C sequestration should be focused on subsoil sequestration. In this way, the potential capacity of large areas of forestlands will be limited due to bedrock impediments to soil depth. Appropriate management practices in croplands and shrublands, which have deep soil profiles with low organic C saturation, seem to be a win-win option for sequestering atmospheric CO_2 and improve soil productivity.

More research, at regional scale, seems to be necessary to implement appropriate public policies able to manage the synergies between climate change, soil degradation and sustainable development in different environmental and socio-economic conditions.

Chapter 4. *Effect of afforestation techniques on C sequestration*

4.1. Introduction

In recent centuries, soils have released large amounts of CO_2 as a consequence of land use changes (e.g. conversion of forests to agricultural lands), soil degradation, and desertification (Lal, 2005; Jandl et al., 2007). This has led to a significant decline of soil organic carbon (SOC) pools and increased concentrations of greenhouse gases (GHG) in the atmosphere. This unfavorable scenario could be worsened, particularly in semiarid ecosystems, by current trends of climate change. In a study of the impact of climate change on SOC in southeast Spain, Albaladejo et al. (2013) reported decreases of SOC stocks with increased temperature and reduced precipitation, as expected in semiarid areas. Traditional approaches to ecosystems restoration have considered afforestation to be an important tool to rehabilitate the capacity of ecosystems to produce goods and services and increase C sequestration (Nosetto el al., 2006; Cao el al., 2010). However, despite the considerable SOC-sequestration potential of afforestation, many studies have reported contradictory findings: afforestation resulted in either a decrease (Wiesmeier et al., 2009) or an increase (Fernandez-Ondoño et al., 2010) in the SOC stocks, or had a negligible effect (Laganière et al., 2010). There are many abiotic factors affecting the extent of change in soil C, including site preparation, previous land use, climate, soil texture, site management, and harvesting (Paul et. al., 2002). In addition, although several studies have estimated the contribution of afforestation to C sequestration (Laganière et al., 2010), there are very few that discuss the mechanisms of C stabilization (Six et al., 2002b; De Gryze et al., 2004), particularly in semiarid areas.

The fractionation of organic matter into different parts that are thought to be functionally homogeneous with respect to physic-chemical properties and turnover rates is an increasingly-used methodology in mechanistic studies of C sequestration (De Gryze et al., 2004). One of the most-promising approaches to obtain functional SOC pools is a combined physical and chemical fractionation method proposed by Zimmermann et al. (2007). This method isolates five soil fractions which can be combined into three functional pools: the active, the intermediate, and the passive SOC pool. The active pool is mainly composed of fresh plant residues, root exudates, and faunal and microbial residues with a short turnover time of 1-10 years (von Lützow et al. 2008). The intermediate and passive pools are characterized by organic matter (OM) with considerably-longer turnover times of 10-100 and >100 years, respectively (Figure 4.1). In these pools OM is stabilized against microbial mineralization by occlusion within soil macro- and microaggregates, selective preservation of recalcitrant compounds, and the formation of organo-mineral associations (Sollins et al., 1996; Christensen, 2001; Kögel-Knabner et al., 2008). The determination of functional SOC pools allows a precise evaluation of the C sequestration in afforested soils.

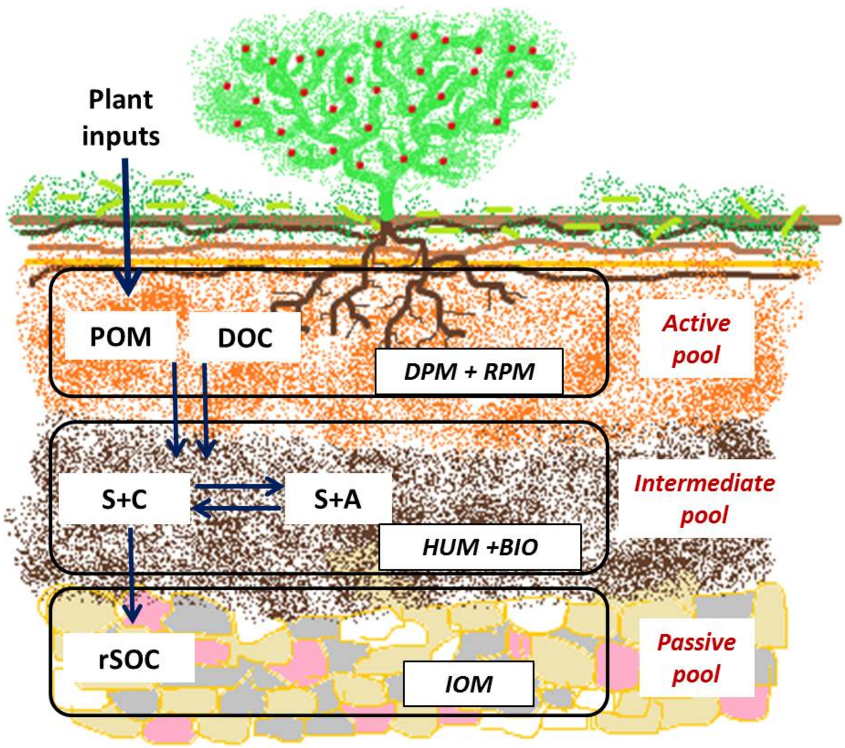


Figure 4.1. Adaptation of conceptual SOC fractions (POM: particulate organic matter; DOC: dissolved organic carbon; S+C: silt and clay; S+A: sand and aggregates; and rSOC: oxidant resistant organic carbon) and pool (active, intermediate and passive) model according to Zimmermann et al. (2007). DPM and RPM (decomposable and resistant plant material, respectively), HUM (humified organic matter), BIO (microbial biomass) and IOM (inert organic matter).

The restoration of degraded semiarid lands by the reintroduction of woody species has become increasingly important (Maestre and Cortina, 2004). However, divergent results caused a lack of consensus about the strategies required to implement the restoration programs. In fragile ecosystems, such as semiarid lands, the increase of forest cover could exacerbate environmental degradation when the management of afforestation ignores climate, pedological, hydrological, and landscape factors that would make a site unsuitable for afforestation (Cao et al., 2011). In dry areas, the introduction of woody species is strongly limited by biotic and abiotic factors (Cortina et al., 2011). Abiotic constraints are likely to increase as temperatures increase and rainfall becomes scarcer (Giorgi and Lionello, 2008). These limitations could be diminished by suitable afforestation techniques.

In this study, we test the hypothesis that C sequestration, following afforestation in semiarid areas, can be increased by suitable techniques which aim to improve soil conditions and resource availability. In a long-term (20 years) experiment, we analyzed the effect of two afforestation techniques on C sequestration by directly measuring the net C accumulation in the afforested ecosystems. The two techniques tested were: (1) terracing, in order to improve the water efficiency, and (2) terracing combined with soil amendment, to improve the water efficiency and the physical and chemical soil fertility. The site was located on a degraded shrubland in the Iberian southeast, one of the areas most vulnerable to desertification (Fons-Esteve and Páramo, 2003), and the results may be a reference for extrapolation to other semiarid areas. The environmental conditions, such as scarce and irregularly-distributed rainfall, erodible soils, and steep terrain, make these areas unable to recover spontaneously (Albaladejo et al., 1998; Puigdefabregas and Mendizabal, 1998). Due to the widespread extent of degraded ecosystems and to the limited funds available, the selection of the areas to be restored is one of the major challenges faced by scientists and practitioners worldwide (Maestre and Cortina, 2004). This study may contribute to an increase in our knowledge of the suitability of afforestation with the purpose of C sequestration in these low-productivity ecosystems.

Initial results of this experiment were reported in Roldan et al., (1996a) and Querejeta et al., (2000). The specific objectives in this long-term study were to: (1) assess the impact of the afforestation technique on soil rehabilitation, (2) determine the changes in the organic carbon stocks for the different components of the ecosystems, according to the afforestation technique, and (3) increase our knowledge of the mechanisms involved in organic carbon accumulation and stabilization in afforested ecosystems under semiarid conditions.

4.2. Material and methods

4.2.1. Study area

The experimental area was located in the Sierra de Carrascoy, southeast Spain (37° 53'N, 1° 15'W, 180 m a.s.l.). The climate is semiarid with an average annual precipitation of 300 mm, which occurs mostly in spring and autumn. The mean annual temperature is 18°C, and the mean annual potential evapotranspiration is 900-1000 mm yr^{-1}. The soils are classified as *Haplic Calcaric Leptosol* with inclusions of *Leptic Calcisol and Haplic Calcisols* (FAO, 2006). The lithology of the area is constituted by hard and compact limestone rocks. The dominant vegetation is composed of characteristic species of Mediterranean shrublands (e.g. *Rosmarinus officinalis* L., *Thymus vulgaris* L., and *Anthyllis cytisoides* L.) with scattered stands of Aleppo pines (*Pinus halepensis* Miller).

4.2.2. Experimental design

In October 1992, an area of 1800 m^2, consisting of three plots of 20 x 30 m, was established on an east-facing hillside (25% mean slope) to test the following afforestation techniques (Figure 4.2): mechanical terracing with soil organic amendment and *Pinus halepensis* plantation (AT), mechanical terracing and *Pinus halepensis* plantation without organic amendment (T) (Figure 4.3a and 4.3b, respectively), and Mediterranean shrubland adjacent to these reforested plots, which was considered as the control plot (S). The mechanical terraces (4 m wide and 30 m long) were excavated with a bulldozer. The organic amendment consisted of the organic waste of urban solid refuse (USR) from the Murcia Municipal Treatment Plant. The USR, with a C content of 253 g C kg^{-1}, was incorporated into the top 20 cm of the soil in the AT treatment, in a single application of 10 kg m^{-2} at the beginning of the experiment. The analytical characteristics of this organic amendment can be found in García et al. (1998). The Aleppo pine seedlings were planted in both treatments - in 40-cm-wide, 40-cm-deep pits - at least 1 m apart in a single row per terrace. The experiment was developed under strictly-natural conditions, without any watering or weeding treatments.

Figure 4.2. General overview of the study area in 1992 with S (control plot); T (mechanical terracing and *Pinus halepensis* plantation without organic amendment); and AT (mechanical terracing with soil organic amendment and *Pinus halepensis plantation*) immediately after of the starter of *P.halepensis* plantation (Photo courtesy of Víctor Castillo).

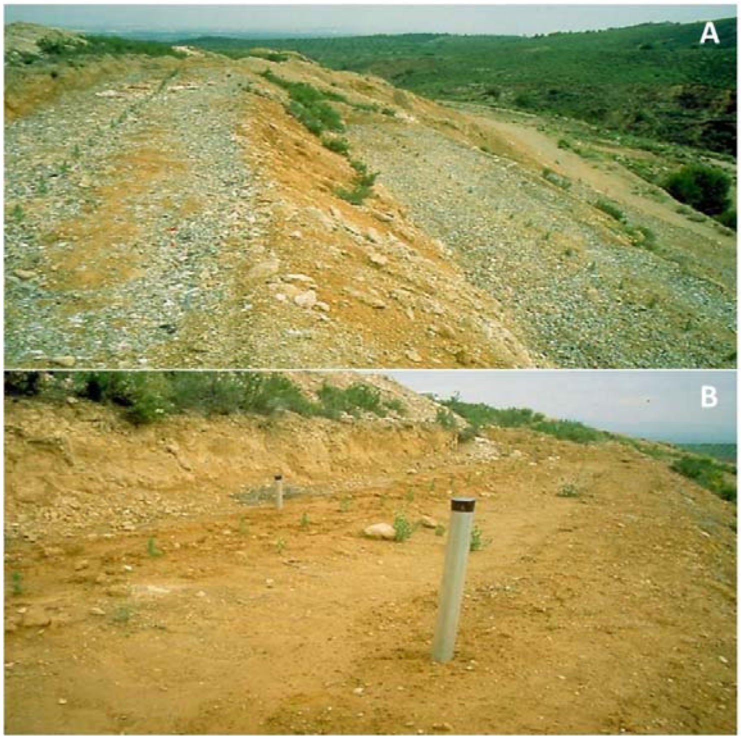


Figure 4.3. Mechanical terraces: **(a)** soil organic amendment and *Pinus halepensis* plantation corresponding to AT and **(b)** *P.halepensis* plantation without organic amendment corresponding to T (Photos courtesy of Victor Castillo).

4.2.3. Soil sampling

At the start of the experiment, six soil samples (0-5 cm) per plot were randomly collected in the terraces and adjacent shrubland for an initial soil characterization. In April 2012, after 20 years of afforestation, a randomized soil-sampling trial was designed to assess the effects of the tested factors (Figure 4.4). Six sampling sites were selected at each plot (18 sampling sites). At each sampling site, soil samples were collected from three depths: 0-5 cm, 5-20 cm, and 20-25 cm (54 sampling points for the whole experiment). At each sampling point, disturbed as well as undisturbed samples were collected. The disturbed samples consisted of a composite of three subsamples collected randomly. For the undisturbed soil samples, steel cylinders with a diameter and height of 5 cm were used.

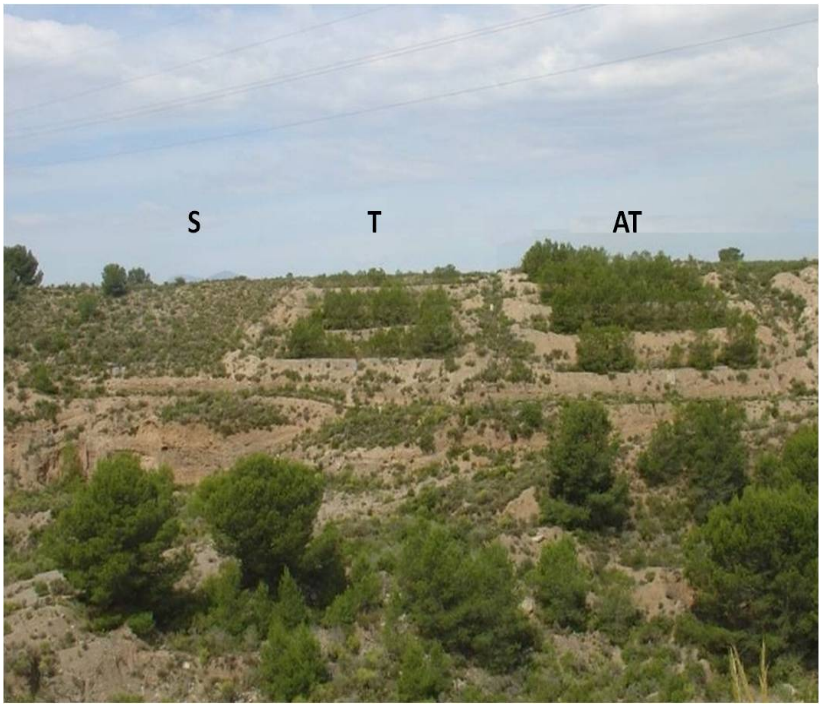


Figure 4.4. General overview of the study area in 2012 showing the state of the vegetation in the experimental plots. Control plot (S), mechanical terracing (T) and mechanical terracing combined with soil amendment (AT).

4.2.4. Determination of soil properties

The soil samples were air-dried, sieved to <2 mm, and analyzed in the laboratory in triplicate. The organic carbon and total N contents were determined using an Elemental Analyzer (LECO TRUSPEC C/N, Sant Joseph, USA). The carbonate concentration was determined using a *Bernard Calcimeter* (Duchaufour, 1975), exchangeable calcium (Ca^{+2}) was measured by the method of compiled by Duchaufour (1975), and the pH was determined in water (1:5) with a pH-meter (CRISON 20). Available phosphorus (P) was extracted with sodium bicarbonate (Olsen et al., 1954) and available potassium (K) was extracted with ammonium acetate: the concentrations of both elements were determined by ICP-OES (ICAP 6500 DUO THERMO, Tennessee, USA). Soil texture was determined by laser diffraction using a Laser Particle Sizer (Coulter LS200, Miami, USA) on air-dry samples, after dispersion. The soil water holding capacity (WHC) at matric potentials of -33 kPa and -1500 kPa was determined using pressure ceramic plate extractors (Soil Moisture Equipment Corp., Santa Barbara, CA). The available water content (AWC) was calculated as the difference in soil moisture content at field capacity (-33 kPa) and wilting point (-1500 kPa). Soil bulk density was calculated from the oven-dried mass (105°C, 24 h), being corrected for the coarse fragments content (Robertson and Paul, 2000). The mean weight diameter (MWD) of the water-stable aggregates was calculated by adding the products of the aggregate fraction weight and the mean diameter of the aggregate classes (Kemper and Rosenau, 1986). Mineralogical analysis of the clay fraction was performed by X-Ray diffractometry using a Philips PW 1710, (Philips, Eindhoven, Holland).

Microbial C was determined by chloroform fumigation extraction, using the calibration factor K_{EC} = 0.38 (Vance et al., 1987), with some minor modifications as reported in Goberna et al. (2007). Soil basal respiration was calculated as the average C content respired daily per kg of soil under controlled conditions. Soils (15 g) were moistened to 60% WHC prior to incubation at 28°C in 125 cm^3 airtight containers. The CO_2 (%) evolved in the containers was measured over 28 days at 3-5 day intervals, with an infrared analyzer (CheckMate II, PBI Dansensor). After each measurement, the stoppers were removed for 1 h to balance the atmosphere inside and outside the bottles.

Soil β-glucosidase and alkaline phosphatase activities were determined colorimetrically as the amount of *p*-nitrophenol (PNP) produced after incubation of 0.5 g of soil (37°C, 1 h) in 2 mL of modified universal buffer (MUB; pH 6) and 0.5 mL of 0.025 M *p*-nitrophenyl-β-D-glucopyranoside or MUB (pH 11) (Tabatabai and Bremner 1969). Soil urease was quantified colorimetrically as the NH_4^+ produced after incubating (37°C, 2 h) 1 g of soil in 4 mL of borate buffer (pH 10) and 0.5 mL of 0.48 % urea (Kandeler and Gerber 1988).

4.2.5. Fractionation of functional SOC pools

Bulk soil samples were separated using a combined physical and chemical fractionation method, according to Zimmermann et al. (2007). Thirty grams of soil (< 2 mm) were added to 150 mL of water and dispersed using a calibrated ultrasonic probe-type with output energy of 22 J mL^{-1}. Application of more energy may disrupt the coarse sand-sized SOM (Amelung and Zech, 1999). The dispersed suspension was then wet-sieved over a 63-µm-aperture sieve until the rinsing water was clear (Figure 4.5a y 4.5b).

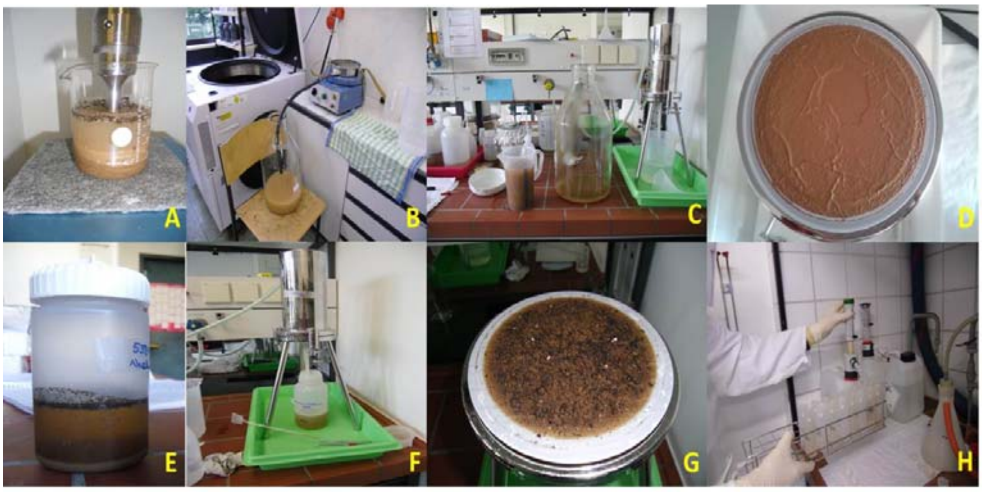


Figure 4.5. Steps of OC pool fractionation according to Zimmermann et al (2007) method: (A) ultrasonic, (B) wet-Sieving, (C) and (D) filtration to obtain S+C fraction, (E) and (F) density separation with SPT = 1.6 g cm^{-3}, (G) POM fraction separated to S+A fraction (H) chemical oxidation with NaOCl.

The fraction > 63 µm, containing the sand fraction and stable aggregates (S+A) together with particulate organic matter (POM), was dried at 40°C and weighed. The suspension < 63 µm was filtered through a 0.45 µm aperture nylon mesh and the material > 0.45 µm was dried at 40°C and weighed (Figure 4.5c). The POM was separated by stirring the fraction > 63 µm with sodium polytungstate at a density of 1.6 g cm^{-3} (Cerli et al., 2012). The mixture was centrifuged at 1000g for 15 minutes and the light fraction was decanted (Figure 4.5d, e, f, g). Both fractions were washed with deionized water to remove all sodium polytungstate, dried at 40°C, and weighed. A chemically-resistant carbon fraction (rSOC) was extracted from the fraction < 63 µm (S+C) by NaOCl oxidation (Figure 4.5h). One gram of S+C was oxidized for 18 hours at 25°C with 50 mL of 6% NaOCl adjusted to pH 8 with concentrated HCl. The oxidation residue was centrifuged at 1000 g for 15 minutes, decanted, washed with deionized water, and centrifuged again. This oxidation step was repeated twice. The residue was dried at 40°C and weighed. The OC and N concentrations were determined for every fraction using an Elemental Analyzer (LECO TRUSPEC CN, Michigan, USA).

4.2.6. Organic C stock

The SOC density (kg m^{-2}) in each layer interval (0-5, 5-20, and 20-25 cm) was determined by the product of the SOC concentration, thickness of the layer interval, and bulk density in each layer of the soil profile. The SOC density was also corrected for the coarse-particle content of each soil layer (see Equation 3.1 in Chapter 3).

The aboveground biomass C stocks of the shrubs and litter were estimated from plot-based measurements. Thirty-six 1 x 1 m plots (18 each for the shrubs and litter) were located in the shrublands (N=6+6), T treatment (N=6+6), and AT treatment (N=6+6) Figure 4.5. The total aboveground shrub biomass and litter were removed from every plot, dried at 50°C for seven days, and weighed. The biomass from the pines was estimated using the following equation:

$$\textbf{Pine biomass (kg)} = (\tfrac{1}{e^2}.142) \text{ x } (\mathrm{DBH}^2.411) \qquad \textit{(4.1)}$$

This pine-specific allometric equation was developed from destructive samples of 20 *Pinus halepensis* trees growing in a forest close to our study area (Barbera et al., 2005). For each treatment (AT and T), 20 pines were measured for diameter at breast height (DBH). The C content in the measured biomass was estimated to be 48% of dry weight.

4.2.7. Statistical analyses

The results are reported as means ± standard errors. All parameters were analyzed with a one-way ANOVA, followed by Tukey's test as a post hoc test. Prior to the analyses, the data were examined for normality by the Shapiro-Wilk test and for homogeneity of variances by the Levene test. The β-glucosidase activity was log-transformed to meet the ANOVA assumptions. All analyses were performed using SPSS 19.0 (Chicago, IL, USA).

4.3. Results

4.3.1. Soil properties as affected by afforestation

Twenty years after the afforestation, significant differences were found for most soil variables in the soil surface layer (0-5 cm) (Table 4.1). The SOC contents increased significantly in AT, compared to the S and T treatments. No significant difference was found between S and T. Total N was higher in AT than in T, but there were no differences between S and the afforested soils. The C/N ratio increased at the afforestation sites, particularly at AT. Likewise, available P was higher in AT than in S and T, with significant differences among the treatments. Available K was higher in S than in the afforested soils. No changes in pH were found among the treatments. The percentages of carbonates and exchangeable Ca^{2+} were higher in the afforested soils. A significant decrease in bulk density was observed in AT, relative to S and T. No differences were found between S and T (Table 4.2). With regards to structural stability, the MWD was higher in AT and S than in T. The soil WHC at field capacity (-33 kPa) was higher in S and AT than in T, with no difference between S and AT. No differences were found among the treatments in terms of water retention at the wilting point (-1500 kPa). The available water, measured as the difference between the field capacity and wilting point water contents, was significantly higher in S and AT than in T. No significant differences were found between S and AT.

4.3.2. Changes in microbiological parameters

The microbial carbon was marginally higher in S, compared to the AT and T treatments. However, no differences were found with regard to the basal respiration (Table 4.3). Well-defined differences were found in the enzymatic activities. Carbon oxidation and nitrogen mineralization were higher in shrubland soil´s than in the afforested areas. Urease activity was higher in S than in AT and T, with significant differences among the treatments (Table 4.3). The β-glucosidase activity was significantly higher in S than in T, but no differences were found between AT and the other two treatments. There were no differences among treatments regarding the phosphatase activity.

Table 4.1. Chemical properties (mean values ± standard errors) of the topsoil (0-5 cm depth) at control plot (S), mechanical terracing (T) and mechanical terracing combined with soil amendment (AT).

Chemical properties	Treatments		
	S	T	AT
Organic carbon (g kg^{-1})	12.8 ± 0.7a	12.5 ± 0.7a	22.6 ± 2.5b
Total N (%)	0.19 ± 0.01ab	0.15 ± 0.02a	0.23 ± 0.02b
C/N	6.8 ± 0.5a	8.3 ± 1.1ab	9.8 ± 0.9b
Available P (mg kg^{-1})	8.9 ± 0.1b	4.8 ± 0.1a	24.9 ± 1c
Available K (meq $100g^{-1}$ soil)	0.72 ± 0.03c	0.39 ± 0.01a	0.57 ± 0.02b
pH	8.1 ± 0.18a	8.0 ± 0.1a	7.9 ± 0.1a
Carbonates (%)	29.5 ± 1.1c	41.4 ± 1.5b	47.2 ± 1.3a
Exchangeable Ca (%)	8.3 ± 1.0b	12.0 ± 1.0a	13.0 ± 1.0a

Different letters in rows means significant differences between treatments (Tukey's test, p <0.05).

Table 4.2. Physical properties (mean values ± standard errors) of the topsoil (0-5 cm depth) at control plot (S), mechanical terracing (T) and mechanical terracing combined with soil amendment (AT).

Physical properties	Treatments		
	S	T	AT
Bulk density (g cm^{-3})	1.13 ± 0.06a	1.26 ± 0.01a	0.84 ± 0.19b
Mean weigth diameter (mm)	1.84 ± 0.02b	1.71 ± 0.02a	1.87 ± 0.02b
Water holding capacity (%)			
Field capacity (-33 kPa)	21.9 ± 1.4b	16.2 ± 0.6a	22.0 ± 1.0b
Permanent wilting point (-1500 kPa)	10.5 ± 1.2a	10.8 ± 0.7a	12.7 ± 0.6a
Available water content (%)	11.5 ± 1.6b	5.4 ± 0.5a	9.3 ± 0.5b
Clay (%)	7.0 ± 1.1a	7.3 ± 1.0a	9.3 ± 1.1a
Silt (%)	43.0 ± 3.0a	47.5 ± 3.1a	51.3 ± 1.0a
Sand (%)	50.0 ± 3.1b	45.2 ± 4.0ab	39.4 ± 2.1a
Clay mineralogy	I+(10-14M), I, K	I+(10-14M), I, K	I+(10-14M), I, K

Different letters in rows means significant differences between treatments (Tukey's test, p <0.05). I, illite; M, montmorillonite; and K, kaolinite.

Table 4.3. Biological properties (mean ± standard errors) of the topsoil (0-5 cm depth) at control plot (S), mechanical terracing (T) and mechanical terracing combined with soil amendment (AT).

Biological properties	Treatments		
	S	T	AT
Microbial carbon (mg kg^{-1})*	983 ± 440a	278 ± 200b	443 ± 169b
Basal respiration (mg C-CO_2 kg^{-1} d^{-1})	39 ± 16a	23 ± 5a	22 ± 10a
Urease (µmol N-NH^+_4 g-1 h^{-1})	2.49 ± 0.15c	0.34 ± 0.12a	0.70 ± 0.11b
Phosphatase (µmol PNP g^{-1} h^{-1})	7.97 ± 2.45a	4.54 ± 1.58a	4.59 ± 1.37a
β- Glucosidase (µmol PNP g^{-1} h^{-1})	3.26 ± 1.37b	1.03 ± 0.25	1.74 ± 0.57ab

Different letters in rows means significant differences between treatments (Tukey´s tes, p< 0.05). * For microbial carbon p< 0.06.

4.3.3. Changes of organic carbon stocks and their allocation patterns

In this study, the ecosystem carbon stock comprised the organic carbon stored in the mineral soil profile (0-25 cm depth) and the aboveground biomass carbon (trees, shrubs, and litter). Note that, as root biomass was not measured, the values of total ecosystem carbon stocks are underestimated, mainly in the afforested ecosystems. Twenty years after the afforestation, important changes in the distribution pattern of C storage among the different ecosystem components were observed (Table 4.4). The OC stock in the mineral soil profile was decreased in T compared to the S and AT treatments, and no differences were found between S and AT. Likewise, the effect of afforestation on C accrual in the aboveground biomass was related to the afforestation approach.

Table 4.4. Distribution of organic carbon stocks (kg m^{-2}) (mean values ± standard errors) among the different components of the ecosystem at control plot (S), mechanical terracing (T) and mechanical terracing combined with soil amendment (AT).

Ecosystem component	Treatments		
	S	T	AT
Mineral soil (0-25 cm)	3.6 ± 0.1b	2.9 ± 0.1a	3.5 ± 0.1b
Trees	0*	0.4 ± 0.2a	1.6 ± 0.9b
Scrhubs (understory)	0.4 ± 0.06b	0.05 ± 0.01a	0.05 ± 0.01a
Litter	n	0.05 ± 0.01	0.2 ± 0.04
Total	4.0	3.4	5.3

Different letters in rows means significant differences between treatments (Tukey's test, p <0.05) n: negligible. * no trees in control plot.

In AT, the total aboveground biomass increased more than three-fold with respect to the shrubland, while no considerable change was observed for the T treatment. So, although there was a decrease in shrubs as a result of the terracing, the total aboveground biomass C increased from 0.4 kg m^{-2} in S to 1.85 kg m^{-2} in AT and accounted for 0.5 kg m^{-2} in the T treatment. This increase in AT was mainly related to the planted trees (Table 4.4), associated with a significant amount of litter (0.2 kg m^{-2}). The growth of pines and the litter production were much lower in T.

4.3.4. Carbon sequestration

Organic carbon stocks in the soil and biomass in T and AT plots were compared with those of the shrubland to estimate the effectiveness of afforestation with regard to terrestrial carbon sequestration (Table 4.4). Differences between the shrubland system and the afforested systems after 20 years of establishment serve as an indicator of the C sequestration rates. The OC stock in the soil decreased 20% in the T treatment, relative to the control S, while there were no differences between S and AT. In contrast, the C stock in the aboveground biomass increased by 362% in AT and no significant change was observed in T with respect to the shrubland. This led to a total increase in the OC stocks of 32% in AT compared with the S plots, equivalent to a C sequestration of 1.3 kg m^{-2} in the AT treatment. However, in T a decrease of 15% occurred, equivalent to a loss of C stock in the ecosystem of 0.6 kg m^{-2}.

4.3.5. Changes of functional SOC pools

Significant differences were found among the treatments and soil depths for all the functional SOC pools (Figure 4.6). In the surface soil layer (0-5 cm), the OC stock of the POM fraction (OC-POM) was highest in AT, followed by T, and was lowest in S, with significant differences among all the treatments. In the 5-20 cm layer, no differences were found in OC-POM among the treatments, while in the deepest layer (20-25 cm) the OC-POM was lower in S than in AT and T. The OC-POM decreased with depth in all the treatments. The OC stock in the S+A fraction (OC-S+A) at the surface (0-5 cm) was higher in AT than in S and T, with no differences between S and T. In the 5-20 cm layer, it was significantly lower in T, without differences between AT and S, and in the deepest soil layer (20-25 cm) no differences were found among the treatments. The distribution pattern of the OC stocks in the stable S+C fraction (OC-S+C) was different to those of the more-labile fractions. Along the total soil profile, the OC-S+C was significantly higher in S than in AT and T, and no differences occurred between the AT and T treatments. The oxidation-resistant organic carbon (rSOC) showed the same pattern as the S+C fraction (Figure 4.6). These results show that there was more stabilized OC in the shrubland, but greater inputs of fresh plant debris in the afforested soils, mainly in the AT treatment. In the surface soil layer, most of the SOC of the shrubland was found in the S+C fraction (38%), while in the afforested soils the POM fraction accounted for most of the total C content (55% and 53% in AT and T, respectively). Below 5 cm depth, the POM fraction decreased in all treatments, more drastically in AT and T, and the greatest percentages of SOC were allocated in the S+A and S+C fractions, showing an increased stabilization of organic C with depth in all the treatments.

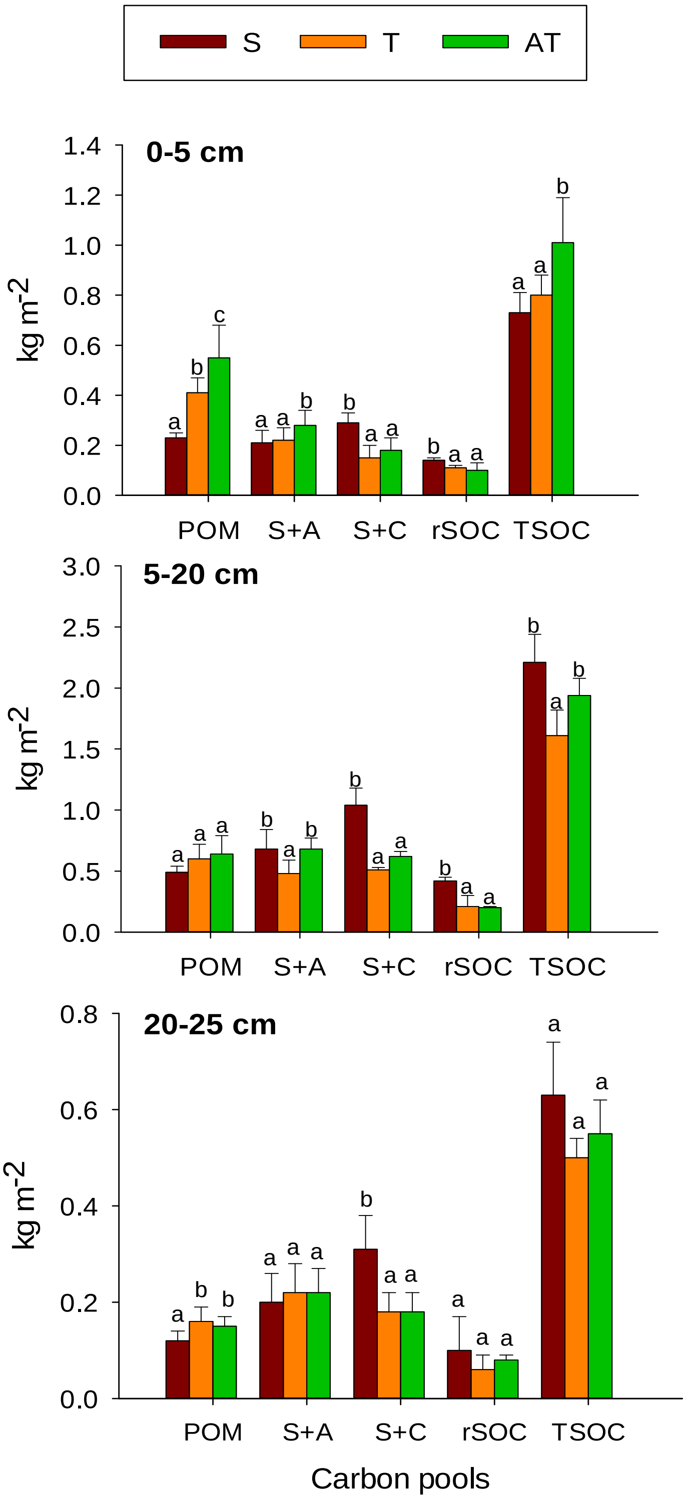


Figure 4.6. Organic carbon stock (mean ± standard errors) in the particulate organic matter (POM), sand and stable aggregates (S+A), silt and clay (S+C), resistant soil organic carbon (rSOC) pools and total SOC (kg m^{-2}) at 0–5, 5–20 and 20–25 cm depth soil layer at control plot (S), mechanical terracing (T) and mechanical terracing combined with soil amendment (AT). Different letters in bars means significant differences between treatments (Tukey´s test, $p < 0.05$) within each pool.

4.4. Discussion

4.4.1. Impact of afforestation on soil properties

There is no agreement in the literature in relation to the effects of afforestation on soil properties. In general, most authors reported losses in soil quality when comparing plantations with natural forests or shrublands (Liao et al., 2012). By contrast, improvements in soil characteristics have been reported when comparing plantations with bare soils or open, degraded scrublands (Maestre and Cortina, 2004; Fernández-Ondoño et al., 2010). The results reported in the literature vary widely depending on different factors, mainly environmental factors such as climate and mineral substrate. Our results clearly show that 20 years after the plantations were set up, the afforestation technique had a distinct impact on soil properties.

The afforestation of the soil terraces with an organic amendment (AT treatment) led to a significant improvement of the chemical and physical soil properties, but also to a slight deterioration of the biological properties, when compared with the dynamic of unplanted shrubland. However, the afforestation approach through terracing without soil amendment (T treatment) led to a pronounced decrease of soil fertility and soil physical and biological properties. Still the used species may have a substantial effect on C incorporation (Jeddi et al., 2009), our results clearly showed the key role of site preparation in the afforestation in semiarid areas. Likewise, Maestre and Cortina (2004) reported that the effects of *P. halepensis* plantations on soil properties are highly dependent on the planting technique employed.

Mechanical terracing modifies the natural conditions of the soil as the epipedon is removed. As a result, soil fertility as well as physical and biological properties sharply decrease (Finkel, 1986; Barber and Romero, 1994). Our results show decreases in the OC, total N, available P and K, water retention capacity, structural stability, and microbial activity, as well as soil compaction, following the terracing. This decline of soil fertility slowed down the growth of trees in the T treatment, leading to low net primary production and reduced plant debris inputs into the soil. This prevented offsetting of the negative impact of the terraces 20 years after planting. In spite of this, it must be noted that the increase in the collection of rain water, due to terracing, enabled the survival of the stands. Losses of soil fertility in pine plantations - with respect to adjacent shrublands - in semiarid areas were also reported by Castillo et al. (2002) and Goberna et al. (2007). In turn, Ruiz-Navarro et al. (2009) did not observe changes in soil fertility after 30 years of shrubland afforestation, as the planted pines offset the terracing disturbance but did not improve the previous soil fertility. However, other studies found that the soil fertility in pine plantations was higher than in open shrublands (Maestre et al., 2003; Fernández-Ondoño et al., 2010). This apparent contradiction seems to be due to the previous level of soil fertility of the afforested shrubland and the plantations age.

In the AT treatment, the decrease in soil fertility caused by the mechanical works disturbance was initially offset by the organic amendment. This soil fertilization enabled greater and faster growth of the planted trees and, over time, greater litter input into the soil, which fostered the improvement of soil characteristics. This was also supported by the results for the SOC pools distribution, discussed later. Our results agree with those of other authors showing the effectiveness of soil organic fertilization in the afforestation of semiarid areas. Ortiz et al. (2012) showed 4-fold higher growth for *P. halepensis* fertilized with sewage sludge and a proportional increase of SOC. Likewise, Barbera et al. (2005) found that organic amendment enhanced the growth of *P. halepensis* in the afforestation of a semiarid Mediterranean pediment ecosystem and emphasize the importance of nutrient supply in semiarid soils.

The changes in the physical soil properties point towards a distinct impact of the site preparation method employed in afforestations. The input of OM in AT promoted the formation of stable aggregates and increased the WHC and available water in the soil, thus reducing the negative effect of terracing on the physical soil properties. Similar results on changes in physical soil properties after organic amendments in semiarid areas were reported in other studies (Barzegar et al., 2002; Albaladejo et al., 2008). At the same time, the greater biomass production in AT delivered larger inputs of plant litter into the soil, which promoted a gradual improvement of the physical soil properties. Other authors also reported improvements in physical soil properties after afforestation (Caravaca et al., 2002; Kahle et al., 2005). Afforestation without soil amendment (T treatment) had a negative impact, as indicated by the increase in bulk density and decreases in WHC and stable aggregates, which was still evident 20 years after planting.

Likewise, Zheng et al. (2005) and Liao et al. (2012) reported degradation of physical soil properties after pine plantation. In semiarid areas, *P. halepensis* plantations usually show lower soil moisture contents when compared with adjacent plant communities (Bellot et al., 2004). The effect of *P. halepensis* on soil moisture may arise from both rainfall interception and water uptake (Maestre and Cortina, 2004). This may result, as in our study, in a lack of recovery of the spontaneous vegetation in the areas afforested with pines trees, which limits their effectiveness. This is a negative aspect, according to the objectives of afforestation in semiarid areas.

The dynamic of biological properties is a key factor in the understanding of soil rehabilitation following afforestation. Our results show a strong decrease in microbial activity in T treatment and a weak change in AT. In agreement with this, Zheng et al. (2005) reported higher microbial carbon (MC) contents in natural forests than in planted forests. However, there are few data in the literature on the effects of semiarid shrubland afforestation on soil microbial activity, and the results are contradictory. Goberna et al. (2007) found that the MC and microbial activity were significantly lower in soils under pine plantations than in those of the adjacent shrubland, but no difference was found between afforested and adjacent grassland soils. In turn, Chen et al. (2008) showed that grassland afforestation leads to lower MC, indicating a decrease in biological fertility, and suggested that this may be associated with lower and less-labile organic inputs into mineral soils under coniferous trees compared with grassland. However, Mao and Zheng (2010) reported an initial decrease in MC following the afforestation of agricultural land in a semiarid area of northeast China, but it then increased with stand development. We found higher MC and biological fertility in control soil S than in the afforested areas. These data, which agree with the related results in the literature mentioned above, indicate an initial negative impact of afforestation on soil biological properties, which recover progressively with stand development. The intensity of the initial impact and the rate of recovery depend on: (i) the previous level of soil quality, and (ii) the factors controlling tree growth (mainly climatic and micro-climatic conditions and soil fertility). Although the differences in the biological variables between AT and T were not very strong, they showed a clear trend towards higher recovery rate of biological properties in AT than in T, as suggested by the enzymatic activity results. This must be due to the initial effect of organic amendment on soil microbial activity and soil fertility. In agreement, other studies (Diaz et al., 1994; Allen and Schlesinger, 2004) found microbial biomass increases in the soil after organic amendments.

In summary, the results for the AT and T treatments suggest that, although water is the main factor affecting tree development, the availability of nutrients is a further key factor in semiarid areas. Likewise, Roldan et al. (1996b) reported that soil fertility levels were significantly correlated with *P. halepensis* seedling growth, particularly the available phosphorus. So, in semiarid areas, soil preparation prior to afforestation, in addition to favoring water harvesting, must maintain or increase soil fertility; technologies involving major disturbance of the soil should be avoided.

Overall, the chemical, physical, and biological changes in the soil following the afforestation show that in the AT treatment the soil disturbance by mechanical terracing was offset; however, the T treatment was still insufficient, after 20 years, to offset the impact of terracing on the soil properties.

4.4.2. Total carbon sequestration in the ecosystems

The difference in total C content between the native shrubland and the afforested lands serves as an indicator of the C gains or losses after land use changes. In our study, the afforestation of semiarid shrubland resulted in losses or gains of OC according to the site preparation technique. Based on the OC contents in the soil and aerial biomass, afforestation with the AT treatment led to an ecosystem gain of 1.3 kg C m^{-2} over 20 years. The average annual rate of C increase was 65 g C m^{-2} $year^{-1}$. The increase of C stocks was related to the accumulation of C in the biomass, mainly in the planted pines, and to a lesser extent in litter. Several studies on C sequestration after afforestation have shown that most of the C was accumulated in the biomass (Grünzweig et al., 2003; Nosetto et al., 2006; Thuille et al., 2006). In contrast, the afforestation without soil amendment (T treatment) led to a decrease in ecosystem carbon of 0.60 kg C m^{-2} over 20 years. All the carbon lost in the ecosystem after the T treatment was from the mineral soil, which represents a concern, in terms of C sequestration, due to the greater stability of the SOC compared with the stocks in biomass (Thuille and Detlefschulze et al., 2006). Both increases and decreases in ecosystem carbon stocks after afforestation have been reported by other authors (see Paul et al., 2002 for a review). A decrease of 24 g C m^{-2} $year^{-1}$ in net ecosystem production in an initial stage (9-23 years) of a pine plantation under semiarid conditions was reported by Paul et al. (2003). Also, Guo and Gifford (2002) found a decrease in soil C stocks of 12-15% in pine plantations on prior pastures, and Wiesmeier et al. (2009) reported a depletion of SOC following plantations of *Pinus taeda* in Brazilian grasslands.

The comparison of the total ecosystem C at the initial state of the afforested areas (after mechanical work for terracing and previous to pine planting) with that 20 years after planting serves to indicate the potential C sequestration capacity of the afforestation. Mechanical terracing involved the removal of all the vegetation and the top 30 cm of the soil, reducing the ecosystem C stock to the C content of the remaining soil. This stock, measured just after terracing was 2.1 kg C m^{-2} in the soil profile (0-25 cm), before the trees were planted. Indeed, the total ecosystem C accumulation produced by the afforestation, over 20 years, was 3.2 and 1.3 kg C m^{-2} for AT and T, respectively, and the annual average was 160 g C m^{-2} $year^{-1}$ and 65 g C m^{-2} $year^{-1}$, respectively. The results for AT were comparable with the mean annual C sink of 150 g C m^{-2} $year^{-1}$ reported by Grünzweig et al. (2003) for an arid-land forest in Israel. However, it must be considered that our results cover only the initial stages of forest development (20 years), while the plantation in Israel was 35 years-old. Several studies on soil C dynamics after afforestation showed that soil C is predicted to decrease between 0 and 10 years, while it is predicted to increase between 10 and 40 years after afforestation (Paul et al., 2003).

Therefore, it is consistent with this that the AT treatment clearly could exceed the accumulation rate found by Grünzweig et al. (2003) for a 35-year-old plantation. However, the values for the T treatment are far below this rate. These results support our finding that soil fertility has a key role in the afforestation of semiarid areas. Several authors reported that nutrient use efficiency (particularly N and P) is a key factor controlling C storage (Conant et. al., 1998; Post and Kwon, 2000). In this study, the organic amendment in the AT treatment increased nutrient availability and gave rise to a significantly-higher C sequestration potential than the T treatment. The increased N-use efficiency was corroborated by an overall increase in the C/N ratio, from 6.8 in S to 8.3 and 9.8 in T and AT, respectively.

4.4.3. Soil carbon sequestration

Since changes in soil C stocks following land-use changes occur mainly in the topsoil (De Gryze et al., 2004), the discussion of C accumulation in the mineral soil has been focused on the 0-5 cm soil layer. For this purpose, we compared the topsoil C stocks (0-5 cm depth) in the terraced soil before the planting of the trees (just after terracing and before soil amendment) with those for treatments T and AT 20 years after planting. Before planting, the OC stock was 0.44 kg C m^{-2}; over 20 years, it increased to 1.0 and 0.78 kg C m^{-2} for AT and T, respectively. This represents a soil carbon sequestration of 0.56 and 0.34 kg C m^{-2} in AT and T, respectively. The average annual sequestration rate was 28 g C m^{-2} $year^{-1}$ in AT and 17 g C m^{-2} $year^{-1}$ in T. Thus, the sequestration rate was 65% higher in AT.

Comparisons of these C sequestration rates with those of other planted forests are associated with uncertainty due to the high number of factors involved. The amount of OM input, which increases with temperature and precipitation, is a major factor determining the rate of accumulation of SOC (Post and Kwon, 2000; Thuille and Detlefschulze, 2006). In a review on soil carbon sequestration and land-use change, Post and Kown (2000) estimated for the world´s afforested systems an average accumulation rate of 30-50 g C m^{-2} $year^{-1}$ in the 0-7 cm soil layer. These values are slightly higher than our estimations for AT (28 g C m^{-2} $year^{-1}$) and twice as high as the value for T (17 g C m^{-2} $year^{-1}$), which seems reasonable due to the lower average precipitation in our study area.

The results show an enrichment in the 0-5 cm soil layer of the AT treatment for both the OC-POM, the younger and more-plant-derived fraction, and the OC-S+A heavy fraction (63-2000 µm aggregates). Similar results for changes in OM pools following afforestation were reported from recently-established plantations in the southeastern US (Garten, 2002). The results may differ according to the time elapsed since planting and the stand development rate. A decrease in POM in the first ten years, due to mechanical site preparation, was found by Gartzia-Bengoetxea et al. (2009) and Mao and Zeng (2010), while from 10-20 years this decrease was overcome by increased plant litter as the trees grew. In old afforested soils, Cromack et al. (1999) found that the POM fraction could constitute as much as 40% of the total C. In our study, the OC-POM reached 55 and

53% of the total SOC stocks (0-5 cm depth) in the AT and T treatments, respectively. This high proportion of POM in our study area may be due to the higher recalcitrance of the lower quality litter of pine stands compared to the shrubland, as shown by C/N ratios of 92 and 65, respectively (data not shown). Our results are in agreement with those of Grünzweig et al. (2007), who reported decreased decomposition of litter and SOC in a semiarid planted forest, when compared to an adjacent shrubland.

4.4.4. Carbon stabilization mechanisms

As shown above, 20 years after the installation of the stand plantations the surface soil layer (0-5 cm) had sequestered 0.56 and 0.34 kg C m^{-2} in AT and T, respectively. Unfortunately, we do not have the stocks of the functional pools in the soil before planting, to determine the changes after afforestation, but we can compare the differences between AT and T in order to identify the distribution of the sequestered C among the different pools. Comparing the SOC stocks (0-5 cm depth) in AT and T (Figure 4.6), the total SOC stock was 0.23 kg C m^{-2} higher in AT than in T. The distribution of this increase was: 0.14 kg C m^{-2} POM (61%), 0.06 kg C m^{-2} S+A (26%), and 0.03 kg C m^{-2} S+C (13%). Considering the total soil profile (0-25 cm), the total increase in OC was 0.6 kg C m^{-2}, with the following distribution: 0.26 kg C m^{-2} S+A (46%), 0.17 kg C m^{-2} POM (30%), and 0.14 kg C m^{-2} S+C (24%). These results suggest that the three main mechanisms of SOC stabilization (Six et al., 2002b; von Lützow et al., 2006) were involved in this study: a) biochemical stabilization due to selective preservation of recalcitrant plant litter (POM), b) physical protection by occlusion in aggregates (S+A), and c) chemical stabilization by interaction with mineral particles (S+C). The relative importance of each process changed with depth. In this study, biochemical stabilization was greatest in the surface soil and sharply decreased with depth, while physical protection and chemical stabilization were more relevant in the subsoil.

The formation of the new aggregates after afforestation, and the accumulation and stabilization of OC by occlusion in the new aggregates, must be favored by the increase in POM, which acts as a binding agent and promotes the formation of soil aggregates (Jastrow, 1996; Wiesmeier et al., 2012). In the AT treatment, the organic amendment also contributed to higher aggregates formation. In the T treatment, the lower input of POM limited the aggregate formation and, as a result, the amount of physically-protected organic C.

The chemical stabilization of OC by associations with silt and clay particles was favored by the composition of the mineral soil matrix. The mechanisms of protection of OM in mineral soils can be mainly attributed to the presence of multivalent cations and surfaces capable of absorbing organic materials in the soil matrix (Baldock and Skjemstad, 2000). The ability of a source of Ca^{2+} cations to protect SOM from mineralization has been well demonstrated (Duchaufour, 1976, Muneer and Oades, 1989). In our study, the high proportion of calcium and minerals with high specific surface area (mainly the inter-stratified illite-montmorillonite) in the soil matrix promoted the formation of organo-mineral associations and the associated stabilization of OC in the S+C fraction. This latter mechanism was slower than the other processes and is indicative of mature and more-recalcitrant OM. In agreement, similar results showing the enhancement of carbon sequestration by physical and physicochemical protection in mineral soils of afforested systems were reported by Six et al. (2002b) and De Gryze et al. (2004).

With respect to the ability of the afforested areas to continue sequestering carbon in the mineral soil over the coming decades, we hypothesize that the potential C storage capacity of both afforested ecosystems is not saturated and that they will continue sequestering carbon as they reach their mature age. There is no mechanistic explanation of a saturation level, if it exists, for the litter and POM fraction (Six et al., 2002b) and thus this process may proceed with increasing biomass production. The degree of soil aggregation is still lower than in the natural forests in these areas and will probably increase with new POM input; this, in turn, would increase the physically-protected C pool. Finally, the mineral-associated C-pool is determined by the mineralogy and size distribution of soil mineral particles (Baldock and Skjemstad, 2000). Since the characteristics of the soil matrix are similar in the shrubland and afforested areas, comparison of the OC allocation in the fractions suggests that the S+C pool is not saturated in the afforested areas, particularly in deeper parts of the soil.

4.5. Conclusions

Our results support the hypothesis that C sequestration, following afforestation in semiarid areas, can be increased by using suitable afforestation techniques. The ecosystems C stock following afforestation was proportional to the increase in plant biomass production, which in turn was determined by soil fertility. Soil organic amendment was very effective in terms of C sequestration. Site preparation techniques involving large soil disturbances are not recommended. The following processes were involved in the SOC sequestration and stabilization: biochemical stabilization due to the higher pine litter production and recalcitrance, physical protection due to improved soil aggregation, and chemical stabilization as a result of the chemical and physicochemical binding between SOC and clay and silt particles. Our results suggest that the protective capacity of the soil in the afforested areas is not saturated, and further litter input will increase the soil OC content.

Chapter 5. *Stabilization mechanisms of SOC under semiarid forest soil use*

5.1. Introduction

Among ecosystem services provided by soils, climate change mitigation through C sequestration is taking growing interest, especially from the suggested emissions limitations based on a C credit trading system in the Kyoto Protocol (Intergovernmental Panel on Climate Change, 1997; Six et al., 2002b). The SOC (soil organic carbon) sequestration may be achieved by means of afforestation and other land-use conversion (De Gryze et al., 2004). However, afforestation does not always cause the expected effects. Despite the considerable SOC sequestration potential of afforestation the results reported by different studies are contradictory (Wiesmeier et al., 2009; Cao et al., 2010; Laganière et al., 2010). A better understanding is needed of the mechanisms and factors controlling the accrual and stabilization of SOC following afforestation.

Various manipulation procedures of plant inputs, many of them inherent to afforestation, are often suggested as methods for enhancing SOC sequestration (Rees et al., 2005). The amount and quality of plant litter inputs is a key factor controlling the accumulation of SOC (Kögel-Knabner et al., 2002), at the same time that promote the processes involved in soil aggregation (Denef et al., 2001; Abiven et al., 2007). Physical soil properties such as soil structure or aggregation regulate many biological and chemical soil processes linked with C sequestration. It has been widely established that the formation of soil aggregates promotes the protection of organic matter against decomposition and oxidation (von Lützow et al., 2006; Jastrow et al., 2007). According to conceptual model of Golchin et al. (1994), the fresh and labile pools of organic matter cause a rapid stimulation of the soil microflora accompanied by a significant increase in macroaggregates formation. Other authors showed significant correlations between the labile C pools and soil aggregation (Bhattacharyya et al., 2012). In addition, the O-alkyl such as carbohydrates have been considered as a major labile organic C source for microbial activity, fostering the binding of clay and silt-size particles and the formation of microaggregates into macroaggregates, increasing the stability of soil aggregates (Jastrow, 1996; Six et al., 2000a). In addition to the substantial role of labile organic matter inputs, many studies have pointed to the important function of soil microorganisms in the formation and stabilization of soil aggregates (Lynch and Bragg, 1985; Diaz et al., 1994; Siddiky, et al., 2012). The microorganisms act in two ways: a) mechanical union of soil particles by fungi hyphae, and b) the exudation of byproducts that promote coalescence of primary particles (De Gryze et al., 2005; Helfrich et al., 2008). Generally, fungi are more important in soil aggregate formation compared to bacteria (De Gryze at al., 2005). Indeed, as pointed Jastrow et al (2007), manipulations for enhanced C sequestration would include shifting the soil microbial community towards increased fungi populations.

The change of microbial structure or the introduction of microorganisms into the soil, with lasting effects, is very difficult to tackle with current technologies (Jastrow et al., 2007). A possible option could be to cause changes in the vegetal cover through afforestation. Several studies suggest a strong effect of vegetation type on microbial community structure (Kuske et al., 2002; Costa et al., 2006). Afforestation is a key land-use change across the world and is considered to be a dominant factor controlling ecosystems functioning and biodiversity, however, the response of soil microbial communities to this change is not well understood (Macdonald et al., 2009). This study help to increase the knowledge on this response using next-generation sequencing techniques to provide a detailed analysis of the structure, diversity and taxonomic composition of both bacterial and fungal communities in natural shrubland and afforested soil under semiarid conditions.

In this study we show the effects of two afforestation techniques on soil aggregation and physical-chemical protection of SOC. We start from the following hypothesis: The change in vegetal cover after afforestation increases fresh litter inputs and promotes shifts in microbial community leading to higher soil aggregation and physical protection of SOC. The study was accomplished under Mediterranean semiarid conditions (Southeast Spain), in an area widely representative of other semiarid areas. Indeed, these results could be extrapolated to wide global surfaces. Previous investigations, at the same experimental site, proved short-term positive effects in increasing biomass production and improving soil characteristics (Querejeta et al., 1998; Querejeta et al., 2000). After 20 years of this afforestation, Garcia-Franco et al. (2014) showed that, in long-term, afforestation of semiarid shrublands can result in either sequestration or loss of SOC depending on site preparation technique used. The specific objectives of this study were to analyze the effects of afforestation of degraded shrublands on: *1)* changes in soil aggregation, *2)* remodeling of microbial community structure, and *3)* physical-chemical processes of SOC protection and stabilization.

5.2. Material and methods

5.2.1. Site description and experimental design

The same study area and experimental design studied in this Chapter are described in the Chapter 4.

5.2.2. Soil sampling design

In April 2012, 20 years after afforestation, six soil sampling sites were randomly selected and samples were collected in the terraces and adjacent shrubland at three soil depths (0-5 cm, 5-20 cm and 20-25 cm), accounting for a total of 54 samples (3 treatments x 3 sampling depths x 6 replicates). The structure of soil bacteria and fungi community was determined at the surface (0-5cm depth) taking three composite samples per treatment.

5.2.3. Analytical methods

5.2.3.1. Functional OC pools of bulk soil

Functional OC pools were separated with a combined physical and chemical fractionation method, according to Zimmermann et al. (2007) (Figure 5.1). Thirty grams of soil (<2000 µm) were added to 150 mL of water and dispersed using a calibrated ultrasonic probe-type with an output energy of 22 J mL^{-1}. Application of more energy may disrupt the coarse sand-sized SOM (Amelung and Zech, 1999). The dispersed suspension was then wet-sieved over a 63 µm aperture sieve until the rinsing water was clear. The fraction >63 µm, containing the sand fraction and stable aggregates (S+A) together with particulate organic matter (POM), was dried at 40°C and weighed. The suspension <63 µm was filtered through a 0.45 µm aperture nylon mesh and the material >0.45 µm was dried at 40°C and weighed. The POM was separated by stirring the fraction >63 µm with sodium polytungstate at a density of 1.6 g cm^{-3} (Cerli et al., 2012). The mixture was centrifuged at 1000g for 15 min and the light fraction was decanted. Both fractions were washed with deionized water to remove all sodium polytungstate, dried at 40 °C, and weighed. The OC concentrations were determined for every fraction using an Elemental Analyzer (LECO TRUSPEC CN, Michigan, USA). The samples were analyzed in triplicate. The OC content at the soil level was calculated with the following equations:

(OC) = $(OC)_{fraction}$*(pool $proportion)_{soil}$ (g C/Kgsoil) (5.1)

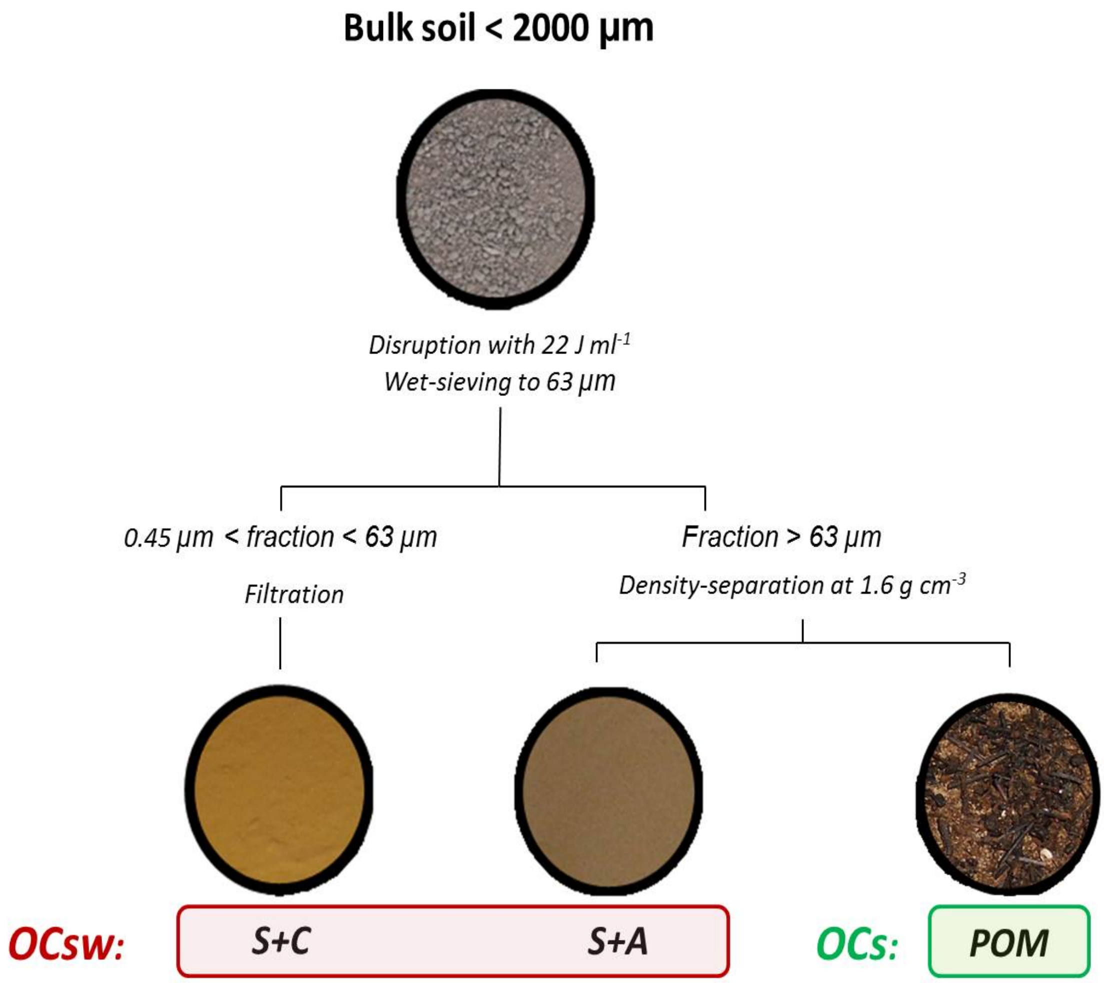


Figure 5.1. Adaptation of diagram which show the fractionation procedure according to Zimmermann et al. (2007): S+C (silt-clay fraction); S+A (sand and stable aggregates); POM (particulate organic matter); OCsw (slow OC pool) and OCs (sensitive OC pool).

In this study, we have processed the OC pools in two main groups according to its turnover rates: i) a sensitive pool formed by particulate organic matter (OCs) and ii) a slow pool constituted by the OC associated to clay and silt and the OC stabilized in aggregates (OCsw).

Characterization of the OCs pool

Molecular composition of the OCs pool at 0-5 cm depth was determined by ^{13}C-NMR spectroscopic analysis using a Varian Unity 300 spectrometer. Samples were filled into zirconium dioxide rotors (7 mm diameter) and spun in a magic angle spinning probe at a rotation speed of 4.0 kHz to minimize chemical anisotropy. A ramped 1H pulse was used during a contact time of 1 ms to prevent Hartmann-Hahn mismatches. The contact time will be 1500 ms. For each sample 20000 running was carried out before the final spectrum. For integration, chemical shift regions were used as follows: i) aliphatic or alkyl-C (0–45 ppm) of lipids, fatty acids, and plant aliphatic polymers; (ii) O-alkyl-C (45–110 ppm) deriving primarily from polysaccharides (cellulose and hemicelluloses), but also from proteins and side chains of lignin; (iii) aromatic or aryl-C (110–162 ppm), deriving from lignin and/or protein; and finally (iv) carbonyl-C (162–190 ppm) from aliphatic esters, carboxyl groups and amide carbonyls. Integration of the peaks within each of the chemical shift regions allowed estimation of the relative C contents expressed as percentages of the total area (Helfrich et al., 2006).

5.2.3.2. Diversity and structure of soil microorganisms

Soil DNA was extracted by using the FastDNA Kit (Qbiogene Inc., USA) and purified on Low Melting Point agarose gel 1.25 (wt/vol) containing with 2% of Polyvinylpyrrolidone (PVP) (Young et al., 1993) DNA extracts were analyzed on 1% agarose gels and their final concentration quantified with a Nanodrop 2000 (Thermo scientific). The universal Eubacterial primers BSF8 (5’-TCAGAGTTTGATCCTGGCTCAG-3’) and USR515 (5’-CACCGCCGCKGCTGGCA-3) were used for amplifying the 16S rDNA *V3 fragment* (Bibby et al., 2010). The *universal eukaryotic primers* nu-SSU-0817-59, (5’TTAGCATGGAATAA TRRAATAGGA-3’) y nu-SSU-1196-39 (5‘-TCTGGACCTGGTGAG TTTCC-3’) was used for amplifying the 18S rDNA V4 fragment (Borneman et al., 2000). Amplifications were performed in a MyCycler Tm Thermal cycler (Bio-Rad Laboratories Inc., USA). The universal Eubacterial primers BSF8 (5’-TCAGAGTTTGATCCTGGCTCAG-3’) and USR515 (5’-CACCGCCGCKGCTGGCA-3') were used for amplifying the 16S rDNA V3 fragment (Bibby et al., 2010). The universal eukaryotic primers nu-SSU-0817-59, (5’TTAGCATGG AATAATRRAATAGGA-3’) y nu-SSU-1196-39 (5‘-TCTGGACCTGGTGAGTTTCC-3’) was used for amplifying the 18S rDNA V4 fragment (Borneman et al., 2000). Amplifications were performed in a MyCycler Tm Thermal cycler (Bio-Rad Laboratories Inc., USA). The PCR conditions used for amplification of 16S rDNA fragment was: 94°C for 5 min, followed by 30 cycles consisting of 30 s at 94°C, 30 s at 56 °C, and 90 s at 72°C, and a final elongation step at 72°C for 10 min. While, the 18S rDNA V4 fragment was amplified using the following method: 94°C for 2 min then 35 cycles of 94°C for 10 s, 56°C for 10 s, and 72°C for 30 s, followed by 72°C for 2 min. The amplicon length and concentration were estimated, and an equimolar mix of all amplicon libraries was used for tag-encoded FLX-titanium amplicon pyrosequencing (Roche, Switzerland).

Processing of 454 sequence data

Sequences were processed using QIIME v. 1.5.0 (Caporaso et al., 2010) by selecting sequences with a quality score >25, containing no ambiguous bases, without any primer mismatches, and a sequence length between 200–400 bp (both bacteria and fungi sequences). After quality checking, counting, sorting and denoising, chimeras were identified using ChimeraSlayer (Haas et al., 2011). Clustering of the sequences into operational taxonomic units (OTUs) was performed using UCLUST (Edgar, 2010) and a cutoff value of 97% sequence identity. The most abundant sequence type within each OTU was selected to represent the respective OTU in further analysis. The taxonomic assignment was performed according to RDP (Wang et al., 2007) and the BLAST data base of NCBI. Chao1 and Shannon indices were calculated to estimate taxon richness and diversity. The community between samples was compared using weighted UniFrac distances in principal coordinate analysis (PCoA) (Hamady et al., 2010).

5.2.3.3. Soil aggregate-size distribution

Aggregate-size separation was performed by a wet sieving method according to Elliot (1986). Briefly, 100 g air-dried (5 mm sieved) soil sample was placed on top of a 2000 µm sieve and submerged for 5 min in deionized water at room temperature. Sieving was manually done by moving the sieve up and down 3 cm, 50 times for 2 min to achieve aggregate separation. A series of three sieves (2000, 250 and 63 µm) was used to obtain four aggregate fractions: i) > 2000 µm (large-macroaggregates; LM), ii) 250 to 2000 µm (small-macroaggregates; SM), iii) 63 to 250 µm (microaggregates; m) and iv) <63 µm (silt plus clay-size particles; s+c). Aggregates sizes classes were oven dried (50°C), weighed and stored in glass jars at room temperature (21°C). Sand correction was performed for each aggregate-size class because sand was not considered to be part of those aggregate (Elliot et al., 1991).

Microaggregates contained within both, large- and small-macroaggregates (LMm and SMm, respectively) were mechanically isolated according to the methodology described by Six et al. (2000b) and Denef et al. (2004). Briefly, a 10 g macroaggregate subsample was immersed in deionized water on top of a 250 µm mesh screen inside a cylinder. Macroaggregates were shaken together with 50 glass beads (4 mm diameter) until complete macroaggregate disruption was observed. Once the macroaggregates were broken up, microaggregates and other <250 µm material passed through the mesh screen with the help of a continuous water flow. The material retained on the 63 µm sieve was wet sieved to ensure that isolated microaggregates were water stable (Six et al., 2000b). OC determination was performed separately for both, each aggregate size class and microaggregates isolated from large- and small-macroaggregates by using an Elemental Analyzer (LECO TRUSPEC CN. Michigan, USA). Samples were analyzed in triplicate. The OC content at the soil level was calculated with the following equations:

$$\mathbf{OC} = (OC)_{fraction} * (agg.proportion)_{soil}\ (g\ C/Kg_{soil}) \qquad (5.2)$$

5.2.3.4. Soil respiration measurements in macroaggregates (> 250 µm)

Basal respiration within macroaggregates was measured as the amount of CO_2-C released daily per kg of soil aggregate during incubation under controlled conditions (Nannipieri et al., 1990). Soil respiration was analyzed by placing 15 g of soil macroaggregates moistened at 60 % of their water-holding capacity in hermetically sealed 125 cm^3 flasks during a 31 day incubation period at 28°C (Bastida et al., 2007). Three repetitions were made per sample. The CO_2 released was periodically measured (every day for the first 4 days and then weekly) using an infrared gas analyzer (Toray PG-100, Toray Engineering Co. Ltd. Japan). After each measurement, stoppers were removed for 1 h to balance the atmosphere inside and outside the bottles.

5.2.4 Statistical analyses

Prior to analyses, the data were examined for normality by Kolgomorov-Smirnov test and for homogeneity of variances by the Levene's test. Data that were not distributed normally (LM, SM, LMm, OC-LM, OC-SM, OC-SM and OC-SMm) were ln-transformed. To compare all soil variables between treatments a GLM procedure was carried out which considered treatment and depth as fixed factor. Pearson correlation analysis was used in order to explore the relationships between functional OC pools (OCs and OCsw) in bulk soil and the distribution of aggregates and their associated organic carbon and respiration; and the relationships between basal respiration and the distribution of aggregates and their associated organic carbon. Analysis were computed with SPSS procedure (SPSS 19.0, Chicago, IL, USA) and significance was set at $p<0.05$.

To compare soil microbial communities between treatments, analysis of variance using distance matrices with the vegan package for R (ADONIS, R Development Core Team 2011; Oksanen et al., 2013) was performed. Statistical significance was tested against 999 null permutations. The effect of the treatments on the relative abundance of bacterial and fungal phyla was analyzed with Generalised Linear Models (GLM) on arcsine-transformed data by using R. To test the influence of soil parameters on the microbial communities we computed correlations between soil physical-chemical and (bacterial or fungal) OTU abundance distance matrices through Mantel tests with the vegan package for R. Similarly, matrix correlations between OTU abundance and aggregate-size distance matrices were performed to test for the influence of the microbial communities on the distribution of soil aggregates.

5.3. Results

5.3.1. Changes in functional SOC pools after afforestation

A significant increase in OCs and OCsw pools at 0-5 cm layer and a significant decrease of OCsw at deeper layers were found in AT compared to S. When afforestation was done without amendment (T) a significant decrease of OCsw content compared to S was obtained through the whole profile, while no changes were observed in the OCs pool (Table 5.1).

In addition, significant differences were found in the chemical composition of the OCs pool at 0-5 cm depth between the afforested (AT and T) and the shrubland (S) soils, showing the former higher percentages of O-alkyl C and lower percentages of aryl C materials than S (Table 2). No differences were detected among treatments as regards to the relative contents of the Alkyl-C or Carbonyl-C materials (Table 5.2).

Table 5.1. Sensitive OC pool (OCs) and slow OC pool (OCsw) concentrations (g kg^{-1}) in AT (afforested + organic amendment), T (afforested) and S (shrubland) at the bulk soil and at 0-5, 5-20 and 20-25 cm depth.

Depth (cm)	**Sensitive pool (OCs)**		
	S	**T**	**AT**
0-5	4.02 ± 0.10aC	5.90 ± 0.78aB	11.65 ± 1.20bB
5-20	2.59 ± 0.21aB	3.10 ± 0.26aA	3.46 ± 0.34aA
20-25	1.74 ± 0.24aA	2.45 ± 0.22aA	1.72 ± 0.31aA
Interaction: *Treatment x depth*	**	**	**
Depth (cm)	**Slow pool (OCsw)**		
	S	**T**	**AT**
0-5	8.78 ± 0.45AbB	5.87 ± 0.26aA	10.92 ± 0.59cB
5-20	9.40 ± 0.73cB	5.09 ± 0.17aA	7.02 ± 0.45bA
20-25	7.28 ± 0.46bA	5.61 ± 0.52aA	5.62 ± 0.35aA
Interaction: *Treatment x depth*	**		**
***Significant at P<0.01*			

Numerical values are means ± standard errors. Different lowercase letters in rows indicated significant differences between treatments at each depth within each OC pool and different uppercase letters in columns indicated significant differences between depths within each treatment (Tukey's test, p <0.05).

Table 5.2. Relative contents (%) of alkyl-C, O-alkyl-C, aryl-C and carbonyl-C in the surface sensitive pool in afforested treatments (AT and T) and shrubland (S) at 0-5 cm depth.

Functional group	**Chemical shift (ppm)**	**Relative content (%)**		
		S	**T**	**AT**
Alkyl-C	0-45	33.2 ± 0.05a	31.7 ± 0.8a	30.3 ± 0.6a
O-Alkyl-C:	45-110	45.1 ± 0.25a	49.2 ± 0.7b	51.1 ± 1.1b
Aryl-C:	110-165	12.5 ± 0.05b	10.2 ± 0.2a	10.4 ± 0.3a
Carbonyl-C	165-220	9.25 ± 0.15a	8.8 ± 0.4a	8.2 ±0.6a

Numerical values are means ± standard errors. Different letter in rows indicated significant differences between treatments (Tukey's test, p <0.05).

5.3.2. Changes in soil microbial community

5.3.2.1. Soil bacterial communities

Bacterial community structure was not significantly different across treatments (Adonis; F = 1.1596, R^2 = 0.279, P = 0.104). No relationship was found between the soil physical and chemical parameters and the bacterial community structure (Mantel test; r = 0.076, P = 0.283). Likewise, the bacterial phylogenetic distance, Chao 1 (species richness), number of observed species (unique OTUs) and Shannon's index did not differ among treatments either (Table 5.3).

The relative abundance of most of the dominant bacterial phyla *(Proteobacteria, Actinobacteria, Chloroflexi, Bacteroidetes, Gemmatimonadetes and Acidobacteria*) was not statistically different among treatments (Figure 5.2). *Planctomycetes* were less abundant in AT than in S, but differences were only marginally significant (P = 0.054). The abundance of other phyla, comprising those with relative abundances below 1 % or not assigned with sufficient confidence to any known bacterial phyla, did not differ significantly among treatments (Figure 5.2).

Table 5.3. Parameters of soil bacterial community at 0-5 cm.

	Soil bacterial community		
	S	**T**	**AT**
PD. whole tree	146.6 ± 6.3a	143.9 ± 4.5a	160.4 ± 4.5a
Observed species	3230 ± 82.8a	3271 ± 138a	3509 ± 57.6a
Chao1	11071 ± 372a	10647 ± 869a	12286 ± 386a
Shannon	10.7 ± 0.1a	10.78 ± 0.2a	11.01 ± 0.1a

Numerical values are means ± standard errors. Different letter in rows indicated significant differences between treatments (Mantel test, p <0.05).

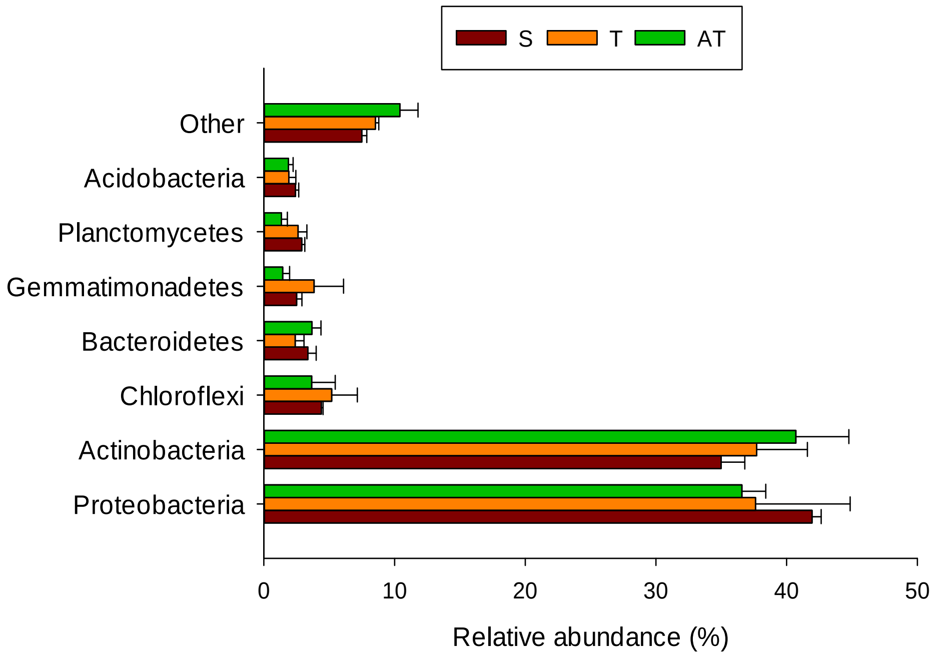


Figure 5.2. Mean ± standard errors (n = 3) for relative abundance of bacterial phyla in the soil surface (0-5cm) in AT (afforested + organic amendment), T (afforested treatment) and S (shrubland). No significant differences were detected among treatments.

5.3.2.2. Soil fungal communities

Fungal community structure differed significantly across treatments (F = 1.602, R^2= 0.348, *P* = 0.015). This was mediated by the differences in the soil physical and chemical parameters (Mantel test; r = 0.468, *P* = 0.013). In addition, fungal species richness was significantly higher in AT compared to S (F = 2.897, *P* = 0.0274), while T did not differed significantly from the other two treatments (Table 5.4). Furthermore, the phylogenetic distance among fungal species was marginally higher in AT compared to S (F = 2.266, *P* = 0.064), T not showing significant differences from AT or S. No differences were found in the number of observed fungal species or Shannon's index (Table 5.4). The relative abundance of the dominant fungal taxa (*Saccharomyceta*) was not statistically different among treatments (Figure 5.3). *Mitosporic Ascomycota* were more abundant in S than in AT (*P* = 0.006), while their abundance in T did not differ significantly from that in S or AT. Other Ascomycota were significantly more abundant in AT compared to T and S (*P* ≤ 0.01). Within *Basidiomycota (Agaricomycotina*) were significantly more abundant in AT than in S (*P* = 0.024), while their abundance in T did not differ significantly from that in S or AT. *Chytridiomycota* were significantly higher in S than in the other treatments (*P* ≤ 0.03). Finally, the abundance of other fungal taxa, comprising those with relative abundances below 1 % (i.e. *Glomeromycota*, and *Neocallimastigomycota*) or not assigned with sufficient confidence to any known fungal taxa, was significantly higher in T and AT than in S ($P \leq 0.03$) (Figure 5.3).

Table 5.4. Parameters of soil Fungal community at 0-5 cm.

	Soil Fungal community		
	S	**T**	**AT**
PD. whole tree	108.7 ± 1.5a	114.2 ± 7.2ab	125.2 ± 5.1b
Observed species	1737 ± 11.4a	1707 ± 138.2a	1934 ± 64.5a
Chao1	4374 ± 243a	5519 ± 498a	6046 ± 439b
Shannon	8.69 ± 0.1a	7.8 ± 0.6a	8.7 ± 0.2a

Numerical values are means ± standard errors. Different letter in rows indicated significant differences between treatments (Mantel test, p <0.05).

Principal coordinate analyses were performed on unweighted Unifrac distance matrices (Louzupone et al., 2006). The Bacterial community structure was different between afforested and shrub treatments (Figure 5.4a). Moreover, the variance in samples from S was lower than in afforested treatments, indicating that bacterial community structure in shrub treatments were more homogeneous. The same trend was found to fungal community structure in AT, T and S treatments (Figure 5.4b).

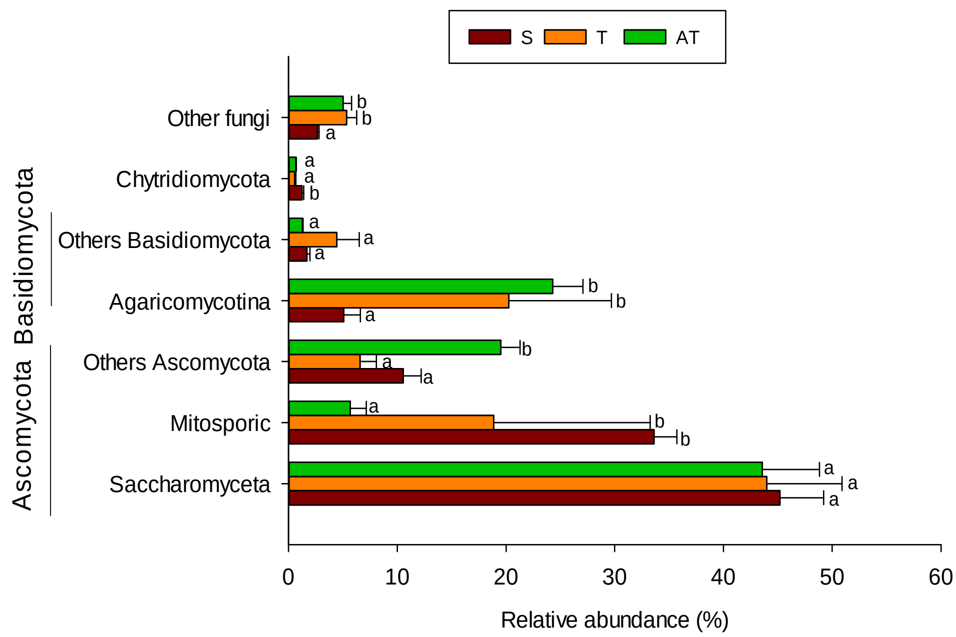
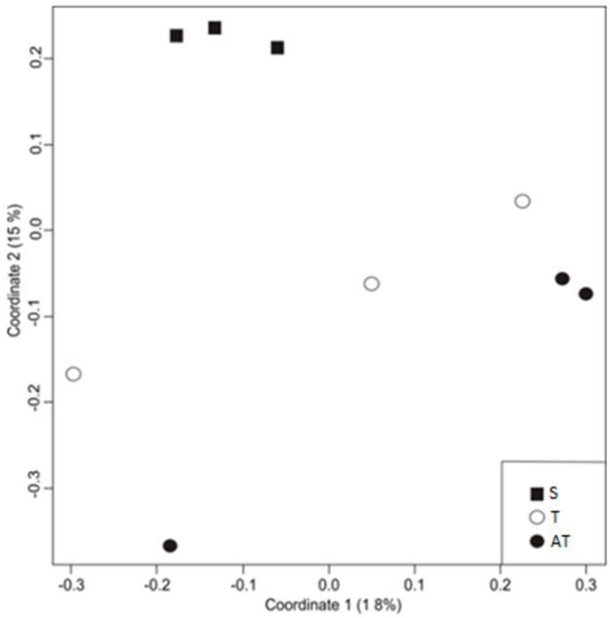


Figure 5.3. Mean ± standard errors (n = 3) for relative abundance of fungal taxa in the soil surface (0-5cm) in AT (afforested + organic amendment), T (afforested treatment) and S (shrubland). Bars with different lowercase letters indicate significant differences between treatments.

a)



b)

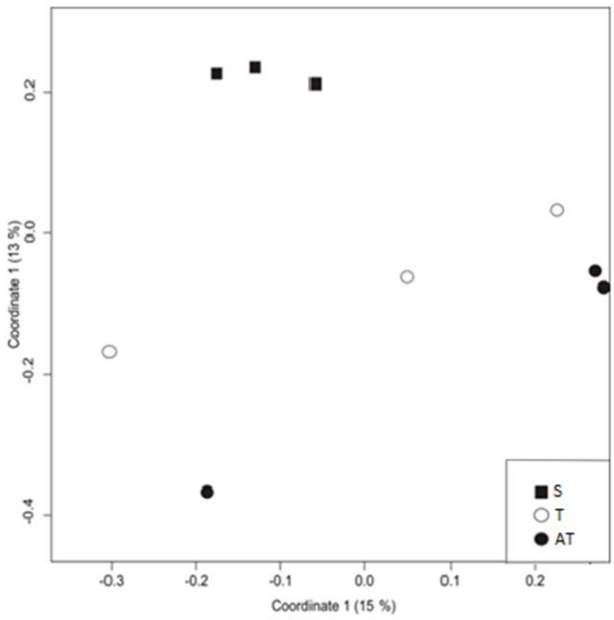


Figure 5.4. Dot-plot obtained from principal coordinate analyses and which represent: **(a)** the bacterial and **(b)** fungal community structure in every treatments: AT (afforested + organic amendment), T (afforested treatment) and S (shrubland).

In addition, the distribution of aggregate-size classes was significantly correlated to community structure of soil fungi (Mantel test; r = 0.378, P = 0.024), but not to that of soil bacteria (Mantel test; r = -0.003, P = 0.517). The same trend with a significant correlation was found between basal respiration measured in soil macroaggregates (RB-

M) and community structure of soil fungi (Mantel test; *r*= 0.462, P=0.011), but not to that of community structure of bacteria (Mantel test; *r*=0.056, P=0.352).

5.3.3. Distribution of water-stable aggregate size classes

Macroaggregates (>250 µm) was the predominant aggregate-size class for all treatments and depths representing between 65 and 80% of the bulk soil. In general, in afforested treatments a decrease of macroaggregates and an increase in microaggregates percentage was observed with depth. Also in S an increase in microaggregates percentage with depth was found. Likewise, silt-clay particles percentage increased with depth in all treatments.

The T treatment led to a reduction in LM and SM compared to S at nearly all depths. On the contrary, in AT similar proportion of LM and SM were observed at surface compared to S, while at deeper depths lower LM and higher SM percentages were found with regard to S (Figure 5.5). Afforested treatments (AT and T) showed lower microaggregates proportion compared to S across depths, while the opposite trend occurred with silt-clay particles, which were always higher in afforested treatment than in the shrubland (Figure 5.5).

5.3.4. Organic carbon associated in aggregate size classes

A reduction in the organic carbon concentrations associated to all the aggregates size classes were found in T compared to S at all depths, while AT presented similar OC-LM and OC-m concentrations than S along the soil profile, but significant higher concentration of OC-SM at the surface layer (Figure 5.6). The organic carbon associated to the silt-clay fraction (OC-s+c) was higher in AT than in T and S in the top layer, whereas AT showed the lowest OC-s+c content below 5 cm depth (Figure 5.6). OC-LM decreased with depth in all treatments, whereas OC-SM contents only decreased with depth in afforested treatments.The OC associated to macroaggregates (> 250µm) represented 47% of the total SOC in topsoil and increased until 76% at the deepest layer (20-25 cm) in AT. The opposite trend was observed in T and S. Thus, OC in macroaggregate reaches to 66% and 73 % of the total SOC in the top soil, decreasing to about 30 % and 50 % below 5 cm depth in T and S, respectively.

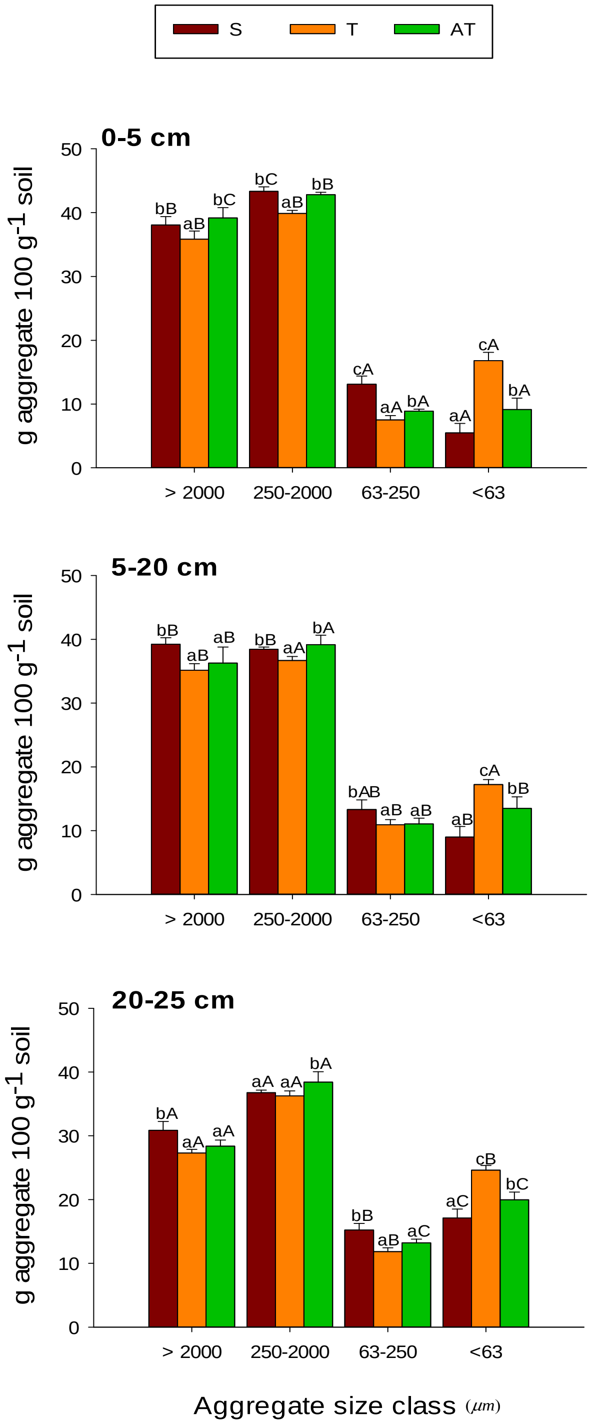


Figure 5.5. Weight percent of water-stable aggregate size distribution (g aggregate 100 g^{-1} soil): > 2000 μm (large-macroaggregates; LM), 250-2000 μm (small-macroaggregates; SM), 63-250 μm (microaggregates; m) and <63 μm (silt+clay fraction; s+c) at 0-5, 5-20 and 20-25 cm soil layers in AT (afforested + organic amendment), T (afforested treatment) and S (shrubland). Numerical values are means ± standard errors. Bars with different lowercase letters indicate significant differences between treatments at each depth and different uppercase letter indicated significant differences between depths within each treatment (Tukey´s test, p <0.05).

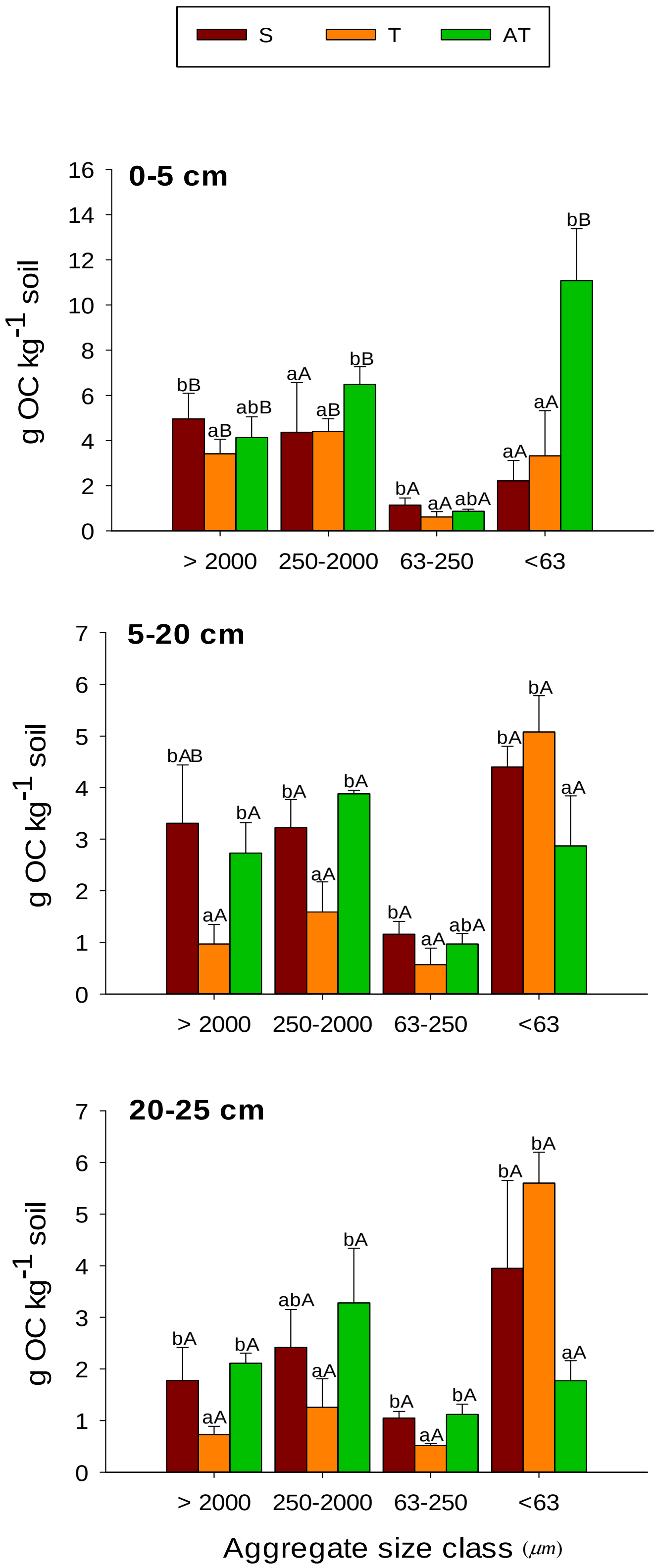


Figure 5.6. Organic carbon content (g kg^{-1} soil) of aggregates: > 2000 µm (large-macroaggregates; LM), 250-2000 µm (small-macroaggregates; SM), 63-250 µm (microaggregates; m) and <63 µm (s+c) at 0-5, 5-20 and 20-25 cm soil layers in AT (afforested + organic amendment), T (afforested treatment) and S (shrubland). Numerical values are means ± standard errors. Bars with different lowercase letters indicate significant differences between treatments at each depth and different uppercase letter indicated significant differences between depths within each treatment (Tukey´s test, $p < 0.05$).

5.3.5. Proportion of microaggregates within large- and small-macroaggregates and associated organic carbon

The AT treatment showed higher proportion of microaggregates within large-macroaggregates (LMm) and microaggregates within small-macroaggregates (SMm) than S and T at 0-5 cm depth. In turn, T treatment did not showed differences compared to the shrubland. Below 5 cm depth, no differences were found between AT and S, while T showed the lowest percentages (Table 5.5).

The percentage of microaggregates occluded within macroaggregates (LMm and SMm) decreased with depth in the afforested treatments while an increase of SM was observed in S. The OC associated to microaggregates within macroaggregates (OC-LMm and OC-SMm) showed the same pattern between treatments than the percentage of microaggregates mentioned above, showing AT higher OC-LMm and OC-SMm contents at surface respect to S and T (Table 5.6).

Table 5.5. Weight percent of microaggregates within macroaggregates (%): LMm (microaggregates within large-macroaggregates), SMm (microaggregates within small-macroaggregates); at 0-5, 5-20 and 20-25 cm soil depth as affected by AT (afforested + organic amendment), T (afforested treatment) and S (shrubland).

Proportion of microaggregates within macroaggregates (%)			
	Treatments		
	S	**T**	**AT**
LMm			
0-5 cm	14.7 ± 2.0aA	17.1 ± 1.5aC	21.6 ± 2.1bB
5-20 cm	24.2 ± 1.8cB	11.7±1.5aB	20.7 ± 2.4bAB
20-25 cm	17.4 ± 1.5bA	3.0 ±0.1aA	17.8 ± 1.4bA
Interaction: Treatment x Depth	**	**	**
SMm			
0-5 cm	14.4 ± 1.5aA	15.4 ± 1.5aA	27.5 ± 1.5bC
5-20 cm	22.3 ± 1.9bB	14.5 ± 1.5aA	20.4 ± 1.0bB
20-25 cm	23.7 ± 1.5bB	14.0 ± 0.9aA	16.4 ± 3.1aA
Interaction: Treatment x depth	**		**

**Significant at P < 0.01

Numerical values are means ± standard errors. Different lowercase letter in rows indicated significant differences between treatments at each depth and different uppercase letter in columns indicated significant differences between depths in every treatments (Tukey's test, p <0.05).

Below 5 cm depth, the T treatment exhibits the lowest OC-LMm and OC-SMm concentrations, while no differences were found between AT an S. A decrease with depth of OC-LMm and OC-SMm were observed in the afforested treatments being this reduction more drastic in T than in AT (Table 5.6).

Table 5.6. Organic carbon concentration (g C kg^{-1}soil) in microaggregates within large-macroaggregates (LMm) and small-macroaggregates (SMm); at 0-5, 5-20 and 20-25 cm soil depth as affected by AT (afforested + organic amendment), T (afforested treatment) and S (shrubland).

OC concentration (g C kg^{-1}) in microaggregates within macroaggregates						
Depth (cm)	*OC-LMm*			*OC-SMm*		
	S	**T**	**AT**	**S**	**T**	**AT**
0-5	1.3 ± 0.2aA	1.3 ± 0.2aB	2.0 ± 0.2bB	1.3 ± 0.2aA	1.4 ± 0.2aB	2.7 ± 0.3bB
5-20	2.1 ± 0.2bB	0.5 ± 0.1aA	1.3 ± 0.1abA	1.9 ± 0.2bA	0.7 ± 0.2AaB	1.8 ± 0.2bA
20-25	1.2 ± 0.2bA	0.1 ± 0.03aA	1.1 ± 0.3bA	1.5 ± 0.3bA	0.5 ± 0.2aA	1.3 ± 0.1bA
Interaction: Treatment x Depth	**	**	*		*	**

***Significant at P < 0.01 * Significant at P< 0.05*

Numerical values are means ± standard errors. Different lowercase letter in rows indicated significant differences between treatments at each depth and different uppercase letters in columns indicated significant differences between depths in every treatments (Tukey's test, p <0.05).

5.3.6. Soil respiration measurements

Significant differences in soil basal respiration were found between treatments. The AT treatment showed higher BR than S and T at all depths (Figure 5.7).

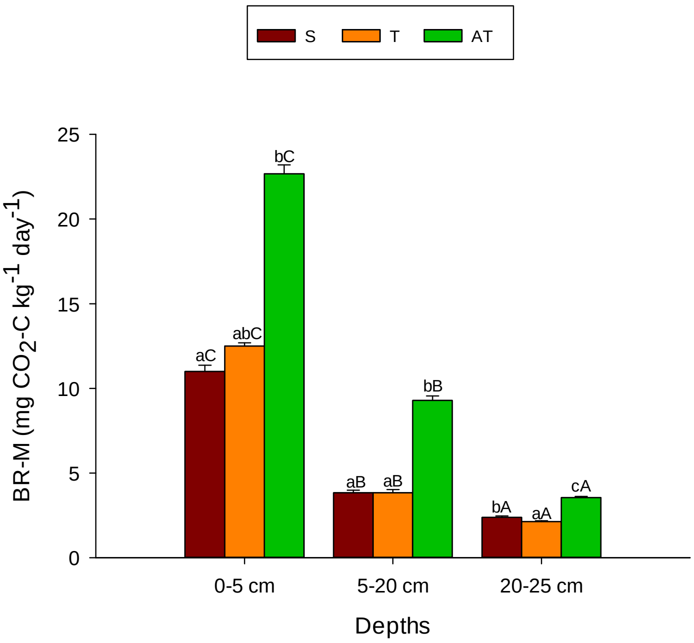


Figure 5.7. Basal respiration (BR: mg CO_2-C kg^{-1} aggregate day^{-1}) in macroaggregates at 0-5, 5-20 and 20-25 cm soil layers as affected by AT (afforested + organic amendment), T (afforested treatment) and S (shrublands). Numerical values are means ± standard errors. Bars with different lowercase letters indicate significant differences between treatments at each depth and different uppercase letter indicated significant differences between depths within each treatment (Tukey test, p <0.05).

At surface, higher BR was observed in T compared to S, while below 5 cm not significant differences were observed between them.

5.3.7 Correlations between functional OC pools, basal respiration and microaggregates within macroaggregates

Due to the similar characteristics and behavior of LM and SM in all the treatments, we are grouped both size-class in a sole of macroaggregates > 250 µm (M), to facilitate the interpretation of the correlations. For these correlations we considered the entire soil profile. Important differences were found between the treatments (Table 5.7).

Table 5.7. Pearson correlation coefficients of the sensitive pool (OCs), slow pool (OCsw) and basal respiration with the aggregates percentage and aggregates associated OC.

	Treatments		
Correlations:	**S**	**T**	**AT**
OCs:			
M (%)	0.816**	0.684**	0.828**
m (%)	ns	-0.714**	-0.836**
Mm (%)	ns	0.683**	0.860**
OC-M (g kg^{-1})	0.780**	0.659**	0.931**
OC-m (g kg^{-1})	ns	Ns	ns
OC-Mm (g kg^{-1})	ns	Ns	0.864**
BR-M (mg CO_2-C kg^{-1} d^{-1})	0.877**	0.784**	0.925**
OCsw:			
M (%)	0.567*	Ns	0.786**
m (%)	ns	Ns	-0.867**
Mm (%)	ns	Ns	0.752**
OC-M (g kg^{-1})	0.552*	Ns	0.743**
OC-m (g kg^{-1})	ns	Ns	ns
OC-Mm (g kg^{-1})	ns	Ns	0.746**
BR-M(mg CO_2-C kg^{-1} d^{-1})	ns	Ns	0.896**
BR-M (mg CO_2-C kg^{-1} d^{-1})			
M (%)	0.798**	0.817**	0.933**
m (%)	ns	-0.922**	-0.918**
Mm (%)	-0.838**	0.855**	0.911**
OC-M (g kg^{-1})	0.719**	0.985**	0.917**
OC-m (g kg^{-1})	ns	Ns	-0.481*
OC-Mm (g kg^{-1})	ns	0.823**	0.819**

*Significant at $P < 0.05$;
**Significant at $P < 0.01$;
ns, not significant.
M: percent of macroaggregates (> 250 µm: LM and SM); m: microaggregates (250-63 µm), Mm: percent of microaggregates within macroaggregates; OC-M: organic carbon content in macroaggregates; OC-m: organic carbon content in microaggregates; OC-Mm: organic carbon content in microaggregates within macroaggregates; BR-M: basal respiration in macroaggregates.

Significant positive correlations were found between OCs-M and OCs-OCM for all treatments. However, between OCsw-M and OCsw-OCM only AT showed a strong correlation. In a similar way, OCs and OCsw were correlated with the percentage of micro within macroaggregates (Mm) and OC associated to this fraction (OC-Mm) only in AT treatment. The OCs pool was correlated with Mm in T treatment and in S no correlations were found between these parameters. Negative correlations between both functional pools and the percentage of microaggregates (m) no occluded within macroaggregates were found in the afforested treatments, mainly in AT.

The BR showed strong correlations with M and OC-M in all treatments. It is important to note the strong correlations between BR-Mm, positive in AT and T treatments and negative in S. In turn, BR and OC-Mm showed good correlations in the afforested soils. As occurred with OCs, the BR was negatively correlated with the percentage of m. Finally, BR and OCs were correlated in all treatments and BR-OCsw only in AT.

5.4. Discussion

5.4.1. Changes in soil aggregation

The results of the changes in the percentage of water stable aggregates, after 20 years from afforestation, showed significant differences among the treatments. The lower percentage of large- and small-macroaggregates in T with respect to AT and S, at the same time that the lack of differences between AT and S, suggest an initial decrease in the percentage of soil aggregates in the afforested soils (AT and T), due to the impact of mechanical disturbance of the terracing works, followed by an active process of formation of new macroaggregates (> 250 µm) in AT, mainly in the 0-5 cm topsoil, which offset the negative impact of terraces. Similar effects on soil structure depletion following mechanical terracing were described by other authors (Finkel, 1986; Barber and Romero, 1994). Likewise, other studies reported increases in soil aggregation due to either: a) soil afforestation (Caravaca et al., 2002; Khale et al., 2005), and b) soil organic amendment (Diaz et al., 1994).

At the same time that the percentage of macroaggregates (> 250 µm) increase in AT, the percentage of microaggregates occluded within macroaggregates and the OC concentration in these microaggregates also was higher in AT than in the other treatments. In addition, all the new microaggregates formed in AT were occluded in macroaggregates and the OC concentration in these microaggregates was higher than in the not occluded microaggregates. Overall, these results suggest a hierarchical order of aggregation in AT, in which macroaggregates are the nucleus for microaggregate formation in the center of macroaggregates (Oades, 1984). Following afforestation with AT treatment, in a first phase, the organic amendment quickly induces the formation of macroaggregates due to: a) an increase in microbial activity, and b) inputs of binding agents like polysaccharides (Tisdall and Oades, 1982; Diaz et al., 1994; Golchin et al., 1994). Over time, this initial effect of organic amendment was gradually compensated by fresh plant material entering the soil as planted vegetation growth (discussed later). In a second phase, inside these macroaggregates, the presence of decomposed organic matter, metabolites and biogenic products, polyvalent cations and other binding agents, promoted the solid-phase reaction between organic matter and clay and silt particles leading to the formation of stable microaggregates (Edwards and Bremmer, 1967; Golchin et al., 1994). Other studies have presented similar models of soil aggregation (see Six et al., 2004, for a review).

In the T treatment the soil conditions: very low soil organic matter, lethargic microbial activity and few biomass inputs, were totally unfavorable for the aggregates formation and the process unfolds very slowly.

5.4.2 Factors controlling changes in soil aggregation

In order to know the relative contribution of each factor to the soil aggregation and carbon stabilization and sequestration capacity in the afforested areas, we have analyzed the changes occurred after each treatment.

5.4.2.1. Quality and amount of soil organic inputs

In AT we must consider, firstly, the initial impact of the organic amendment. As was shown in a previous publication (Querejeta et al., 2000), the organic amendment significantly increased the percentage of stable aggregates in the first years following AT treatments. Similar results have been worldwide reported by many researchers (Metzger et al., 1987; Bartoli et al., 1992; Clark et al., 2009), and specifically under the same environmental conditions and organic materials added (Diaz et al., 1994; Roldan et al., 1996b). But, not all the organic amendments have the same effect on soil aggregation (Clark et al., 2009). The ability to promote the formation of new macroaggregates depends on: a) the content of transient binding agents such as polysaccharides or fresh plant material, and b) the amounts of microbial available C used to promote fungal proliferation (Lucas et al., 2014). In this experiment, the composition of the organic amendment had great importance. The high content in carbohydrates (see Querejeta et al., 1998) was a key factor in the initial stages of macroaggregate formation due to its double action as a binding agent and as a food source for stimulating microbial activity.

Several studies have shown the temporary effects of the soil organic amendments, which appear mainly in the first week after addition (Debosz et al., 2002; Clark et al., 2009). This must be due to the rapid turnover of labile organic pools. Diaz et al. (1994), under similar conditions to this study, reported a rapid decrease of polysaccharides content in a soil amended with comparable organic materials. However, in AT the formation of macroaggregates continue 20 years after the soil amendment. This suggests that the changes occurred with the growth of the vegetation and litter inputs, mainly the changes in functional SOC pools and the structure and activity of microbial community, promote soil aggregation.

With regard to the changes in functional SOC pools, our results showed that afforestation with *Pinus halepensis* and organic amendment led to an increase in soil OCs and OCsw pools at the surface soil layer (0-5 cm), while afforestation without organic addition did not increased the OCs pool but lead to a reduction of OCsw respect to shrubland. These results are due to higher biomass production and litter input in AT than in T and shrubland (Garcia-Franco et al., 2014). Other authors (Jandl et al., 2007; Laganiére et al., 2010; Wang et al., 2011; Wei et al., 2013) obtained similar results after afforestation on abandoned agricultural soils. They pointed out that the higher inputs of plant debris after afforestation had a large potential to increase both SOC concentrations and stocks, especially in the topsoil where the accumulation of soil organic matter is positively related to the C inputs.

Besides quantitative changes on OC pools, the quality of OCs played an important role in soil aggregation and C sequestration (Steffens et al., 2009). The composition of OCs in afforested soils showed an increase in O-alkyl C such as carbohydrates respect to shrub. O-alkyl C compounds were investigated as indicators of organic binding agents, improving the macroaggregate formation (Degens, 1997). Several authors (Kinsbursky et al., 1989; Martens and Frankenberger, 1992) showed that carbohydrates were significantly correlated with aggregate stability in some cases. Likewise, our results were according to Pikul et al. (2007) and Steffens et al. (2009), who found greater aggregate stabilities associated with large contributions of O-alkyl C from labile fraction of soil organic matter.

Indeed, our results suggested that the increase of functional SOC pool, mainly OCs and the higher carbohydrates concentration in these functional pools, after afforestation with organic amendment, led to an increase of soil carbon sequestration capacity induced by new aggregates formation. An important proportion of this OC come from microaggregates enriched in OC occluded within macroaggregates as can be deduced by the positive correlation obtained between this occluded OC and both functional pools mainly in OCs (Table 5.6). Other authors (Bhattacharryya et al., 2012) also obtained positive correlations between C labile pools and macroaggregates and the MWD.

5.4.2.2. Changes in microbial activity

With regard to the microbial community implications in soil aggregate formation and C sequestration, we must discuss two aspects: a) microbial activity, and b) community structure. Due to basal respiration (soil respiration measured *in vitro*) is a very accurate indicator of the total microbial activity in the soil (Vanhala et al., 2005), we used this parameter in this discussion. The AT treatment showed higher BR than S and T in all depths, which proved a significant increase of soil microbial activity, after afforestation with organic amendment. However, a decrease in BR, respect to S, occurred with T treatment. Similarly, we found in the literature different responses of soil microbial activity to afforestation. Several studies showed decreases in microbial activity in the afforested areas (Zheng et al., 2005; Goberna et al., 2007; Chen et al., 2008) and, on the contrary, other authors reported increases in microbial activity after afforestation (Mao and Zheng, 2010). This apparent discrepancy is mainly due to the previous soil use, the afforestation method, or the stand age. In this study, the different behavior between AT and T was due to the use or no of the amendment.

The strong correlations between BR and the percentage of macroaggregates (> 250 mμ) in all treatments proved that microbial activity was an important factor in the new macroaggregates formation. In accordance with these results, many studies have proved the key role of microbial populations in soil aggregate formation (Lynch and Braggs, 1985; Siddiky et al., 2012; Daynes et al., 2013). However, the correlations between BR - Mm and BR – OC Mm showed a very different behavior between the native shrubland and afforested treatments, with respect to aggregation processes and C sequestration. The strong positive correlations BR-Mm and BR-OC Mm in AT and T were indicators of an active process of microaggregates formation inside larger aggregates, much more active in AT as was discussed above, which increases its turnover time and protect the OC associated with these microaggregates, leading to present C sequestration in the afforested ecosystems. In addition, the negative correlation between BR –m and BR-OCm supporting all the above and suggest that a process are occurring where these free microaggregates are being occluded into macroaggregates. Paradoxically, the increase in BR, measured as CO_2 emissions from the soil, leads to an increase of OC in the soil. This suggests that the process of OC-enriched microaggregates occlusion in macroaggregates is a self-protection system developed by the soil to offset the potential effect on SOC degradation derived from the microbial activity increase. This process is activated in the soil when BR increases occur and, in this study, was associated to the increase in the inputs of fresh plant residues. Our results suggested the importance of soil microbial activity enhancement in the afforestation methodologies, in semiarid areas, with the purpose of C sequestration.

On the contrary, in the shrubland a negative correlation between BR and Mm was found. This suggests that, in the shrubland, a process of aggregate breakdown and loss of organic C is happening. We hypothesized for this aggregate disintegration a similar model, but in reverse, to the aggregation model of Denef et al. (2007): Due to the reduction of fresh litter inputs, derived from shrubs degradation, microbial activity decreases leading to a reduction in biological binding and macroaggregate disruption; this diminishes the microaggregate protection and accelerates the mineralization of associated OC. Therefore, the key factor, for the activation of the self-protection organic carbon system (SPOCS) or the aggregate disruption model, is the inputs of vegetal debris into the soil, as they regulate the presence of biotic and abiotic binding agents.

According with this discussion, we think that the correlations between microbial activity (i.e. measured as basal respiration) and percentage of microaggregates within macroaggregates and OC concentration in microaggregates within macroaggregates, could be a valid indicator of present soil degradation or improvement. Positive correlations indicate soil improvement and negative correlations indicate soil degradation processes. The advantage of this indicator is that the measurement at a point in time allows determining the trend of a dynamic process. Long periods of time are not needed to establish soil dynamic in the ecosystem.

5.4.2.3. Changes in microbial community structure

The afforestation treatments, mainly AT, invoked long term shifts in the fungal community structure, diversity and relative abundance of several major fungal taxa, compared to the reference shrubland. However no significant differences were detected in the soil bacterial communities across treatments. The changes in the fungal community structure detected in this study seem to be mediated mainly by the variations in plant cover due to the afforestation with pine trees, as suggested the increase in the relative abundance of *Agaricomycotina*. As is well known, most of this fungal group form ectomycorrhizas with *Pinus halepensis* (Roldan and Albaladejo, 1994). This is in agreement with previous studies which reported that bacterial populations primarily respond to changes in the physical-chemical characteristics of the substrate, while fungal communities are more sensitive to land uses changes (Innerebner et al., 2006; Ros et al., 2006; Allison et al., 2007; Macdonald et al., 2009; Yu et al., 2011).

In general, fungi have stronger influence on soil aggregation than bacteria (Beare et al., 1997; Bossuyt et al., 2001; De Gryze et al., 2005; Zhang et al., 2012) and promote the storage of soil C because metabolize and transform more efficiently low quality substrates (Six et al., 2006) than bacterial (Holland and Coleman, 1987; Griffith and Bardgett, 2000; Allison et al., 2005). However, there is a big gap in the scientific literature about the specific functions and the disposition of each species of fungi to promote the formation of soil aggregates.

Faced with the following question: “were the changes in fungi community structure, after afforestation, involved in the promotion of soil aggregation?”. Our findings suggest an affirmative response. We found the following changes in afforested areas as regard to shrubland: 1) an increase in *Agaromycotina* a fungal group, which can produce considerable amounts aggregates stabilizing mycelium (Caravaca et al., 2002), 2) a decrease in *Chrytridiomycota* an unicellular fungus unable to produce mycelia, 3) an increase in richness species. We think that these changes increased the amount and diversity of biotic bonding agents and fostered the formation of macroaggregates. This affirmation may be supported by the significant correlations found between fungal community structure and size aggregate distribution, and between fungal community structure with basal respiration, at the surface layer, in all the treatments tested.

5.5. Conclusions

Our results confirmed the starting hypothesis. The qualitative and quantitative changes in OC pools linked to the shifts in microbial activity and fungal community structure after afforestation with AT treatment, promoted the formation of macroaggregates which acted as the nucleus for the formation of OC-enriched microaggregates inside. The accrual and physic-chemical stabilization of OC in this hierarchical structure is a key aspect to increase or maintain soil C stocks.

In addition, we suggest that the correlations between basal respiration and percentage of microaggregates within macroaggregates could be an accurate indicator of soil degradation or rehabilitation processes. Further research is needed to validate this indicator.

Chapter 6. *Stabilization mechanisms of SOC in agricultural areas: Effect of management practices*

6.1. Introduction

The soil organic matter (SOM) is a dynamic factor responsible for soil structure development (Beare et al., 1994). The amount of plant residues and the degree of SOM decomposition are vital factors in the formation and stabilization of aggregates, which in turn improves soil structure and drives soil organic carbon (SOC) sequestration (Haynes and Beare, 1996). However, the mechanisms of interaction between the soil structure and SOC dynamics are still not well understood (Blanco-Canqui and Lal, 2004). It is known that agricultural management practices modify the soil structure, with subsequent profound impacts on soil carbon (C) sequestration. In agricultural soils, tillage and traffic operations are major factors involved in soil structure degradation, due to aggregate disruption, with the loss of aggregate-occluded SOM (Paustian et al., 1997a) through fragmentation and compaction processes (Kay, 1990). In fact, the negative effects of conventional tillage (CT) and the benefits of using conservation agriculture measures (such as crop rotations, reduced tillage, cover crops, and no tillage), in terms of SOC sequestration, as well as suitable techniques for greenhouse gas (GHG) mitigation, have been demonstrated widely (Paustian et al., 1997b; Álvaro-Fuentes et al., 2010; Abdalla et al., 2014; López-Garrido et al., 2014).

No tillage (NT) is one of the conservation agriculture measures that have been applied in the last three decades all over the world (Dimassi et al., 2014), however, contradictory results have been obtained in relation to the utility of this technique from the point of view of C sequestration (Edward et al., 1988; Baker et al., 2007; Virto et al., 2012; López-Garrido et al., 2014). Green manure incorporation has also been demonstrated to improve the soil structure and increase SOC accumulation, besides helping to control erosion (Gómez et al., 2009; Higashi et al., 2014). Nevertheless, it is not a common practice in semiarid agroecosystems because of a fear of competition over water resources between the green manure and the main crop (Unger and Vigil, 1998; Ramos et al., 2010, Almagro et al., 2013). Most of the experimental studies focused on the impacts of these two sustainable agriculture management practices on SOC dynamics have been performed under extensive cereal and irrigated crops in temperate areas (Denef et al., 2004; Six et al., 2004; Virto et al., 2012; Dimassi et al., 2014); and under Mediterranean conditions (Hernanz et al., 2002; Álvaro-Fuentes et al., 2009, 2012b; Macinelli et al., 2010; Plaza-Bonilla et al., 2010, 2013; 2014; López et al., 2012; Das et al., 2013; López-Garrido et al., 2014). However, very few studies have been carried out under rainfed tree crops in semiarid areas - where these crops represent a significant proportion of the total agricultural production (Gómez et al., 2009; Nieto et al., 2012; Almagro et al., 2013). Moreover, only few of the above mentioned studies were orientated to determine the effects of the conservation agriculture practices on OC pools and on the way in which the OC interacts physically and chemically with aggregates and the mineral fraction. In fact, the physical protection of SOM by aggregates (Denef et al., 2001) and the physical-chemical stabilization through the adsorption and chemical binding of OC onto mineral surfaces are considered to be important mechanisms of soil C stabilization (Krull et al., 2003). Furthermore, the study of different SOM pools within soil aggregates (free LF: free light fraction, occurring between aggregates; iPOM: intra-aggregate particulate OM, occurring within aggregates; and mineral-C: OC associated with the mineral soil fraction) can be used as an early indicator of soil changes by management practices (Six et al., 2002c). Besides, an identification of those pools, may help to improve our understanding of how aggregates store and interact with OC. This could help us to choose the best sustainable land management (SLM) practices with regard to the enhancement of SOC sequestration in Mediterranean areas. In this study we evaluate the effects of different SLM agricultural practices on OC stabilization by soil aggregates in an organic rainfed almond (*Prunus dulcis* Mill.) orchard under Mediterranean semiarid conditions. Our hypotheses were: i) SOC associated with aggregates increases with green manure addition and/or with the cessation of tillage, and ii) physical-chemical protection of OC is responsible for the accumulation of OC under these SLM practices. To test these hypotheses, we isolated different aggregate size classes (large and small macroaggregates, microaggregates free and within macroaggregates; and silt plus clay) and the intra-aggregate particulate OM fractions (Free LF-C, coarse iPOM-C, and fine iPOM-C) and mineral-associated carbon (mineral-C) contained within these classes, in order to investigate the role of each pool in OC sequestration under each treatment and at different depths (0-5, 5-15, 15-30 cm).

6.2. Material and methods

6.2.1. Site description and experimental design

The experimental area was established on 21 October 2008 in an organic, rain-fed almond (*Prunus dulcis Mill.*) orchard located in Cehegín in the Northwest of the province of Murcia, in Southeast Spain (38° 3’ 15’’ N, 1° 46’ 12’’ W). The altitude of the orchard is 633 m.a.s.l. and the average slope is lower than 7%. The climate is semiarid Mediterranean with an average annual precipitation of 370 mm, concentrated in the spring and autumn months, but with a great inter- and intra-annual variability. The mean annual temperature is relatively high, 16.6 °C, and the mean potential evapotranspiration reaches 800 mm $year^{-1}$, so the mean annual water deficit, calculated by the Thorntwaite method, is 430 mm. July and August are the driest months. These conditions result in aridic soil moisture content and a thermic soil temperature regime (ICONA, 1988). The soil is a *Petrocalcic Calcisol* (FAO, 2006) with the following main properties for the 0-30 cm soil layer (average values): electrical conductivity (1:5): 168.3 ± 23.3 μScm^{-1}; carbonates percentage: 55.5 ± 1.2%; calcium exchangeable percentage: 18.9 ± 0.2 and 19.6 ± 0.6%; pH: 8.8 ± 0.02; sand (2000–50 µm), silt (50–2 µm), and clay (<2 µm) contents: 430 ± 120 g kg^{-1}, 415 ± 90 g kg^{-1}, and 155 ± 42 g kg^{-1}, respectively; and a mixed-clay mineralogy dominated by kaolinite (1:1 layer type) and illite (2:1 layer type).

For 14 years, the habitual soil management in the study area was reduced tillage (RT), to control weeds. In 2008, two alternative SLM practices were applied: i) reduced tillage plus green manure (RTG) and ii) no-tillage (NT) (Figure 6.1). The experimental design consisted of 12 plots in a randomized-block design, with three replicates for each treatment (for more details, see Martínez-Mena et al., 2013). The tillage consisted of chisel plowing to 0.15 m depth using a cultivator, twice a year (autumn/spring) to control weeds. The green manure consisted of a mix of common vetch (*Vicia sativa* L.) and common oat (*Avena sativa* L.) in a proportion of 3:1, applied manually during early autumn at 150 kg ha^{-1}. The green manure (or cover crop) is planted yearly, in the autumn, and cut off in May. After cutting, it is incorporated into the soil using a cultivator. In the NT treatment, the weeds are cut manually in May and left on the soil surface. No addition of OM or manure was performed.

Figure 6.1. Different management practices in the study area: **(A)** Reduced tillage (RT), **(B)** No tillage (NT), **(C)** and **(D)** Green manure in two contrasting years according to the pluviometry (P) - **(C)** 2009: P = 345 mm; **(D)** 2011: P = 230 mm). Note the difference in the planted green manure in 2009 (wet year; C) and 2011 (dry year; D).

6.2.2. Soil sampling and analysis

Soil samples were taken from three soil layers (0–5, 5-15, and 15-30 cm) in October 2012, following crop harvest. The soils were sampled in the rows between the trees, 3.5 m away from the tree trunk. Five disturbed soil samples were taken in each treatment, per block and depth, and pooled to make two composite samples in each treatment, per block and depth (2 x 3 x 3 x 3). A total of 54 composite samples were taken to the laboratory for further analysis.

Determination of soil properties

The soil samples were air-dried, sieved to <2 mm, and analyzed in the laboratory, in triplicate. The soil carbonate content was determined using a Bernard Calcimeter (Duchaufour, 1975) exchangeable calcium (Ca2+) was measured by the method of Duchaufour (1975), and the pH (1:5 soil: water) was determined with a pH meter (CRISON 20). The soil electrical conductivity (EC) was measured for the same suspension, using an EC meter. The textural characteristics were determined using a Coulter LS200 'Laser particle sizer'. Mineralogical analysis of the clay fraction was performed by X-Ray diffractometry, using a Philips PW 1710 model (Philips, Eindhoven, Holland). Microbial C was measured with a respirometer (MicroTrack 4200-Sy Lab, Gomensoro, Madrid, Spain) and calculated according to Anderson and Domsch (1978).

Separation of water-stable soil aggregates.

Aggregate-size separation was carried out on each sample using a modified wet sieving method adapted from Elliott (1986). Briefly, a 100-g sample of air-dried soil (5-mm sieved) was placed on top of a 2000-µm sieve and submerged for 5 min in deionized water at room temperature. The sieving was done manually by moving the sieve up and down 3 cm, 50 times in 2 min, to achieve aggregate separation. A series of three sieves (2000, 250, and 63 µm) was used to obtain four aggregate fractions: i) > 2000 µm (large-macroaggregates; LM), ii) 250 to 2000 µm (small-macroaggregates; SM), iii) 63 to 250 µm (microaggregates; m), and iv) < 63 µm (silt plus-clay-sized particles; s+c). The aggregate-size classes were oven dried (50°C), weighed, and stored in glass jars at room temperature (21°C) (Figure 6.2A). Protected microaggregates contained within macroaggregates were determined in the small-macroaggregates class because not enough large-macroaggregates were obtained to carry out the microaggregate isolation procedure described by Six et al. (2000b) and Denef et al. (2004). Briefly, a 10-g small-macroaggregate subsample was immersed in deionized water on top of a 250-µm mesh screen inside a cylinder. The small-macroaggregates were shaken together with 50 glass beads (4 mm diameter) until complete macroaggregates disruption was observed. Once the small-macroaggregates were broken up, microaggregates and other <250 μm material passed through the mesh screen with the help of a continuous water flow to the sieve. The material retained on the 63-μm sieve was wet sieved, to ensure that the isolated microaggregates were water stable (Six et al., 2000b). These microaggregates obtained from small-macroaggregates were oven-dried, at 50°C (24 h) in aluminium trays, and weighed (Figure 6.2B).

Density fractionation of soil aggregates.

Since not enough water-stable large-macroaggregates (>2000 μm) were obtained from the wet sieving procedure, the subsequent OC fractionation by density was performed in the small-macroaggregates (250-2000 μm) and microaggregates (63-250 μm). Different pools of OC contained within the small-macroaggregates and microaggregates were further separated by density according to Six et al. (1998), using sodium polytungstate (SPT) at a density of 1.60 g cm^{-3}. A subsample (10 g) of oven-dried material was put into a 50-ml centrifuge tube together with 35 ml of SPT. Briefly, the free light fraction (free LF) was isolated by density flotation. The liquid was gently stirred - to avoid breaking up the aggregates - and the floating material (free LF) was aspirated, filtered using a 20-μm nylon filter, rinsed with distilled water, transferred into aluminium tins, and dried at 50°C. The remaining material, the heavy fraction (HF: iPOM+sand), in the centrifuge tube was washed with distilled water and then centrifuged. The heavy fraction was then dispersed in 5 g l^{-1} sodium hexametaphosphate. After shaking for 18 h, the dispersed heavy fraction was passed through 2000-, 250-, and 63-μm sieves, depending on the aggregate size being analyzed (Álvaro-Fuentes et al., 2009). The intra-aggregate (iPOM) material and sand retained on the sieves - coarse iPOM (250-2000 μm) and fine iPOM (63-250 μm) - and the material passing through the 63-μm sieve (mineral particles) were allowed to settle, centrifuged (3000 rpm, 20 min), and oven-dried at 60°C before samples were weighed and ground (Schwendenmann and Pendall, 2006) (Figure 6.2C).

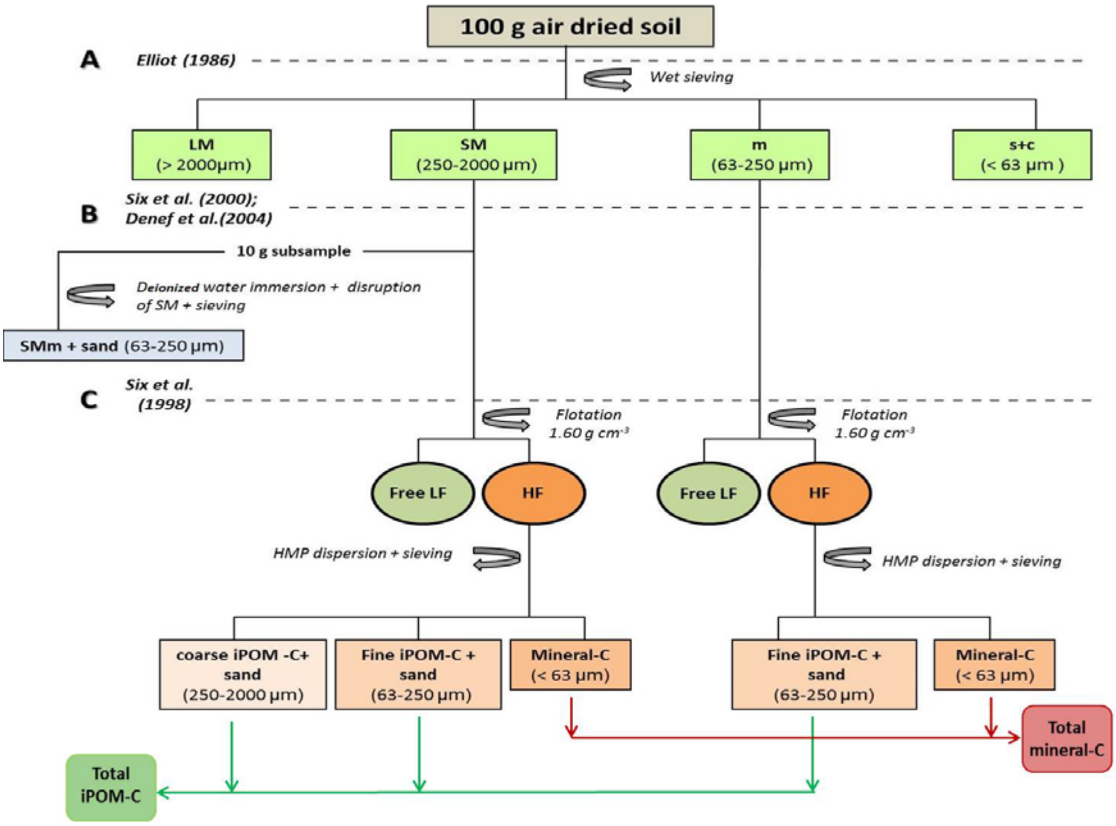


Figure 6.2. Scheme of the fractionation methods used: **(A)** Wet-sieving (Elliot et al., 1986): large-macroaggregates (LM: >2000 µm), small-macroaggregates (SM: 250-2000 µm), microaggregates (m: 63-250 µm), and silt plus clay particles (s+c: <63 µm); **(B)** Separation of microaggregates within macroaggregates (Denef et al., 2004 and Six et al., 2000b): microaggregates within small-macroaggregates plus sand (SMm: 63-250 µm); and **(C)** Density fractionation (Six et al., 1998): free light fraction (free LF, density < 1.60 g cm^{-3}), heavy fraction (HF, density > 1.60 g cm^{-3}), coarse intra-aggregate particulate organic matter (coarse iPOM-C: 250-2000 µm), fine intra-aggregate particulate organic matter (fine iPOM-C: 63-250 µm), and mineral fraction occluded into aggregates (Mineral-C: <63 µm).

Carbon and nitrogen determinations

The soil organic C and total N concentrations were determined in the bulk soil and separately for water-stable aggregates and density fractions using an Elemental Analyzer (LECO TRUSPEC CN, Michigan, USA). The samples were analyzed in triplicate. The OC content at the soil level was calculated with the following equations:

$$OC = (OC)_{fraction}*(agg.proportion)_{soil}\ (g\ C/Kg_{soil})$$

$$OC = (OC)_{fraction}*(density\ pool\ proportion)_{soil}\ (g\ C/Kgsoil)$$

Sand correction was performed in each aggregate-size class and in each density fraction, because sand was not considered part of those aggregates (Elliot et al., 1991).

6.2.3. Statistical analyses

To compare the effects of treatments and soil depths a GLM procedure was carried out which considered treatment and depth as fixed factors and block as a random variable. When significant, differences among treatments and depths were identified at the 0.05 probability level of significance using Tukey´s test. Prior to the analyses, the data were examined for normality by the Kolmogorov–Smirnov test and for homogeneity of variances by the Levene's test. Data that were not distributed normally (coarse iPOM-C, fine iPOM-C and mineral-C from small-macroaggregates) were ln-transformed. Pearson correlation analysis was applied in order to explore the relationships between all the study variables within treatments across depths. All analyses were carried out using IBM SPSS statistics 19.0 software (SPSS Inc., Chicago. Illinois).

6.3. Results

6.3.1 SOC, C/N ratio and microbial C in the bulk soil.

The SOC concentration in the bulk soil was significantly greater in RTG than in RT or NT at 0-5 cm depth, while no differences were observed among treatments below 5 cm depth (Table 6.1). In all treatments, slightly higher values of bulk SOC were found at the surface compared to deeper layers, but no significant differences were found between depths within treatments. No differences in the total N concentration of the bulk soil were found among treatments (data not shown). The C/N ratio oscillated between 9.6 and 12.1, depending on the treatment and depth, and did not differ among treatments or depths, although slightly higher values were obtained in RTG and NT with respect to RT.

The soil microbial C was significantly higher in RTG than in RT or NT at 0-5 cm (Table 6.1). While we did not detect differences between treatments at 5-15 cm depth, below 15 cm both tillage treatments (RTG and RT) gave higher microbial C than NT.

Table 6.1. The SOC, C/N ratio, and microbial carbon values of the bulk soil at three depths under distinct sustainable land management practices: RT (reduced tillage), RTG (reduced tillage with green manure), and NT (no tillage).

		Soil management practices		
Soil properties	**Depth (cm)**	**RT**	**RTG**	**NT**
OC Bulk soil (g kg^{-1})				
	0-5	11.8 ± 0.5aA	13.7 ± 0.6bA	10.2 ± 0.4aA
	5-15	11.1 ± 1.1aA	11.3 ± 1.6aA	9.7 ± 1.6aA
	15-30	11.1 ± 0.6aA	8.9 ± 0.6aA	8.8 ± 0.9aA
C/N Ratio				
	0-5	9.6 ± 0.7aA	11.56 ± 0.3aA	11.2 ± 1.8aA
	5-15	9.5 ± 0.8aA	11.6 ± 2.2aA	10.1 ± 0.8aA
	15-30	9.7 ± 2.1aA	8.4 ± 0.8aA	12.1 ± 1.8aA
Microbial C (mg kg^{-1})				
	0-5	1410.1 ± 207.5aB	2012.5 ± 194.8bB	1407.1 ± 185.4aB
	5-15	977.8 ± 131.5aA	1055.6 ± 152.7aAB	855.1 ± 125.3aAB
	15-30	618.3 ± 51.2bA	713.3 ± 51.2bA	425.9 ± 48.7aA

Numerical values are means ± standard errors. Different lower-case letters in rows indicate significant differences between treatments at each depth in some soil properties and different upper-case letters in columns indicate significant differences between depths within each treatment (Tukey's test, $p < 0.05$).

6.3.2 Aggregate size distribution and associated organic carbon.

6.3.2.1 Water-stable aggregates

The distribution of soil water-stable aggregates (WSA) was different according to the treatment and depth. At 0-5 cm depth, total macroaggregates (>250 µm) was the most-representative aggregate-size of the soil in RTG and NT (41% and 46%, respectively), while silt-clay-sized particles was the most-representative class in RT (48%), (Figure 6.3). Large-macroaggregates (> 2000 µm) and small-macroaggregates (250-2000 µm) were increased by about 30% in NT, compared to RT. However, RTG showed higher proportion of large-macroaggregates than NT while no differences were obtained in small-macroaggregates between both treatments (Figure 6.3). At 5-15 cm depth, total macroaggregates (> 250 µm) was the most-representative class in all treatments and no differences in the proportion of LM were observed among them. However, the SM proportion was higher in RTG than in RT and NT (Figure 6.3).

The percentage of macroaggregates increase at this depth under both reduced tillage treatments (LM and SM in RT and LM in RTG) while no differences in percentage of macroaggregates between depths were observed in NT.

The proportion of microaggregates (m) was higher in RTG, compared to RT and NT, at the surface layer. At 5-15 cm the proportion of microaggregates in both tillage treatments (RT and RTG) was about 30% higher than for NT. Below the plow layer (15-30 cm depth), differences among treatments in the soil size-aggregates distribution decreased (Figure 6.3).

The OC concentration in silt-plus clay-sized particles was the most representative fraction of the organic C contents of the bulk soil profile (between 44% and 58.5% of the total SOC at 0-5 cm depth and between 33 and 45% at deeper layers, depending on the treatment), while the OC within large macroaggregates represented the lowest percentage (<3%) of the total SOC in all treatments. A decrease in the aggregate-associated OC concentration with soil depth was also observed in most of the treatments, with the exception of the OC in large-macroaggregates (OC-LM) and in microaggregates (OC-m) for NT (which did not change with depth) and the OC contained in small-macroaggregates (OC-SM) for RT - which was greater at 5-15 cm depth than in the upper or lower layers (Figure 6.4). No changes in OC concentration associated to silt-clay-sized with depth were observed of all treatments. Over the whole profile, higher OC concentrations associated with macro (large and small) and microaggregates were observed under RTG than RT treatment, these differences being more accentuated at the surface than in the deepest layer. Thus, the conversion from RT to RTG led to an increase of 72% in the OC in total macroaggregates and of 45% in the OC associated with microaggregates, in the surface layer. The conversion from RT to NT led to a gain of about 23% in the OC associated with SM at the surface layer, while no differences were observed in other aggregate sizes. At 5-15 cm depth, the OC in microaggregates was higher in both tillage treatments (RTG and RT) than in NT. Below the plow layer, no differences in the aggregate-associated OC concentration were observed among all treatments, except that the OC-m was about 15% higher in RTG than in RT (Figure 6.4).

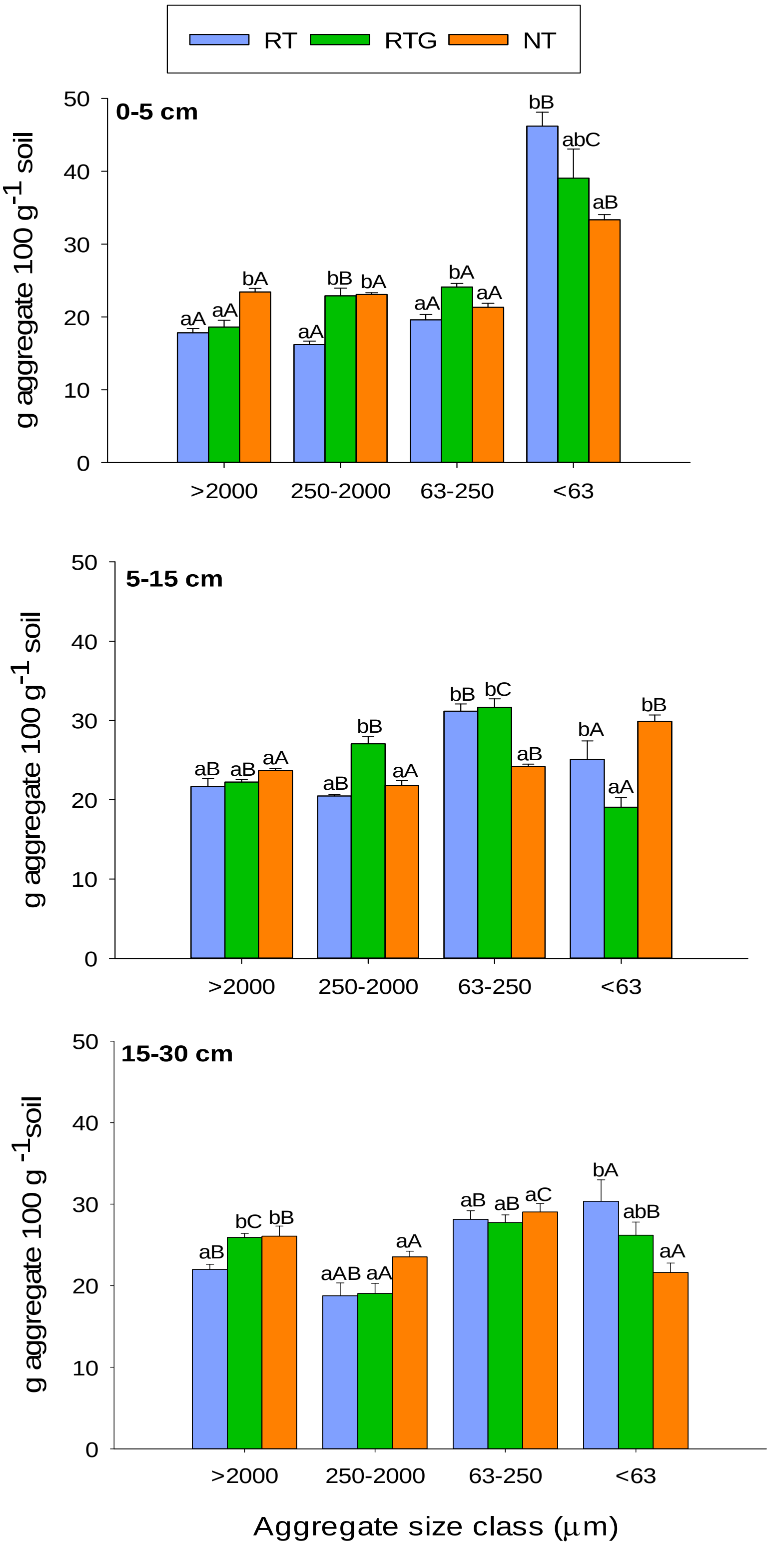


Figure 6.3. Percentages by weight of the water-stable aggregate size distribution (g aggregate 100 g^{-1} soil): > 2000 µm (LM), 250-2000 µm (SM), 63-250 µm (m), and <63 µm (s+c) in the 0-5, 5-15, and 15-30 cm soil layers for RT (reduced tillage), RTG (reduced tillage plus green manure), and NT (no tillage). Numerical values are means ± standard errors. Different lower-case letters in bars indicate significant differences between treatments for each aggregate size at $p < 0.05$. Different upper-case letters in bars indicate significant differences between depths within each treatment (Tukey's test, $p < 0.05$).

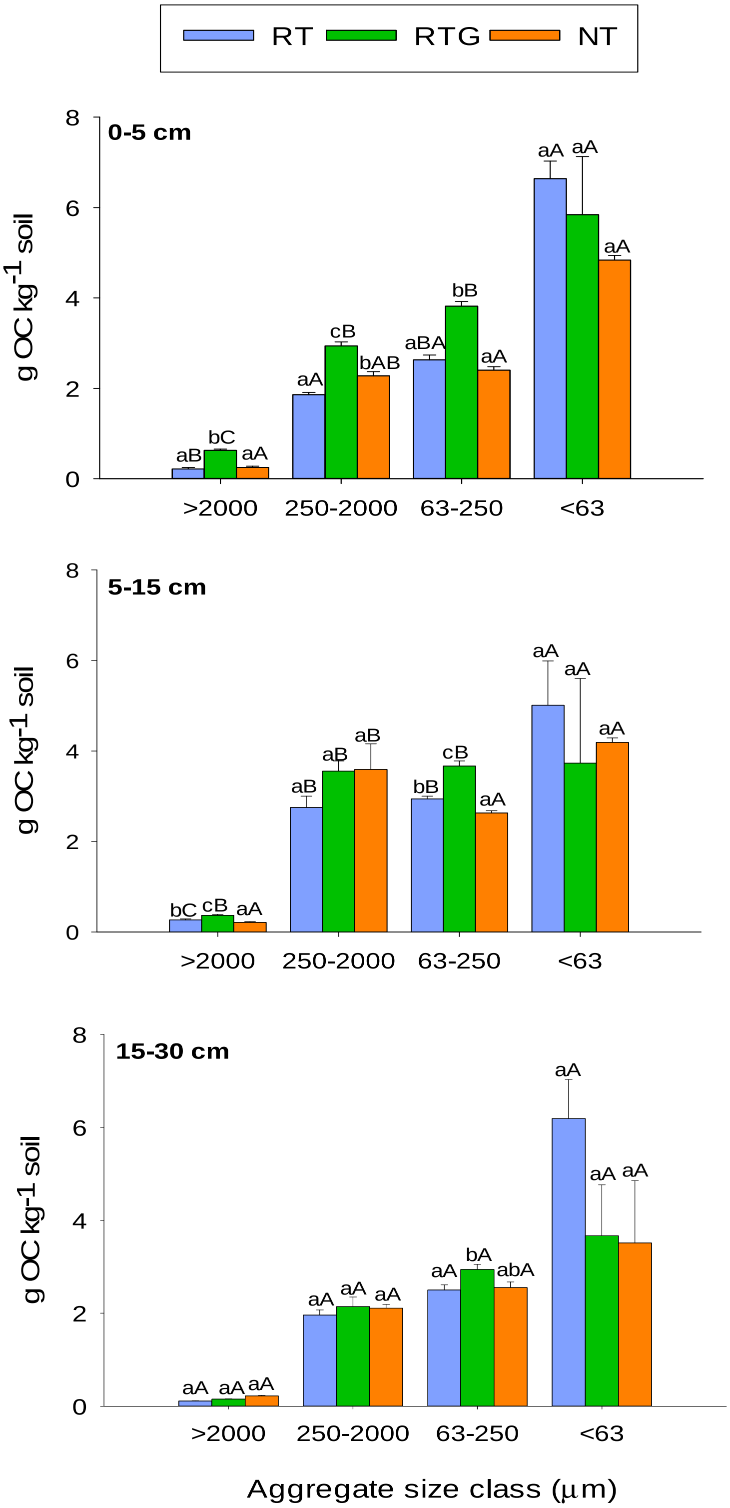


Figure 6.4. Organic carbon content (g kg^{-1} soil) of aggregates: > 2000 µm (LM), 250-2000 µm (SM), 63-250 µm (m), and <63 µm (s+c) in the 0-5, 5-15, and 15-30 cm soil layers, for RT (reduced tillage), RTG (reduced tillage + green manure), and NT (no tillage). Numerical values are means ± standard errors. Different lower-case letters in bars indicate significant differences between treatments for each aggregate size at $p < 0.05$, whereas different upper-case letters in bars indicate significant differences in depths within each treatment (Tukey's test, $p < 0.05$).

6.3.2.2 Microaggregates within small-macroaggregates

The percentage of microaggregates within small-macroaggregates (SMm) was relatively low in these soils and ranged between 5% and 12%, depending on the treatment and depth (Table 6.2). RTG was the treatment with the highest percentage of SMm and associated OC (OC-SMm), at all depths, compared to RT and NT. At the soil surface, the conversion from RT to RTG led to an increase of 121% in the percentage of SMm and of 180% in the associated OC (OC-SMm). However, at 5-15 cm the differences between RT and RTG decreased with respect to those in the surface layer (RTG gave an increase of 47% in SMm proportion and of 84% in the OC-SMm, relative to RT). Below the plow layer RTG showed higher percentage of SMm than RT, but no differences were found in their associated OC. Conversion from RT to NT led to an increase of 30% in SMm but did not result in an increment of the OC-SMm at the surface layer. At 5-15 cm, however, RT produced higher percentages of SMm and OC-SMm than NT (Table 6.2). Below the plow layer, no differences between RT and NT treatments were observed. Interestingly, no changes with depth, neither in the percentage of SMm nor in the associated OC, were observed in treatment RTG, while in RT an increase with depth in both SMm and OC-SMm was observed. For NT, the SMm proportion increased with depth but not its associated OC (Table 6.2).

Table 6.2. Weight percentage and organic carbon concentration (g kg^{-1} soil) of the SMm (microaggregates within small-macroaggregates) in the 0-5, 5-15, and 15-30 cm soil layers, as affected by treatments (RT, reduced tillage; RTG, reduced tillage plus green-manure; NT, no-tillage).

	Soil management practices		
Depth (cm)	RT	RTG	NT
SMm (%)			
0-5	4.96 ± 0.23aA	11.00 ± 0.41cA	6.55±0.19bA
5-15	7.98 ± 0.21bB	11.38 ± 0.40cA	6.46±0.34aA
15-30	8.45 ± 0.50aB	11.96 ± 0.29bA	8.60±0.71aB
OC –SMm (g kg^{-1})			
0-5	0.76 ± 0.02aA	2.13 ± 0.27bA	0.75 ± 0.10aA
5-15	1.12 ± 0.04bB	2.07 ± 0.13cA	0.70 ± 0.13aA
15-30	1.19 ± 0.01abB	1.88 ± 0.30bA	0.93 ± 0.09aA

Numerical values are means ± standard errors. Different lower-case letters in rows indicate significant differences between tillage treatments at each depth. Different upper-case letters in columns indicate significant differences between depths within each treatment (Tukey´s test, p <0.05).

6.3.3 Distribution and organic carbon concentration of density fractions

The OC associated with the free light fraction (free LF-C) represented between 3% and 11% of the total SOC, according to the treatment and depth (Table 6.3). At the soil surface, free LF-C was 1.5-times higher in RTG treatment, compared to RT or NT treatments. However, below 5 cm no differences were found between RT and RTG treatments: both had more than twice the concentration of free LF-C found for treatment NT (Table 6.3). Moreover, in tillage treatments (RT and RTG) a significant increase in OC content in the free LF-C from 0-5 to 5-15 cm while no change was observed in NT.

The OC concentration in the total intra-aggregate particulate organic matter (total iPOM-C) represented higher percentages of the total SOC in RTG and NT (about 20%) than in RT treatment (about 12%), in the whole soil profile. Treatments RTG and NT gave increases of 114% and 33%, respectively, in the total iPOM-C concentration, compared to RT, at the 0-5 cm depth (Table 6.3). A significant decrease with depth in total iPOM-C was observed in RTG while no differences in this pool through the soil profile was obtained in RT or NT (Table 6.3). No differences in total iPOM-C among treatments were observed below 15 cm.

The OC associated with the total mineral fraction occluded within small-macroaggregates and microaggregates (total mineral-C) differed in the order RTG ≥ RT ≥ NT at 0-5 cm depth. Below 5 cm, no differences between treatments were observed. For both RTG and NT, the total mineral-C was significantly higher in the 5-15 cm layer than at the other depths (0-5 and 15-30 cm), whereas no differences were observed with depth in RT.

According to Figure 6.5, where a more detail distribution of the different iPOM (coarse and fine) and mineral associated C occluded within aggregates (small-macroaggregates and microaggregates) is presented, no differences were observed in the coarse iPOM-C among treatments at 0-5 cm depth. However, at 5-15 cm, while RTG did not differ from RT and NT, the latter showed an increase of 310 % in this pool compared to RT.

Both alternative treatments (RTG and NT) experienced an increase in fine iPOM-C occluded in both, small-macroaggregates and microaggregates respect to RT mainly at the surface layer. Thus, RTG gained 63% iPOM-C in SM and 139% in m respect to RT. On the other hand, NT gained 118% iPOM-C in SM and 57% in m respect to RT. The total mineral-C (occluded within SM and m) represented 23-34% of the total SOC, depending on the treatment and depth. Differences in the total mineral-C concentration among treatments were only observed at the 0-5 cm depth - where RTG showed the highest values followed by RT and NT (Table 6.3). Interestingly, in RTG and NT, but not in RT, the concentration of total mineral-C increased from 5 to 15 cm soil depth. Mineral-C occluded in SM was the fraction mainly responsible for the gains in total mineral-C in RTG, compared to RT, in the plow layer. Furthermore, the OC in mineral-C occluded in SM was about 50% greater in NT, with respect to RT, at 5-15 cm (Figure 6.5).

Table 6.3. The organic carbon concentration (g C kg^{-1} soil) of freeLF-C, total intra-aggregate particulate organic matter (iPOM-C within small-macroaggregates + iPOM-C within microaggregates), and total mineral-associated soil organic C (mineral-C within small-macroaggregates + mineral-C within microaggregates) in the 0-5, 5-15, and 15-30 cm soil layers, as affected by treatments (RT, reduced tillage; RTG, reduced tillage plus green manure; NT, no-tillage).

	Soil management practices		
Depth (cm)	**RT**	**RTG**	**NT**
Free LF-C			
0-5	0.45 ± 0.02aA	0.71 ± 0.04bA	0.46 ± 0.01aA
5-15	1.21 ± 0.11bB	1.21 ± 0.12bB	0.53 ± 0.05aA
15-30	0.58 ± 0.07bA	0.58 ± 0.02bA	0.37 ± 0.05aA
Total iPOM-C			
0-5	1.36 ± 0.08aA	2.91 ± 0.11cC	1.81 ± 0.07bA
5-15	1.55 ± 0.07aA	2.20 ± 0.10aB	2.40 ± 0.72aA
15-30	1.52 ± 0.08aA	1.70 ± 0.13aA	1.64 ± 0.10aA
Total mineral-C			
0-5	2.70 ± 0.13abA	3.20 ± 0.14bA	2.31 ± 0.14aA
5-15	3.07 ± 0.44aA	3.80 ± 0.23aB	3.34 ± 0.19aB
15-30	2.38 ± 0.14aA	2.94 ± 0.27aA	2.66 ± 0.10aA

Numerical values are means ± standard errors. Different lower-case letters in rows indicate significant differences between treatments at each depth within each aggregate size and different upper-case letters in columns indicate significant differences between depths within each treatment (Tukey´s test, $p < 0.05$).

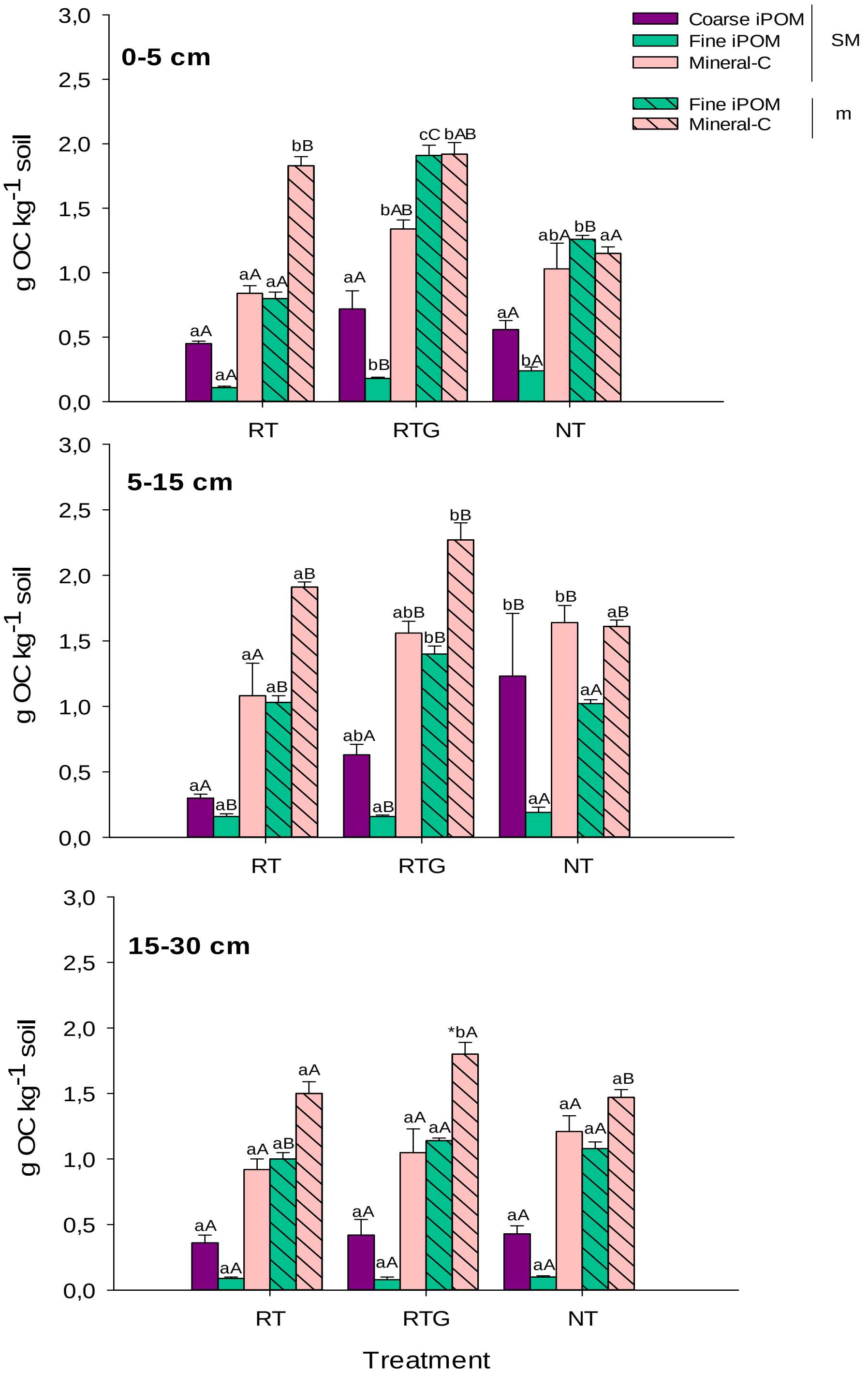


Figure 6.5. Organic carbon content (g kg^{-1} soil) in subpools of small-macroaggregates and microaggregates: intra-aggregate particulate organic matter (iPOM C): coarse iPOM-C, fine iPOM-C, and mineral-C from SM (small-macroaggregates) and m (microaggregates), at 0-5, 5-15, and 15-30 cm depth in RT (reduced tillage), RTG (reduced tillage + green manure), and NT (no tillage). Numerical values are means ± standard errors. Different lower-case letters in bars indicate significant differences between treatments at each depth within each density fraction (Tukey's test, p <0.05; with * p< 0.06), whereas different upper-case letters in bars indicate significant differences between depths within each treatment.

6.4. Discussion

6.4.1. Aggregate-size distribution and associated carbon

As mentioned earlier, previous to the establishment of the experiment these fields had been plowed for 14 years. It is well known that plowing disturbs the soil, affecting the soil aggregate stability and distribution of soil aggregates - which promotes the breakdown of macroaggregates into microaggregates and silt-plus clay-sized particles enhancing OC mineralization in macroaggregates (Tisdall and Oades, 1982; Beare et al., 1994; Six et al., 1999; Denef et al., 2001; Bossuyt et al., 2002). In our soils, even when reduced tillage was applied, the aggregate rupture by tillage was reflected in the higher percentage of silt-clay-sized particles at the surface layer, together with their significant decrease with depth in the tillage treatments, compared to no tillage (NT). Other authors have obtained, at surface, a significant increased in silt plus clay particles in tillage treatments (included reduced tillage) compared to no tillage treatments (Andruschkewithsch et al., 2014). However, the incorporation of green manure reduced the effect of tillage on macroaggregate rupture. Thus, while in RT the percentages of large-macroaggregates and small-macroaggregates were reduced significantly by tillage, in RTG only a reduction of the proportion of large-macroaggregates was observed, compared to NT at 0-5 cm depth (Figure 6.3). Other authors obtained a high proportion of macroaggregates after the addition of fresh organic residue (Olchin et al., 2008; Yan et al., 2012). The incorporation of organic material from green manure in RTG treatment resulted in an accumulation of OC in all soil aggregate size classes at the surface layer (0-5 cm), with an increment of 186% for the OC associated with the large-macroaggregates, 58% in small-macroaggregates, and 45% in microaggregates, relative to RT (Figure 6.4). Moreover, in RTG an increase of the total OC concentration in the bulk soil of around 14% was observed in the surface layer, with respect to RT (Table 6.1).

The higher OC concentration in microaggregates under RTG, compared with RT, is beneficial to SOC sequestration in the long-term because microaggregates have a longer turnover time and higher stability than macroaggregates (Balesdent et al., 2000; Huang et al., 2010). In addition, the green manure also increased the OC content of microaggregates within small macroaggregates, this gain being higher (180%) at 0-5 cm than at 5-15 cm (86%) with respect to RT. At 0-5 cm depth, about 69% of the total SOC gained in RTG with respect to RT was accounted for by the change in microaggregates within small macroaggregates. Other authors obtained percentages close to 50%, at 0-20 cm in semiarid soils (Álvaro-Fuentes et al., 2009). The higher content of OC in microaggregates within small macroaggregates in RTG with respect to RT indicates the potential of green manure for achieving high SOC stabilization. Indeed, higher aboveground biomass values in RTG than in RT (182 g m^{-2} and 114 g m^{-2}, respectively) (Almagro et al., 2013) together with the higher C concentration of free-LF in the top soil (an indicator of the input of plant material) for the former are consistent with the higher C concentration of the microaggregates within macroaggregates observed in RTG than RT treatments (see Table 6.2). In addition, the green manure incorporation resulted in an increase in microbial C in the surface layer, relative to RT. All together, these results indicate the important role of the quantity and quality of the plant material input in the stimulation of the soil microflora, in relation to the production of binding agents that increase the OC and aggregates formation and stabilization - as has been reported by other authors (Tisdall and Oades, 1982; Abiven et al., 2007; Helfrich et al., 2008; Tang et al., 2011). The rapid aggregation (after 84 days of incubation) in soils with high contents of oat (*A. sativa*) and the increase of soil aggregation and OC concentration with the residues of legumes, often rich in the labile organic fraction, have been previously reported by other authors (Martens et al., 2000; Blanco-Canqui and Lal et al., 2004).

Previous work showed the importance of the incorporation of plant residues by tillage (rather than leaving them on the surface: NT), which could be a result of better contact with soil particles and soil microbes - hence improving the physico-chemical protection of SOC (Blanco-Canqui and Lal, 2004; Christopher et al., 2009; Zhao et al., 2012). In our case, the both reduced tillage treatments (RT and RTG) showed high positive correlations between the percentage of small-macroaggregates and C concentration of the free light fraction, an indicator of plant material input, (r= 0.76, $p<0.01$ and r= 0.45, $p<0.05$, for RTG and RT, respectively). This means that the incorporation of plant material into the soil promotes the formation of small-macroaggregates, enhancing the permanence of the OC in these microaggregates within small-macroaggregates - as reported by other authors (Oades, 1984; Golchin et al., 1994; Angers et al., 1997).

The positive effect of tillage - promoting the incorporation of OC into the soil - was also evidenced by the fact that the free LF-C content was higher in the 5-15 cm soil layer than at the surface for the tillage treatments, while no change with depth was observed for NT. Therefore, tillage might favor the mixing of OM with mineral particles, hence reducing the mineralization of OC and promoting the migration and accumulation of OC through the soil profile. Thus, the addition of fresh and easily-decomposable OM in the RTG treatment and its incorporation by tillage led to a slight change in the OC aggregate distribution respect to RT. Thus, while in RT a decrease in OC concentration with increasing aggregate size (free s+c > m > SM > LM) was observed through the soil profile (Figure 6.3), in RTG the OC content within small macroaggregates was equal to that within microaggregates (free s+c > m = SM > LM) at 0-5 cm. Below 5 cm, OC contained in both, m and SM were equal to the OC in the silt plus clay-sized particles (free s+c = m = SM > LM).

The shift from RT to NT did not result in a gain of OC concentration in the bulk soil, but favored the permanence of OC in small-macroaggregates at 0-5 cm depth - indicating the protection of this OC once tillage had ceased. However, due to the relatively-short duration of this experiment (14 years of RT followed by 4 years of NT), the conversion from RT to NT was not was not reflected in the formation of microaggregates within macroaggregates - as can be deduced by the lack of effect of RT on OC-SMm in this layer, even when NT produced greater aboveground biomass (183.5 g m^{-2}) than RT (Almagro et al., 2013). Furthermore, below 5 cm depth, OC SMm was greater in RT than in NT. Unlike in the tillage treatments, a lack of correlation was found in NT between the plant biomass input (as free LF-C) and SM formation. All these findings together indicate that the accumulation of the residues at the surface under NT might have accelerated the mineralization (vegetal material more exposure to photodegradation) of this material and slowed down its incorporation on the soil (Bronick et al., 2005). Furthermore, in a previous study we detected that a lack of tillage leads to higher bulk density and shear strength of the soil profile compared to tillage treatments (Martínez-Mena et al., 2013), which might help to inhibit the leaching of this material through the soil profile and migh limite the oxygen content restrict the access of microorganisms to the subsoil (De Gryze et al., 2005). The greater reduction of the microbial C below the plow layer (15-30 cm) in NT compared to the tillage treatments supports this suggestion (Table 6.1). In fact, other authors reported higher biological activity and greater accumulation of microbial-C at the surface under NT treatments (Doran, 1987; Balesdent et al., 2000). Previous work that compared NT with conventional tillage in semiarid agroecosystems and under different crops (mainly cereals) found neither an increase in the OC associated with microaggregates within-macroaggregates nor differences in the total SOC levels, in the short-term (after five years of NT) (Lal et al., 1998; West and Post, 2002; Denef et al., 2007). By contrast, Plaza-Bonilla et al. (2013), in longer experiments, found an increase in the proportion of macroaggregates and an enrichment of the C concentration within microaggregates under NT. It is well known that accumulation of SOC under NT is a slow and gradual process (Denef et al., 2007).

6.4.2. Changes in OC associated with density fractions

The formation of new aggregates and the subsequent OC occlusion in RTG, commented on before, are supported by the greater occlusion of fine iPOM-C within small-macroaggregates at the surface layer in this treatment, relative to RT. From these results we hypothesize, according to the theory of Six et al. (1998), that the organic matter input from green manure formed coarse iPOM-C which was decomposed and fragmented into fine iPOM-C. Finally, the fine iPOM-C was sequestered mainly in the newly-formed microaggregates within macroaggregates. In fact, in RTG, there was a high, positive correlation between the OC associated with the total intra-aggregate particulate organic matter (total iPOM-C) and the microbial C (r=0.705, $p<0.01$). This suggests that the total iPOM-C might be a C source for the microorganisms which are inductors for the union of silt-plus clay-sized particles and microaggregates within macroaggregates (Courtier-Murias et al., 2013). In this treatment, however, the continual rupture of aggregates by tillage led to the loss of coarse iPOM-C and so to the lack of differences between RTG and RT in this fraction in the short-term. In RT, the OM input was not enough to offset the loss of fine iPOM-C due to the faster macroaggregate turnover and breakdown caused by tillage.

In NT treatment, the fine iPOM-C within small-macroaggregates was also greater than in RT. However, this increment was not enough to form new aggregates and to produce an increment in the total OC-SMm, due to the short-term nature of this experiment. In this sense, the fact that OC-SMm concentration in NT is not different from RT might be an indicator of the high stability of this fraction being less affected by tillage than other OC fractions occluded in larger aggregates. Other authors obtained significantly-higher fine iPOM-C concentrations in small-macroaggregates in superficial soil layers when conventional tillage was shifted to no tillage in long-term experiments (Six et al., 1998; Alvaro-Fuentes et al., 2009). In our case, however, at 5-15 cm depth the coarse iPOM-C content of SM was higher in NT than in RT, while no differences in fine iPOM-C were observed: this might have been determined by the inaccessibility of the OM remaining in the subsoil (when tillage ceased, 4 years ago) to the microorganisms responsible for its transformation into fine iPOM-C. Irrespective of tillage and/or amendment, the much–higher values of mineral-C - compared with free or intra-aggregate particulate OC pools - in our soils indicate that the mineral-C has a higher storage capacity, being less susceptible to mineralization (Courtier-Murias et al., 2013; Plaza-Bonilla et al., 2014). The mixed-clay mineralogy of these soils, dominated by kaolinite (1:1 layer type) and illite (2:1 layer type) and, in general, with high specific surface area in the soil matrix promoted the formation of organo-mineral associations and the stabilization of OC in the silt-plus clay fraction, due to the ability of a source of Ca^{2+} ions to protect the SOM from mineralization (Duchaufour, 1976; Muneer and Oades, 1989). These stabilization mechanisms are usual in Mediterranean areas and have been found in other sites, close to the study area (Garcia-Franco et al., 2014).

In addition, mineral-C was the most-relevant pool for OC sequestration in SM, representing about 45% of the total OC-SM in all treatments. Similar results were obtained by other authors (Denef et al., 2001; De Gryze et al., 2004; Álvaro-Fuentes et al., 2009) and suggest that a significant part of the C stabilization in SM is due to physical-chemical protection of SOM by minerals (Jastrow, 1996; Six, 1999; Krull et al., 2003; Bronick and Lal, 2005). In fact, when the fresh organic matter input was added in the RTG treatment, a positive correlation (*r*=0.656, $p<0.05$) between the free-LF-C and total mineral-C (from SM and m) was obtained, indicating that a relevant proportion of the fresh and labile OC, as free LF-C, was adsorbed by mineral particles.

Nevertheless, and despite the fact that the greatest storage of C in these soils occurs in the mineral-C pool, a significant part (17-25%, depending on the treatment and depth) occurs in the intra-aggregate POM fraction. Hence, relative to treatment RT, RTG gave increases of 138.8% and 36% at 0-5 and 5-15 cm depth, respectively, in the fine iPOM-C occluded in free microaggregates, while in NT an increase of 57.5% was found at 0-5 cm depth. These results indicate that, in these treatments, new microaggregates could have been formed free in the soil, with the incorporation of fine iPOM-C. This supports the idea that the incorporation of new C into free microaggregates is an important factor contributing to C sequestration (Denef et al., 2001), since the C contained in free microaggregates has a slower turnover than that in macroaggregates (Jastrow et al., 1996). In fact, in RTG, the fine iPOM-C occluded in free microaggregates represented 56% of the increment in the total bulk SOC at the surface layer, compared to RT.

6.5. Conclusions

Despite the short-term nature of this experiment, we found differences in all OC density pools due to the alternative SLM practices (RTG and NT), mainly at the surface layer. Moreover, our results show that in these soils both the mineral-C and iPOM-C fractions are responsible for the OC accumulation in the bulk soil, suggesting that this joint physical-chemical mechanism of OM stabilization is enhanced when amendment is added and/or tillage is ceased. The greatest increases in the total SOC and OC associated with the aggregates were found with the combination of green manure and reduced tillage (RTG), suggesting that this practice is a good option for OC sequestration since: i) green manure represents a continuous OM input which activates the physical protection of OC through new aggregates formation and physico-chemical stabilization in mineral particles, and ii) minimum tillage is necessary because it favors the incorporation of plant material into deeper layers, promoting the formation of new aggregates in these layers. The relevance of OC sequestration in the subsoil in Mediterranean agricultural areas has been pointed out by other authors (Albaladejo et al., 2012). No tillage favored OC accumulation in the fine iPOM-C occluded within the small-macroaggregates and microaggregates at the surface layer and in the coarse iPOM-C at 5-15 cm depth, but four years of cessation of tillage was not enough to increase the total OC in the bulk soil. Nevertheless, taking into account the characteristics of these soils, the adoption of NT as an isolated strategy might not produce the expected positive results with respect to soil C accumulation.

In future studies, soil fractionation combined with techniques to determine which fungal and bacterial communities are involved in the accumulation and stabilization of SOC could be necessary to obtain more-complete and detailed knowledge of all the mechanisms of interaction among the physical, chemical, and biological soil properties implicated in C sequestration. This would facilitate the selection of the most-adequate SLM practice in these semiarid Mediterranean agroecosystems.

General conclusions

1. The type of land use was shown to be the factor with greatest weight in the control of the concentration of organic carbon (OC) in the soil in semi-arid ecosystems. The conversion of forest into farmland areas implies a considerable reduction in the soil organic carbon (SOC) content (about 70% in the 0-20 cm depth), so it should be severely restricted.

2. The factors controlling the changes in the SOC content vary depending on the type of use and the depth of the soil. In relation to the use, while in forest ecosystems the major control factors were mean annual precipitation and texture, in shrubland and agricultural ecosystems they were temperature and lithology. With respect to depth, its increase reduces the relative importance of climatic factors (temperature and precipitation) and the texture becomes the most important factor, regardless of the land use. These results suggest that, in future scenarios of climate change, changes in temperature will have greater impact on agricultural soils, while precipitation changes will affect forest soils more greatly. Our results predict a decrease in the contents of SOC, in a scenario of climate change with an increase in temperature and a decrease in precipitation - as is expected in semi-arid areas. For all uses, fine-textured soils will be more resistant to the changes than coarse-textured soils.

3. The analysis of environmental factors shows that the impact of climate change will be greater at the soil surface than at depth, so actions intended to achieve carbon sequestration should focus on sequestration in the subsoil. In this sense, the traditional reforestation in mountainous areas is potentially limited by the presence of bedrock at shallow depth. The distribution of carbon stocks in the soil profile (Chapter 3) suggests that actions in agricultural areas (to encourage management practices appropriate to these objectives) are potentially more effective and probably more profitable economically and socially, since, in addition to reducing the atmospheric CO_2, they increase the productivity of the soil and the agricultural yields.

4. The potential sequestration of C in semi-arid reforested areas depends largely on the techniques used for reforestation. The C stocks in reforested

ecosystems are directly proportional to the amount of biomass produced, which, in turn, is determined by the productivity of the soil. Therefore, methods that improve the productivity of the soil must be used. The addition of organic amendments to the soil, prior to planting, was very effective in terms of C sequestration. The cessation of practices involving major disturbance of the soil horizons is recommended, since, in addition to reducing soil productivity, they suppose the emission of high amounts of CO_2 to the atmosphere.

5. The chemical stabilization of OC, through the formation of complexes with silt and clay particles and its physical protection in microaggregates formed within macroaggregates, was the main mechanism of C sequestration in the soils studied, both in agricultural and forest areas.

6. The chemical stabilization was promoted by the mineral composition of the soil matrix, particularly the high concentration of multivalent cations, mostly Ca^{2+}, and the presence of mineral surfaces with a high capacity for absorption of organic compounds, such as the inter-stratified illite-montmorillonites - which have a very high specific surface area.

7. The physical protection of SOC was promoted by the changes, both qualitative and quantitative, in the plant contributions to soil. In both the forested area, especially for the treatment involving organic soil amendment, and in the agricultural area where green cover was implemented, an increase in the labile pool of OC in the soil occurred. This increase promotes the formation of macroaggregates, in two ways: a) directly, by acting as a binding agent between soil particles, and b) indirectly, by activating the microbiological activity, especially that of the fungi - which "package" the particles with their hyphae. The establishment of these new macroaggregates favors the formation of microaggregates that occlude organic matter inside and make it inaccessible to the microorganisms.

8. In the agricultural soils of these semi-arid areas, minimum tillage seems necessary, since it promotes the incorporation of plant material into deeper layers, promoting the formation of aggregates and therefore OC occlusion within them.

9. The changes in the vegetation cover induce changes in the community structure of the fungi in the soil which, in the case of reforestation with *Pinus halepensis*, favor the formation of macroaggregates, due to an increase in the proportion of hyphae-forming species.

10. The strong correlations between basal respiration and the percentage of microaggregates within macroaggregates, positive in the reforested soils and negative in the degraded shrubland, suggest that: a) the formation of microaggregates, rich in OC, within macroaggregates is a self-defense mechanism of the soil, for the protection of the OC against the increased microbiological activity, and b) these correlations could serve as indicators of processes of improvement (positive correlation) or degradation (negative correlation) of the soil.

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