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APORTACIONES AL CONOCIMIENTO DE LA DINÁMICA DE LA MATERIA ORGÁNICA EDÁFICA EN SUELOS CALIZOS DEL PÁRAMO CASTELLANO-LEONÉS

CONTRIBUTIONS TO THE KNOWLEDGE OF THE DYNAMICS OF SOIL ORGANIC MATTER IN SOILS OF LIMESTONE MOORLAND OF CASTILLA Y LEÓN

TESIS DOCTORAL

Mireia Llorente Sánchez
Palencia, 2011

Universidad de Valladolid

E.T.S.II.AA.

EDAFOLOGÍA Y QUÍMICA AGRÍCOLA

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CONTRIBUTIONS TO THE KNOWLEDGE OF THE DYNAMICS OF SOIL ORGANIC MATTER IN SOILS OF LIMESTONE MOORLAND OF CASTILLA Y LEÓN

Presentada por MIREIA LLORENTE SÁNCHEZ para optar al grado de
Doctora por la Universidad de Valladolid

Dirigida por:
MARÍA BELÉN TURRIÓN NIEVES

Palencia, 2011

La Dra. **María Belén Turrión Nieves**, Profesora Titular de la Universidad de Valladolid,

CERTIFICA:

Que la Licenciada en Biología **Mireia Llorente Sánchez** ha realizado bajo su dirección el trabajo que, para optar al Grado de Doctor, presenta con el título "APORTACIONES AL CONOCIMIENTO DE LA DINÁMICA DE LA MATERIA ORGÁNICA EDÁFICA EN SUELOS CALIZOS DEL PÁRAMO CASTELLANO-LEONÉS".

Y que mediante este Trabajo de tesis, se solicita el reconocimiento del Doctorado europeo de la doctoranda, quien realizó una estancia de cuatro meses en el Institute of Soil Science and Soil Geography, University of Bayreuth, Alemania, bajo la supervisión del Dr. Bruno Glasser (Profesor titular de dicha Universidad).

Y para que así conste a los efectos lo firmo en Palencia, a 20 de octubre de 2011.

_____

María Belén Turrión Nieves

A mi padre, Santos, que desde que mi manita no abarcaba ni su palma, me ha enseñado a amar la naturaleza y a festejar la vida, dos de mis tesoros más preciados.

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A Gus y a Hebe, mi familia.

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RESUMEN

A lo largo del siglo pasado, muchos de los bosques naturales de encina (*Quercus ilex)* que ocupaban los suelos cálcareos de Castilla y León fueron roturados para su conversión en tierras de cultivo. Hace unos 40 años, estas tierras agrícolas de muy bajo rendimiento volvieron a reforestarse principalmente con *Pinus halepensis.*

Para entender cómo estos cambios de manejo han afectado a la dinámica de la materia orgánica de estos suelos, hemos estudiado parámetros tanto de cantidad como de calidad de la materia orgánica edáfica bajo los 3 usos del suelo mencionados: bosque natural del encina, tierra de cultivo y plantación forestal de pinos, en el contexto de las características físicas, químicas y bioquímicas del suelo.

El fraccionamiento por densidad combinado con la dispersión ultrasónica de los agregados del suelo permite separar fracciones de la materia orgánica basándonos en los mecanismos de estabilización física de la misma, pudiéndose diferenciar: la fracción no protegida (fracción lábil); la materia orgánica ocluida (fracción ocluida) y; la materia orgánica estabilizada a través de complejos órgano-minerales (fracción órgano-mineral).

En este trabajo hemos separado dichas fracciones de densidad y hemos determinado en ellas: C orgánico, inorgánico y total, N total, relación C/N, el contenido en *Black Carbon* (BC), la abundancia natural de algunos isótopos ($\delta^{13}C$ y $\delta^{15}N$) y el contenido y composición en carbohidratos.

En un periodo de 100 años, aproximadamente el 67% del C del suelo (considerando los primeros 10 cm del suelo) se perdió al roturar el bosque de encinas y convertirlo en tierras de cultivo. La reforestación con pinos durante 40 años consiguió la recuperación del 71% del C respecto a la situación inicial (considerando los primeros 10 cm del suelo).

A pesar de las diferencias en el contenido de C orgánico del suelo bajo los distintos usos comparados, los mecanismos de estabilización de la materia orgánica actuaron de forma similar. En los suelos calcáreos estudiados el mecanismo de estabilización principal fue la formación de complejos órgano-minerales. La facción más afectada por los cambios de uso del suelo fue la fracción lábil de la materia orgánica.

El BC, que representó entre el 1,2 y el 2,3% del contenido de C orgánico de estos suelos se ubicó en mayor proporción en la fracción ocluida. La proporción de BC respecto al contenido de C orgánico del suelo se mantuvo independientemente del cambio de uso de suelo, sugiriendo un equilibrio entre las entradas y salidas de BC en el mismo. Estos resultados nos llevan a sospechar que probablemente el BC es menos resistente a la biodegradación de lo que se da por supuesto en la bibliografía.

Además, se compararon algunas fracciones lábiles del C como indicadoras de cambio de uso del suelo: C microbiano, C extraíble con K_2SO_4 (C_{K2SO4}) y C mineralizado acumulado

en 21 días de incubación a 28ºC ($C\text{-}CO_{2(21d)}$). La biomasa y la actividad micorbianas resultaron bajas coincidiendo con el alto contenido de carbonatos. Como indicadores de cambio de uso del suelo, C_{K2SO4} and $C\text{-}CO_{2(21d)}$ parecieron ser indicadores al menos tan sensibles como el C microbiano.

Las emisiones de C respirado se midieron para el studio de la cinética de la mineralización del C en estos suelos. El ajuste al modelo cinético de primer orden $C_m=C_o(1 - e^{-kt})$ fue muy bueno ($R^2 > 0.94$).

Se aplicó un Análisis de Componentes Principales para sintetizar las relaciones entre los parámetros microbiológicos estudiados. A partir de este análisis se observó que la eficiencia en el uso de la materia orgánica del suelo por parte de los microorganismos disminuye a medida que aumenta la cantidad y la labilidad del sustrato.

Los carbohidratos del suelo representaron entre el 6 y el 10% del total del C orgánico del suelo. El cultivo del suelo supuso una pérdida significativa de la proporción de C representado por carbohidratos y también se observó como significativas las diferencias en cuanto a composición de dichos carbohidratos al comparar los distintos usos del suelo. Al comparar las distintas fracciones de densidad también se observan diferencias significativas en la composición en monosacáridos aunque se mantiene constate la proporción del C orgánico que queda representado por C monosacárido.

Para cualquiera de las fracciones de densidad y de los usos del suelo, la glucosa siempre es el monómero de azúcar más abundante, seguida por la manosa y la xilosa. Existe una correlación positiva y significativa entre el contenido de C representado por monosacáridos y el C orgánico, la relación C/N, el C microbiano y la relación C/N en la biomasa microbiana.

Dada la demostrada importancia de la medición de la biomasa microbiana como indicador temprano de la dinámica de la materia orgánica en el suelo, la presente Tesis incluye un capítulo final que pretende ser una aportación al método de estimación del C y el N de la biomasa microbiana mediante el uso de absorbancia ultravioleta, método que supone un sigificativo ahorro de tiempo y esfuerzo respecto al método de fumigación-extracción. Las estimaciones del C y el N microbianos a 260 nm mostraron las mejores correlaciones con las obtenidas por el método de fumigación-extracción.

ABSTRACT

In the last century, many calcareous soils in Castilla León (northwestern Spain) have been transformed from natural *Quercus ilex* forest to cropped land. Reforestation with *Pinus halepensis* has been taking place during the past 40 years.

In order to obtain a better understanding of how these disturbances affect ecosystem functioning, we studied the quantity and quality of soil organic matter (SOM) under the mentioned land uses.

Density fractionation combined with ultrasonic dispersion enables separation and study of soil OM fractions, considered on the basis of the mechanisms of physical protection: non-physically protected OM (FF), OM occluded into aggregates (OF), and OM stabilized in organo-mineral complexes (DF).

We separated SOM density fractions and determined the concentrations of C and N, C:N ratio, Black Carbon content (BC), the natural isotopic abundance ($\delta^{13}C$ and $\delta^{15}N$ values) and neutral sugar content.

About 67% of the total C in the topsoil was lost as a result of converting the natural*Quercus ilex* forest to cropped land, 100 years ago. An average recovery of 71% of the previously lost OC had been recovered, after 40 years of pine plantation. Despite the different OC contents of soils under different land use, OM stabilization mechanisms were not significantly different. In calcareous soils, accumulation of SOC and N is mainly due to organo-mineral associations. The changes in OC stocks affected mainly the free fraction (FF).

Black carbon represented between 1.2 to 2.3% of the TOC of soil with the highest concentrations in OF. The maintenance of BC proportion through land uses suggests equilibrium between inputs and outputs, and leads to the suspicion that BC could be less stable and less resistant to biodegradation than is often taken for granted.

Also, labile C fractions: microbial biomass C (MBC), K_2SO_4 extractable C (C_{K2SO4}) and the cumulated mineralized C in 21 days incubation at 28ºC ($C\text{-}CO_{2(21d)}$), were compared as land use indicators. Microbial biomass and activity were found to be low and coincided with high carbonate contents. As indicators of land use, C_{K2SO4} and $C\text{-}CO_{2(21d)}$ showed the same sensitivity as MBC. $C–CO_2$ emissions were measured in order to study C mineralization kinetics. A good fit ($R^2 > 0.94$) to the first-order kinetic model $C_m=C_o(1 - e^{-kt})$ was found. Principal Components Analysis was applied to the data and the relationship among microbial metabolic quotient (qCO_2), labile C pools, and MBC revealed a decrease in efficiency of organic substrate utilization with an increase in availability and lability of the organic matter.

Soil carbohydrates accounted for 6 to 10 % of the total organic C of soils. Cultivation also affected the proportion of soil C present as carbohydrates. Monosaccharide analysis revealed significant differences in carbohydrate composition between land uses. Significant differences in monosaccharide composition were also found among density fractions however, the proportion of the organic C represented by monosaccharide C among fractions was constant. Whatever the fraction and land use considered, glucose was the dominant sugar monomer, followed by mannose and xylose. Stands out the positive and significant correlation between monosaccharide C content in samples and OC, C/N ratio, microbial biomass C, and microbial biomass C/N ratio.

Because the ability to estimate soil microbial biomass C (SMB C) and nitrogen (SMB N) is of fundamental importance for studying a range of soil processes, present thesis includes a chapter assesses the relationship between SMB C and SMB N measured by conventional fumigation-extraction method and the estimates obtained by increases in UV absorbance at 224, 260, 280, and 340 nm of 0.5M K_2SO_4 extracts of fumigated soils. The UV technique provides a simple and adequate way of SMB C and SMB N estimations in calcareous soils, being the increase in absorbance at 260 nm the best estimator of SMB C and SMB N in the studied calcareous soils.

NOTE TO READERS

This thesis is base don five original works, which are published or under revision in different international journals. Each article constitutes one of the studies or chapters of the thesis (Chapters 5 to 9). Authors, coauthors, and the stage of the publication are presented below and also at the first or each chapter. Previously to the presentation of these articles, the readers can find written in Spanish the following chapters a general introduction (Chapter 1), the general objectives in Spanish and English (Chapter 2), a general description of the study area (Chapter 3) and the main methodologies and analyses employed (Chapter 4). At the end, the reader will find the general conclusions of the thesis written in Spanish and English (Chapter 10) and General Bibliography (Chapter 11)

List of original works

Mireia Llorente and María-Belén Turrión. 2010. Microbiological parameters as indicators of soil organic carbon dynamics in relation to different land use management. *European Journal of Forest Research 129: 73-81. DOI: 10.1007/s10342-008-0249-z*

Mireia Llorente; Bruno Glaser; M. Belén Turrión. 2010. Storage of organic carbon and black carbon in density fractions of calcareous soils under different land uses. *Geoderma. 159: 31-38.* :10.1016/j.geoderma.2010.06.011

Mireia Llorente; Bruno Glaser; María-Belén Turrión.2011. Anthropogenic disturbance of natural forest vegetation on calcareous soils alters soil organic matter composition and natural abundance of ^{13}c and ^{15}n in density fractions. European Journal of Forest Research. 129, Issue 6: 1143. DOI 10.1007/s10342-010-0402-3.

Mireia Llorente; Bruno Glaser; María-Belén Turrión. Effect of land use change on contents and distribution of carbohydrates whitin density fractions of calcareous soil. *Science of the Total Environment. (Under revision).*

Mireia Llorente, María-Belén Turrión; Francisco Lafuente Estimation of microbial biomass carbon and nitrogen in calcareous soils by UV-absorbance. *Journal of Environmental Management (Under revision).*

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1. INTRODUCCIÓN GENERAL

1.1. Suelos, cambio de uso del suelo y materia orgánica

Nos encontramos ante una crisis climática cuya dimensión va en aumento. El crecimiento exponencial de la población humana unido a un modelo económico basado en una aceleración constante del ritmo de producción-consumo, nos ha llevado a una situación en la que las tasas de emisión de CO_2 superan con creces las tasas a lo que los ecosistemas pueden secuestrar y, por tanto, a una situación de acumulación creciente de CO_2 en la atmósfera.

Los suelos, suponen uno de los mayores depósitos de C en los ecosistemas terrestres (Eswaran et al., 1993) por lo que los cambios de uso del mismo juegan un papel crucial en el balance global de C. Aunque el uso masivo de combustibles fósiles y la producción de cementos son los principales responsables de las emisiones de CO_2, el manejo agrícola industrial y los cambios de uso de suelo en conjunto suponen en la actualidad más de un 30% del total de las emisiones (IPCC, 2007) (Figura 1). Son abundantes los estudios que identifican la materia orgánica del suelo (MOS) como uno de los compartimentos en el ciclo de C determinante en el Cambio Climático (e.g. Sanderman et al., 2003). Por ello, además de una urgente disminución de las emisiones, en la Conferencia de Kyoto se insistió en la necesidad de profundizar en el conocimiento de las relaciones fuente-sumidero a nivel de ecosistema con dos objetivos principales: conservar el C almacenado en los ecosistemas y mejorar la capacidad de los mismos como sumideros de C.

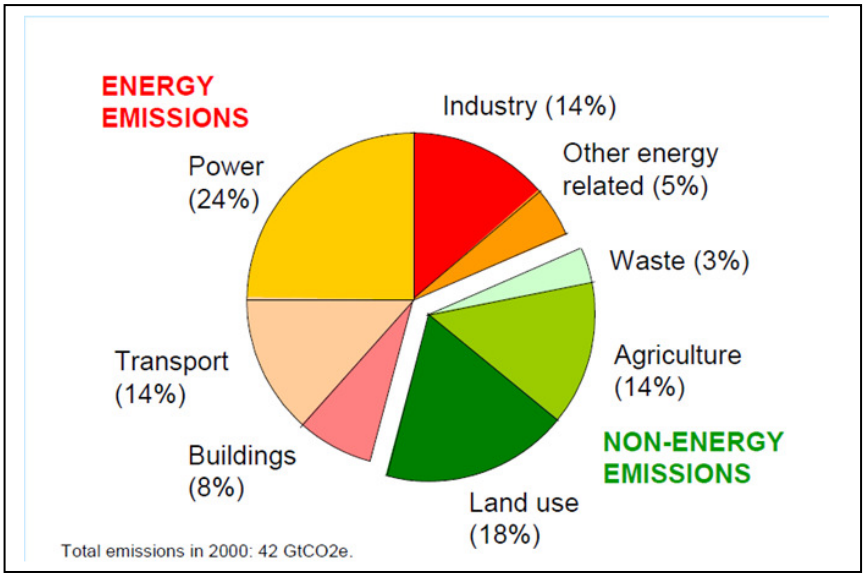

Figura 1

Reparto de las emisiones de GEI por sectores en 2000 (IPCC, 2007)

El estudio del suelo como fuente y sumidero de C ha de abordarse desde el entendimiento de la complejidad del sistema de interrelaciones atmósfera-suelo-vegetación-organismos edáficos donde la MOS y su dinámica juegan un papel clave.

El suelo actúa, tanto de soporte físico para la vegetación, como de reserva de recursos: agua y nutrientes solubles en ella. Además, la MOS juega un papel fundamental en el equilibrio del ecosistema, amortiguado cambios bruscos de humedad, temperatura o pH, así como de fenómenos de degradación edáfica tales como la compactación o la erosión del suelo. Las características del suelo condicionan la instalación, la supervivencia y la productividad de las comunidades vegetales (Doran et al., 1996). A su vez, la vegetación supone un factor esencial de formación del suelo. Por una parte, la vegetación es la principal fuente de MOSy, por otra, el sistema radical de las plantas sujeta el suelo, protegiéndolo de fenómenos de erosión, mejorando su estructura y su aireación (Quilchano, 1993). En este contexto, numerosos trabajos se han dirigido al estudio de cómo la vegetación supone cambios tanto en las propiedades físicas (Goss et al., 1992) como en las propiedades químicas (Rauschkolb, 1971) del suelo.

En cuanto a la interrelación de los organismos del suelo y la cubierta vegetal, existe una fuerte interdependencia nutricional entre ambos compartimentos del ecosistema a través de su acción en distintos puntos del ciclo de la materia orgánica (MO). La vegetación existente determinará, en gran medida, la cantidad y calidad de la MO aportada al suelo, que supone la principal fuente de entrada de nutrientes para los organismos edáficos. Los organismos del suelo, a su vez, son los principales responsables de la disgregación y descomposición de la MO (Palma et al. 2000), poniendo de nuevo a disposición de la vegetación los nutrientes que necesita. Por otra parte, la estructura de la vegetación va a determinar condiciones microclimáticas (luz, temperatura, magnitud de las oscilaciones térmicas y humedad) que tienen una gran importancia en la comunidad de organismos del suelo (Priha et al., 1999). La extensión y distribución de las raíces determinadas, tanto por la especie vegetal, como por las características del suelo, es un factor muy importante en la distribución de muchos de los organismos edáficos.

En el suelo, se albergan una gran variedad de especies, integrando todas las categorías tróficas de un ecosistema por lo que su estudio taxonómico es complejo y suele recurrirse para su conocimiento a la estima de ciertos parámetros metabólicos. La composición y el comportamiento de la comunidad de seres vivos del suelo dependen de factores tales como la cantidad y calidad de la MOS, humedad y aireación del suelo, temperatura, pH.

Por todo lo anterior, los cambios en la cubierta vegetal y en la forma de manejo del suelo suponen cambios en todo el sistema edáfico que afectan al ciclo de la MO y, con ello, al comportamiento del suelo como fuente o como sumidero de C.

Johnson (1995) modeliza cómo la cantidad de MO que retiene el sistema depende de la relación entre la tasa de incorporación MO nueva y la tasa de descomposición de la misma. Ante un cambio de uso del suelo, ambas tasas se ven alteradas y el sistema se comportará como exportador o como importador de C hasta alcanzar un nuevo estado de equilibrio (Figura 2). La pérdida de C del suelo que se produce al provocar un

cambio de uso de suelo se llama Deuda de Carbono y en el caso de un bosque o un pastizal que se transforma hacía uso agrícola la pérdida oscila entre el 30 y el 70 % del C inicial (e.g., Burke et al., 1989).

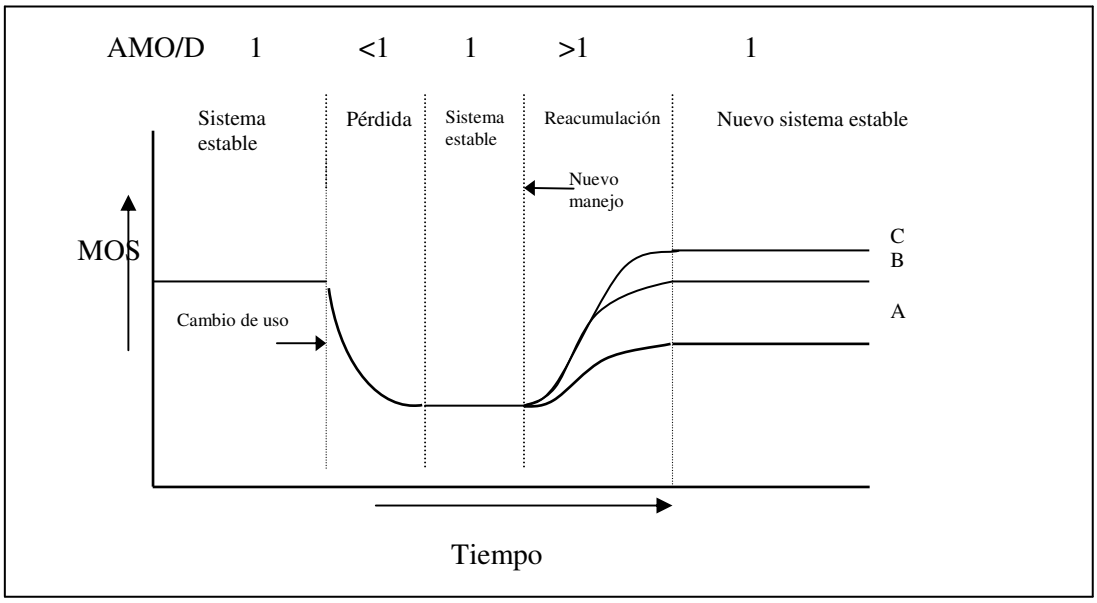

Figura 2

Modelo conceptual de pérdida y reacumulación de materia orgánica en el suelo (MOS) tras una perturbación (modificado de Johnson, 1995). AMO: acumulación de materia orgánica; D: descomposición.

1.2. Actividad microbiana y descomposición de la materia orgánica

Las tasas de descomposición de la MO y, por tanto, la velocidad a la que el C regresa a la atmósfera, depende de muchos factores tales como la calidad del sustrato y su disponibilidad de nitrógeno, las características físicas y químicas del suelo, las condiciones físicas del sitio, todos ellos relacionados con la actividad de los microorganismos en los procesos de descomposición de la MO (Mary et al., 1996; Ryan and Law, 2005).

El estudio y la medición de los parámetros biológicos del suelo tienen una gran importancia, tanto por el papel fundamental que los organismos edáficos juegan en el ciclo del carbono y en la nutrición vegetal, como porque sirven como indicadores sensibles a los cambios en las características de la cubierta vegetal o a la forma de manejo del sistema (García et al., 1997; Goberna, 2006). Han et al. (2007), señalan la utilidad de la medición de la biomasa microbiana para el entendimiento de los procesos de secuestro-liberación de C en el suelo. Asimismo, el estudio de la actividad microbiana y de la tasa metabólica de la comunidad microbiana (qCO_2), se utilizan como indicadores de la eficiencia de los microorganismos en el uso del sustrato (Goberna

et al., 2006). También se utilizan medidas de otros pooles de C lábil del suelo, tales como el C extraíble en K_2SO_4 o el C respirado acumulado tras un cierto periodo de incubación, para una mejor comprensión de la dinámica de la descomposición de la MOS.

El uso de modelizaciones matemáticas es útil para mejorar la interpretación del significado ecológico de algunos parámetros microbiológicos (Paul and Voroney, 1984; Murwira et al., 1990). Los modelos que mejor se han ajustado a la cinética de la actividad microbiana son modelos de primer orden que asumen que las tasas de mineralización sólo depende del sustrato de partida, considerando uno o varios pools de carbono. Pero se las tasas de mineralización pueden depender de otros factores por lo que es necesario validar estos modelos para las distintas condiciones de sitio (Grant et al., 1993).

1.3. El fraccionamiento de la materia orgánica del suelo

Para el estudio y modelización de la dinámica del C en el suelo, es necesario tener en cuenta que la MOS no es un componente homogéneo si no que en ella puede diferenciarse fracciones de distinta estabilidad y, por tanto, a las que se asocian distintas tasas de descomposición, que pueden oscilar desde unos pocos días a cientos de años (Schimel et al., 1985).

La localización de la MOS respecto de la matriz edáfica es el factor que más determina la estabilidad de la misma (Balesdent, 1996). En base a esta localización se pueden distinguir dos mecanismos de estabilización física de la MOS frente a los procesos de descomposición. Uno de estos mecanismos es que la MO quede protegida por la agregación de partículas minerales que la protegen frente a los agentes descomponedores (estabilización por oclusión). El otro mecanismo de estabilización consiste en la formación de complejos órgano-minerales por interacción química entre partículas minerales y partículas orgánicas.

Los métodos de fraccionamiento físico de la MO permiten separar y estudiar la dinámica, estructura y función de las distintas fracciones de la MOS (Golchin et al., 1994; Six et al., 2001).

En concreto, el fraccionamiento de la MOS por densidad, combinado con dispersión ultrasónica, permite separar y estudiar tres fracciones en base a los mecanismos de estabilización anteriormente expuestos: MO libre, MO ocluida en agregados, y MO en interacción con el sustrato mineral (Sohi et al., 2001).

Diversos estudios que utilizan el fraccionamiento de la MOS señalan la MO libre como la fracción más lábil del suelo, asociada a tasas de descomposición muy rápidas, del orden de días. En contraste, la fracción ocluida y la fracción órgano-mineral de la MOS son fracciones más estables con tasas de descomposición que pueden oscilar desde décadas hasta siglos (e.g. Golchin et al., 1994; John et al., 2005). Por todo ello, cabe señalar que las distintas fracciones de la MOS se ven afectadas de forma diferente por los cambios de uso o manejo del suelo y que el estudio de las mismas puede acercarnos a un mejor entendimiento de los efectos del cambio de uso de los suelos sobre la MO edáfica.

1.4. *Black Carbon* ¿una fracción realmente tan estable?

Algunas publicaciones en torno a Black Carbon (BC) del suelo dan por sentado que éste puede tener una gran relevancia en el ciclo global del carbono al representar una forma de MO tan estable que podría considerarse permanente y que, por tanto supone, un sumidero de C a largo plazo (Kuhlbusch, 1998; Schmidt and Noack, 2000). Basándose en estas afirmaciones, desde algunos sectores se pretende promover la incorporación al suelo de BC sintetizado de forma artificial, al que llaman Biochar, como forma de aumentar su papel como sumidero de C. De hecho, en las últimas negociaciones internacionales por el clima empieza a discutirse esta tecnología para su posible incorporación como MDL (Mecanismo de Desarrollo Limpio).

En contraste, existe un creciente número de publicaciones que muestran como el BC puede presentar tasas de descomposición no demasiado lentas (Iswaran et al., 1980) ya que puede ser degradado tanto por causas bióticas como abióticas (Cheng et al., 2006). Existen también diversos estudios que muestran que la adición de BC puede aumentar las emisiones normales de C en algunos ecosistemas debido a la proliferación de microorganismos especializados en la degradación de C vegetal (Hammer et al., 2004) y al aumento de la actividad microbiana debida a la presencia de carbón vegetal que supondría una aceleración en la biodegradación de la MO preexistente en el suelo (Reijnders, 2009).

La realidad es que, a día de hoy, no se cuenta con estudios suficientes sobre el comportamiento de BC como para poder generalizar la afirmación de que BC puede jugar un papel clave en la estabilización del C en el suelo y es necesario ahondar en los factores que determinan su tasa de descomposición. En el caso de suelos de la región mediterránea y en particular sobre suelos calizos, no conocemos de la existencia de estudios anteriores al publicado como parte de esta tesis.

1.5. Composición isotópica de las fracciones orgánicas del suelo

El análisis de los isótopos estables de la MOS es un análisis relativamente barato y sencillo que, sin embargo, permite obtener información muy útil sobre algunos cambios químicos en la MO (Glaser, 2005).

Esta técnica se apoya en el hecho de que la abundancia natural relativa de los isótopos pesados y ligeros (por ejemplo, ^{13}C y ^{12}C) en la MO varía debido a que durante los procesos biológicos se produce una discriminación a favor de los isótopos más pesados (Andreux et al., 1990). Esta discriminación isotópica queda muy influenciada por factores tales como la química del suelo, la mineralogía o el tipo de MO que se incorpora al suelo (Krull and Skjemstad, 2003)

El uso de análisis de isótopos estables del carbono ($\delta^{13}C$) y, aunque en menor grado, de isótopos estables de nitrógeno ($\delta^{15}N$) para el estudio de la MOS ha sido crecientemente aplicado para el estudio de las tasas de descomposición en C y N (p.e., Balesdent and Mariotti 1988; Sevink et al. 2005) así como para conocer el grado de descomposición de la MOS (p.e., Wedin et al. 1995; Connin et al. 2001).

Aunque hay bastantes estudios sobre la composición isotópica de las fracciones de la MOS (p.e., Shang and Tiessen 2000; Crow et al. 2006) éste tipo de datos son de gran utilidad y los mecanismos que determinan el

cambio en la composición isotópica asociada a los mecanismos de descomposición de la MO aún no están claros (Liao et al. 2006).

1.6. Importancia de los carbohidratos en el suelo

Los carbohidratos del suelo tienen un importante papel en el ciclo de los nutrientes (Cheshire, 1979), representan entre el 5 y el 25% de la material orgáica edáfica (Lowe, 1978) y se presentan en distintas formas, desde restos de plantas y animales sin descomponer hasta productos químicos y bioquímicos de la descomposición y síntesis.

Los carbohidratos del suelo tienen tasas de descomposición rápidas y están implicados en distintas reacciones bioquímicas y órgano-minerales. Represetan la mayor fuente de nutrientes y de energía para los organismos edáficos por lo que los carbohidratos del suelo son un indicador sensible de las propiedades biológicas del suelo. Además los carbohidratos juegan un importante papel en la mejora de la estabilidad estructura del suelo ya que mejoran la estabilidad de los agregados (Feller and Beare, 1997).

2. OBJETIVOS GENERALES

El objetivo general de la presente tesis, enmarcada en suelos calizos del páramo calizo castellanoleonés, es contribuir al conocimiento de la dinámica de la MO del suelo bajo los distintos usos del suelo que existentes en el territorio de estudio: bosque nativo adehesado dominado por *Quercus ilex;* plantaciones forestales de *Pinus halepensis* y tierras agrícolas cerealistas.

Los objetivos específicos del presente estudio son

a) Comparar el contenido en MO de dichos suelo bajo los distintos usos

b) Estudiar la influencia de los cambios de uso del suelo estudiados, la profundidad en el perfil y la cobertura vegetal sobre distintos parámetros microbiológicos: Carbono Microbiano del Suelo (MBC); Carbono extraíble con K_2SO_4 (C_{K2SO4}), Carbono respirado en 21 días de incubación ($C\text{-}CO_{2(21d)}$) y actividad microbiana.

c) Estudiar el efecto del cambio de uso del suelo y la profundidad en el perfil del suelo en el cociente metabólico microbiano (qCO_2).

d) Estudiar y comparar el efecto de estos usos del suelo en la distribución y en las características de la MOS en las distintas fracciones de densidad.

e) Cuantificar y comparar los efectos del uso de suelo en el contenido de Black Carbon tanto en el suelo objeto de estudio como en sus fracciones de densidad.

h) Estudiar el efecto del cambio de uso del suelo y la profundidad en el perfil del suelo en lacomposición isotópica (^{13}C y ^{15}N) de la MO del suelo y de sus fraccciones de densidad.

i) Comparar la cantidad y la naturaleza de los monosacáridos presentes en el suelo y en sus fracciones de densidad

j) Observar la relación entre la material orgánica del suelo, la biomasa microbiana y el contenido de monosacáridos del suelo

k) Estudiar la validez de aplicar mediciones a 260, 280 y 340 nm de absorbancia sobre extractos de suelo como método para estimar el C y el N microbianos.

2´. GENERAL OBJECTIVES

The objective of the present study was to contribute to the understanding of organic matter dynamics in calcareous moor soils under different types of land use -native forest (Quercus ilex), forest plantation (Pinus halepensis) and agricultural land (planted with a cereal crop), in the context of physical and chemical properties.

More specifically, the aims of the study were:

a) to compare the effect of land use on SOM content;

b) to assess the effect of land use, depth and tree cover on the parameters: microbial biomass carbon (MBC), K_2SO_4 extractable C (C_{K2SO4}), the cumulated mineralized C in 21 days of incubation ($C–CO2_{(21d)}$) and the C mineralization activity;

c) to assess the effect of land use, tree cover and depth on microbial metabolic quotient (qCO2);

d) to study and to compare the effect of land use/land cover on the distribution and characteristics of SOM density fractions

e) to quantify and to compare the effect of land use on BC content and distribution on whole soil and SOM density fractions;

f) To assess the effect of land use and depth on OM abundance and on the ^{13}C and ^{15}N isotope composition on whole soil and SOM density fractions;;

g) to assess and compare the amount and nature of monosaccharide whole soil and SOM density fractions;

h) to assess and compare the relationship among SOM, MBC, and monosaccharide content in soils;

i) to investigate the validity of the 260 and 280 nm UV absorbance technique for estimating SMB C and SMB N in soils with high carbonate contents.

3. ÁREA DE ESTUDIO

3.1. Descripción del área de estudio

Esta tesis doctoral se centra en el estudio de los suelos del páramo calcáreo de Castilla y León (UTM: 30T 384465E 4639001N).

Los páramos calizos castellano-leoneses tienen una extensión aproximada de 8.000km^2 que se extienden por las provincias de Palencia, Valladolid, Segovia y Burgos. Los páramos son superficies planas elevadas en el terreno, constituidas por estratos calizos horizontales de origen terciario.

La precipitación media en esta región es en torno a los 400 mm bajo un régimen de humedad xérico y medias térmicas anuales de 12,3 ^{o}C. La Figura 3 muestra el diagrama ombrotérmico elaborado a partir de los datos obtenidos en la estación meteorológica de Santa Espina (Castromonte, Valladolid) por ser ésta la estación meteorológica que, por su ubicación mejor puede representar la región del páramo calizo castellanoleonés.

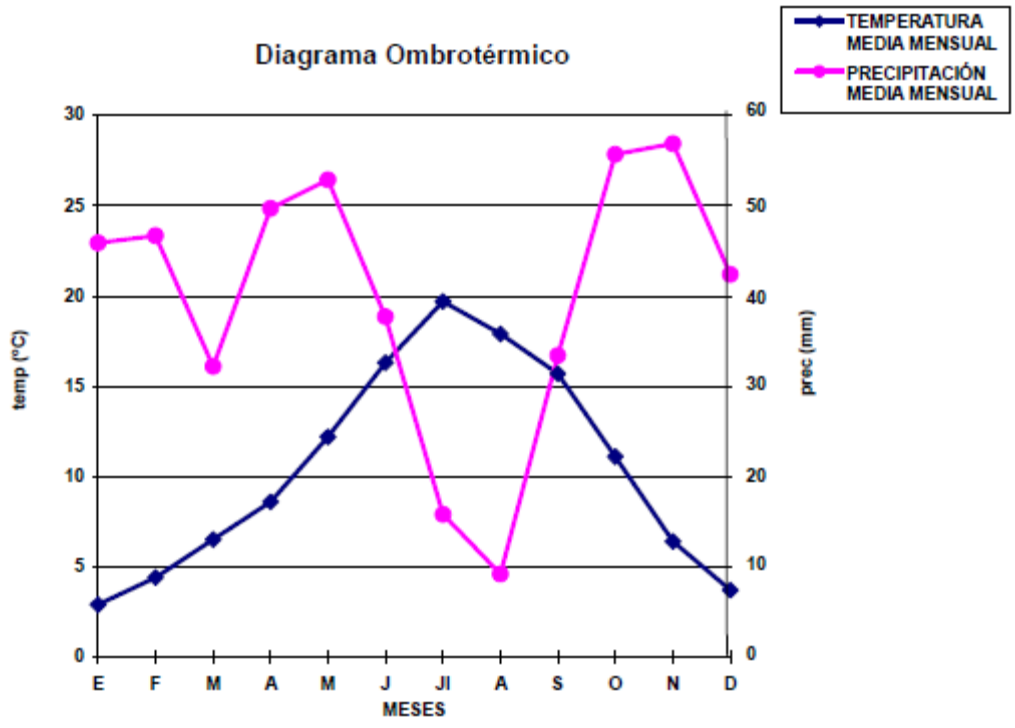

Figura 3

Diagrama ombrotérmico (datos obteidos de la estación meteorológica de Santa Espina)

La altitud del páramo varía entre los 800 y 900 m con pendientes ligeras (<9 %).

Los suelos son Xerepts (USDA, 2006), es decir suelos incipientes poco desarrollados y poco profundos, bastante homogéneos a lo largo del páramo aunque diferentes en cuanto a la historia de uso del terreno.

La vegetación natural de estos páramos corresponde a encinares de *Quercus ilex* subsp *ballota,* no muy densos, con un estrato arbustivo y lianoide mermado. Las plantas acompañantes más destacadas son *Rhamnus saxatilis*, *Crataegus monogyna*, *Bupleurum fruticescens*, Rosa spp., *Lonicera etrusca*, *Salvia lavandulifolia*, *Thymus vulgaris*, *Linum suffruticosum*, entre otras. Especialmente desde la desamortización en el siglo XIX, la mayor parte de la cobertura vegetal de los páramos calizos fue roturada y sustituida por campos de cultivo mayoritariamente cerealistas, de manera que, en la actualidad, el encinar está reducido a manchas aisladas. A partir de los años 50 del pasado siglo, primero la Confederación Hidrográfica del Duero y la Junta de Catilla y León después, llevan a cabo repoblaciones con distintas especies de pino, concretándose en nuestra zona de estudio son plantaciones de *Pinus halepensis*.

3.2. Selección de las parcelas

Partiendo del Modelo de Elevación Digital y el Mapa Geológico (1:50.000) de Castilla y León, se elaboró un Mapa de Páramos Calizos Castellano-leoneses que, a su vez, se interceptó con un Mapa de Usos del Suelo de Castilla y León (1:50.000) obteniendo el Mapa de Usos del Suelo en los Páramos Calizos de Castilla y León. Para la elaboración del mapa se utilizó el programa ArcGis 9.0 para Windows (Figura 4, Figura 5).

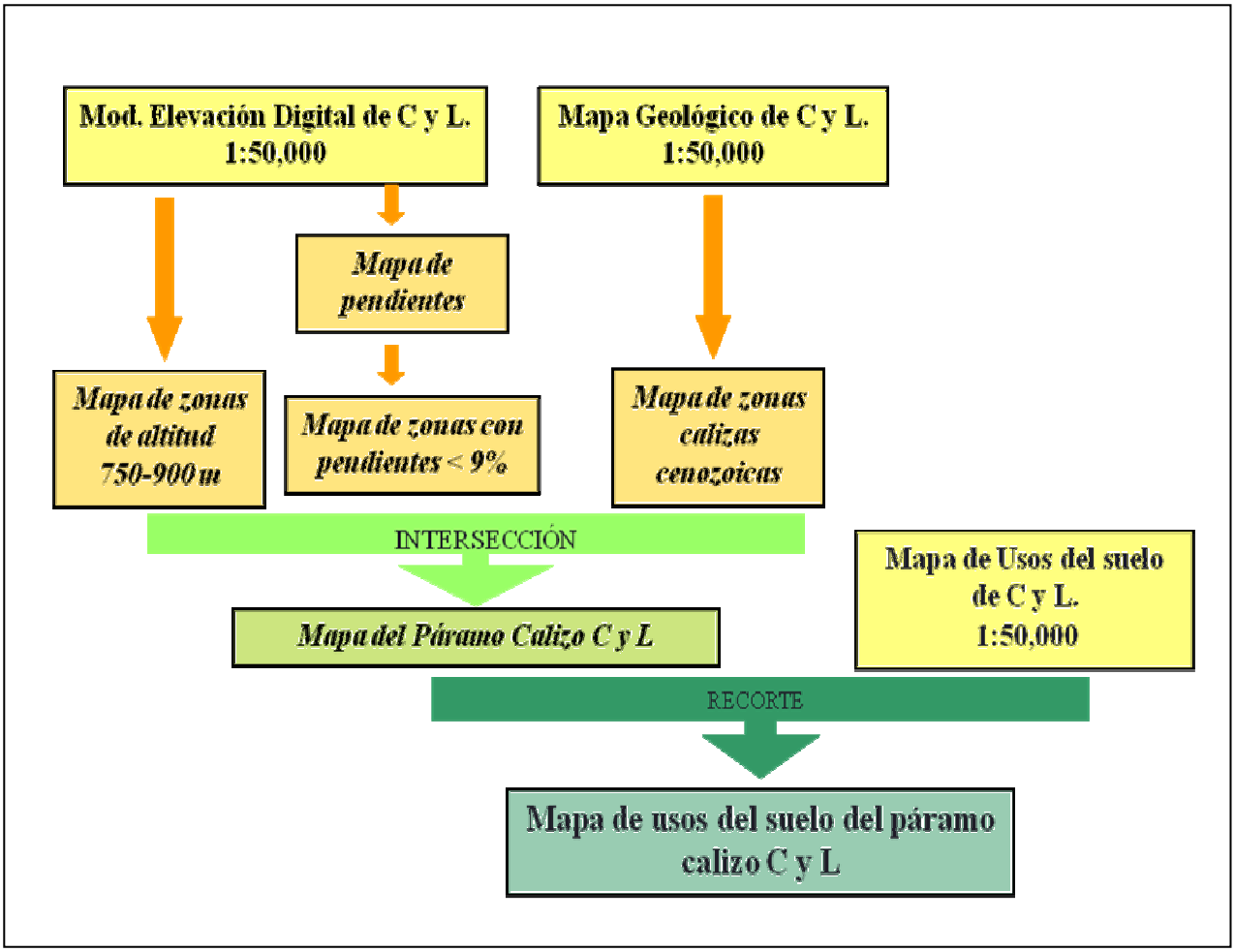

Figura 4

Elaboración del Mapa de Usos del suelo del páramo calizo de Castilla y León

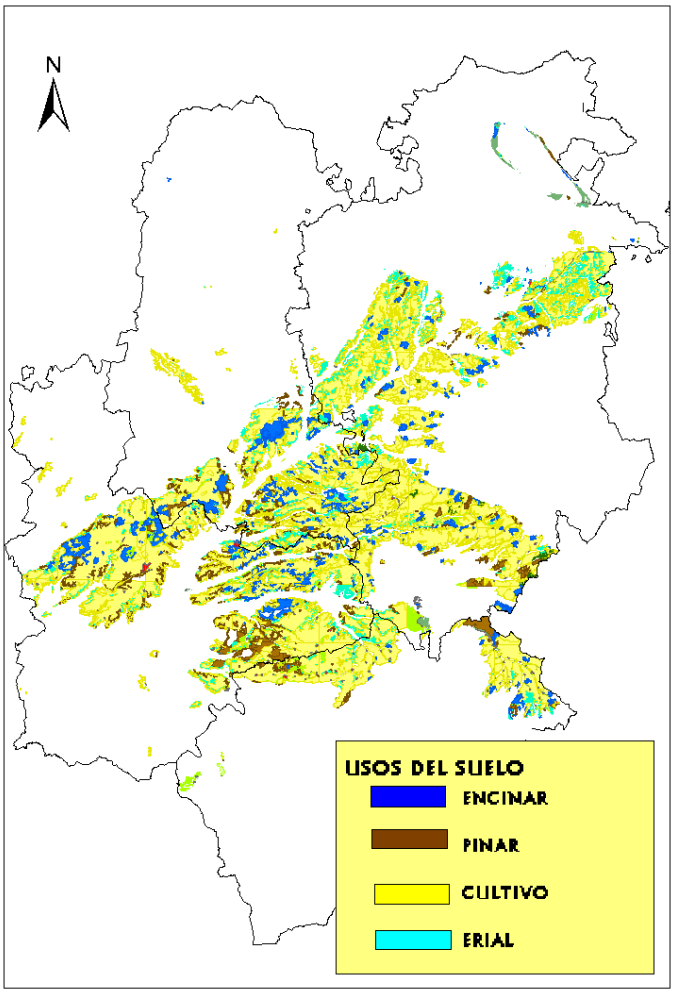

Figura 5

Mapa de Usos del Suelo de los suelos calizos del páramo calizo de Castilla y León

A partir de este mapa generado, se eligieron 3 parcelas de muestreo (Tabla 1) de forma estratificada bajo los siguientes tres criterios: 1) que reunieran los tres usos más representativos de los páramos calizos Castellano-leoneses (formación natural de *Quercus ilex*, cultivo extensivo de cereal y repoblación con *Pinus halepensis*) en subparcelas colindantes entre sí; 2) que cada subparcela tuviese una extensión mínima de 1 ha y; 3) que la antigüedad de cada uso fuera de al menos 30 años.

Tabla 1. Caracterización general de las parcelas de muestreo

	Ubicación	Altitud	Pendiente	Profundidad del perfil del suelo		Área basal	Densidad
Parcela	W 30T UTM	m	%	cm	Subparcela	m^2ha^{-1}	Árboles*ha^{-1}
Cerrato	384525, 4636840	880	6-9	35-40	*Quercus ilex*	14	3156
					Pinus halepensis	52	1164
Monte Viejo	371464, 4646480	897	4-7	40-45	*Quercus ilex*	15	3540
					Pinus halepensis	49	1068
Ampudia	357463, 4638067	874	3-6	35-40	*Quercus ilex*	13	3200
					Pinus halepensis	51	1210

En cada parcela de muestreo se seleccionan 3 subparcelas de 25x25 m correspondientes a cada uno de los usos a muestrear: cultivo extensivo de secano, vegetación natural de *Quercus ilex* y repoblación con *Pinus halepensis*. Estas parcelas se ubicarán lo más próximas entre si tras evitar el efecto borde, que se considera de 15 m del límite de la parcela (Figura 6).

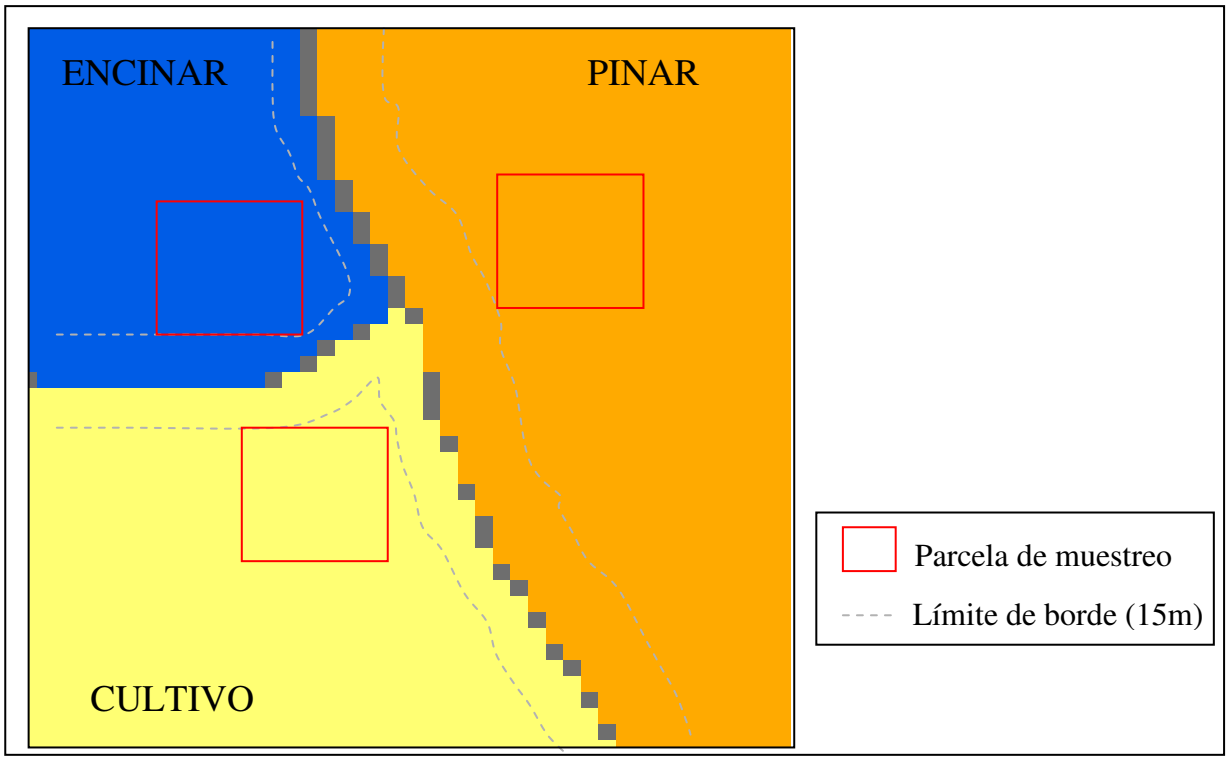

Figura 6
Esquema de ubicación de las subparcelas dentro de la parcela de muestreo

Estableciendo una malla de muestreo, se seleccionan al azar la ubicación de las calicatas. Se establecen y muestrean 4 calicatas en cada subparcela y, por tanto, un total de 36 perfiles son muestreados (3 parcelas x 3 subparcelas x 4 calicatas).

4. MÉTODOS ANALÍTICOS Y ANÁLISIS DE DATOS

Las muestras de suelo fueron secadas al aire y, posteriormente, homogeneizadas y tamizadas con malla de 2 mm, guardando las muestras tamizadas en bolsas hasta su análisis.

Para la caracterización general del suelo se analizaron algunas de sus propiedades físicas y químicas más generales. Las propiedades físicas del suelo analizadas fueron la densidad aparente del suelo; la capacidad de campo, mediante adaptación del método propuesto en los Métodos Oficiales de análisis de Suelos y Aguas (1974); las clases texturales (% arenas, % limos, % arcillas) mediante el Método de la Pipeta, tomando los rangos para las clases texturales propuestos por el ISSS; el % gruesos, considerando gruesos todos los fragmentos minerales de diámetro mayor a 2 mm; y los carbonatos, utilizando el Método de Volumetría de Neutralización (Allison y Moodie, 1965) tratando la muestra con ácido perclórico 1 N y valorando el exceso de ácido con hidróxido sódico 0,5 N.

Las propiedades químicas del suelo analizadas fueron: pH y conductividad eléctrica (C.E.), que se determinaron en suspensión de agua: suelo 1:2,5; C y N totales, mediante combustión seca, utilizando un analizador de C y N Leco (CHN-2000); C orgánico (C org), calculado como la diferencia entre el C total y de C procedente de los carbonatos; y relación entre C org y N total (C/N).

Los análisis anteriormente enumerados forman parte de la caracterización del suelo común a todos los capítulos que conforman esta tesis, sin embargo, según los objetivos específicos perseguidos en cada uno de ellos, se han aplicado sobre las muestras otra serie de análisis más específicos que quedan detalladamente descritos en cada apartado “Material y Métodos” correspondiente.Asimismo, el análisis de datos aplicado también es específico de cada capítulo y se describe detalladamete en él, según corresponda. Sí es común a todo el trabajo de tesis la utilización para el análisis de datos del software Systat 14.0Statistical Software Package (SPSS para Windows).

5. MICROBIOLOGICAL PARAMETERS AS INDICATORS OF SOIL ORGANIC CARBON DYNAMICS IN RELATION TO DIFFERENT LAND USE MANAGEMENT

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Abstract

Labile C fractions: microbial biomass C (MBC), K_2SO_4 extractable C (C_{K2SO4}) and the cumulated mineralized C in 21 days incubation at 28°C ($C\text{-}CO_{2(21d)}$), were compared as land use indicators in a calcareous soil under three different management systems: native *Querqus ilex* forest (under and outside tree cover), a *Pinus halepensis* plantation, and cropped land (with cereals). Microbial biomass and activity were found to be low and coincided with high carbonate contents. As indicators of land use, C_{K2SO4} and $C\text{-}CO_{2(21d)}$ showed the same sensitivity as MBC. $C–CO_2$ emissions were measured in an incubation experiment in order to study C mineralization kinetics. The data for cumulative amounts of $C–CO_2$ released showed a good fit ($R^2 > 0.94$) to the first-order kinetic model $C_m=C_o(1 - e^{-kt})$. The kinetic parameters C_o and C_ok were affected by land use and especially by tree cover. Principal Components Analysis was applied to the data and the relationship among microbial metabolic quotient (qCO_2), labile C pools, and MBC revealed a decrease in efficiency of organic substrate utilization with an increase in availability and lability of the organic matter.

Keywords Microbial biomass- Metabolic quotient- Soil respiration- Calcareous soil

5.1. Introduction

The increase in the concentration of atmospheric carbon dioxide during the past century has drawn attention to the links among the capacity of ecosystems to act as C sinks, changes in land use, and climate change (Briones et al., 2006). In particular, soil organic matter (SOM) is recognised as an important factor in C driven climate change (Sanderman et al., 2003).

The long term storage of C in soil ecosystems is determined by the balance between the rate of incorporation of new organic matter to soil and the decomposition of SOM (Johnson, 1995). An improved understanding of these fluxes is vital to increase our awareness of how soil management affects soil fertility and C sequestration (Kemitt et al. 2007).

The rate of organic matter decomposition (and thus the speed of C return to the atmosphere) depends on several factors, such as quantity and quality of the substrate, climatic conditions and physical and chemical properties of soils, which may invoked as controls over microbial processing of SOM (Mary et al., 1996; Ryan and Law, 2005).

Laboratory studies that measure microbial respiration under optimal conditions of temperature and humidity can be useful for providing information about the influence of land use on microbial activity (Lagomarsino et al., 2006).

The first-order kinetic model used to describe the C mineralization process of SOM ($C_m=C_o(1- e^{-kt})$) (Stanford and Smith, 1972) assumes that the microbial biomass is constant and that the rate of decomposition only depends on the available substrate. The kinetic parameters calculated according to the model -potentially mineralizable C (C_o), mineralization rate (k) and the initial potential mineralization rate (kC_o)- may be of great interest for improving our understanding of SOM balance (Riffaldi et al., 1996).

The total organic carbon content of soil changes slowly and is difficult to measure accurately against the existing background of organic matter (Undersander and Reiger, 1985). However, microbial biomass carbon (MBC) responds much more quickly than SOM to changes in management (Powlson et al., 1987). In addition, labile C fractions, such as K_2SO_4 extractable C (C_{K2SO4}) and the cumulated mineralized C in 21 days of incubation ($C\text{-}CO_{2(21d)}$), have been shown to be early sensitive indicators of land use changes (García et al. 1997; Lagomarsino et al. 2006).

The metabolic quotient (qCO_2) (Anderson and Domsch, 1993) also indicates the relative efficiency of soil microorganisms in utilizing the available resources and the intensity of C mineralization (Moscatelli et al. 2005).

The objective of the present study was to contribute to the understanding of organic matter dynamics in calcareous moor soils under different types of land use -native forest (*Quercus ilex*), forest plantation *(Pinus halepensis)* and agricultural land (planted with a cereal crop), in the context of physical and chemical properties.

More specifically, the aims of the study were: a) to assess the sensitivity of the parameters MBC, C_{K2SO4}, and $C\text{-}CO_{2(21d),}$ to changes in land use; b) to study the effects of land use, tree cover and depth on microbial C mineralization activity; c) to assess the effect of land use, tree cover and depth on microbial qCO_2.

5.2. Methods

5.2.1. Site description

The study was carried out in calcareous moor soils in the region of Castilla y León (north western Spain), UTM: 30T 384465E 4639001N. The mean annual rainfall in the region is below 400 mm under a xeric moisture regime, and the mean annual temperature is approximately 12.3^{o}C. The altitude of the moor varies between 800 and 900 m, with low slopes (<7%). The soils are Inceptisol Xerepts, which are quite homogeneous but differ in their land-use history. The native vegetation in the area is the Holm-oak wood

(*Quercus ilex* subsp *ballota*). In the 19th century most of the forest was converted into agricultural land but since the 1950s, reforestation with *Pinus halepensis* has been carried out on abandoned agricultural land.

5.2.2. Sampling procedures

A Land Use Map of the calcareous moor of Castilla y Leon was elaborated with a GIS (ArcGis 9.0 for Windows). The map was used to select the sampling plots on the basis of the following criteria: a) *Quercus ilex* forest, cropped land, and *Pinus halepensis* plantations in adjacent areas; b) minimum area of each land use, 1 ha, and c) minimum antiquity of land use, 30 years. For this study, one plot per land use and four representative profiles of each land use were selected. Holm-oak wood soil was sampled both under and outside tree cover. A total of 16 profiles were thus sampled. Some characteristics of the selected plots are shown in Table 2.

Table 2. *Forest structural characteristics of the subplots sampled*

Vegetation	*Quercus ilex*	*Pinus halepensis*
Altitude (m.a.s.l.*)	879	885
Basal area (m^2ha^{-1}	13	50
Density (tree ha^{-1})	3200	1184

* meters above sea level

For characterization of each soil type, samples were taken at depths of 0-10, 10-20 and 20-30 cm. Visible plant residues and roots were removed and fresh soil was sieved (< 2 mm) and stored in plastic bags until analysis.

5.2.3. Physical and chemical characterization

The main physical (bulk density, water holding capacity (WHC), % of carbonates, texture, and % of coarse soil materials (Ø > 2 mm), and chemical properties (pH, electrical conductivity (EC), total N, total C, organic C and C/N) were determined.

For bulk density determinations, all of the soil extracted from the soil pit was weighed. The volume of the soil was calculated from the volume of water required to fill the hole (after impermeabilization of the soil pit with plastic sheeting). Three subsamples of the extracted soil were dried in the oven (90ºC, 24 h) and weighed in order to calculate the dry weight of the sample.

The WHC was determined gravimetrically, and soil carbonates were determined by use of $HClO_4$ titrated with NaOH. Particle-size distribution was determined by the International Pipette Method (USDA, 1972), and the % of coarse material by sieving. The pH (soil:water, 1:2.5) was measured with a pH-meter, and EC with an EC-meter. Total concentrations of soil C and N were determined with an automated C/N analyser (CHN-2000, Leco).

5.2.4. Soil respiration and microbial biomass

MBC was determined by the chloroform fumigation extraction method (Vance et al., 1987). K_2SO_4 extractable C (C_{K2SO4}) was measured in the non-fumigated soil extracts.

In order to measure microbial respiration, 50 g of moist soil (at 70 % of water holding capacity) sample were placed in 0.5 l stoppered glass jars and incubated at 28ºC. The CO_2 evolved was collected, after 3, 5, 7, 9, 14, 21, 28, 35, 42, 49, 59, 69, 79, 89, and 98 days of incubation, in 10 ml 0.5M NaOH and determined by titration with 0.5 M HCl (Alef, 1995).

C mineralization kinetics were determined following a first-order kinetic model $C_m=C_o(1 - e^{-kt})$, where C_o is the potentially mineralizable C and k is the mineralization rate (Stanford and Smith, 1972). The accumulated mineralized C recovered after incubation for 21 days ($C\text{-}CO_{2(21d)}$) was considered (according to Xu et al., 2006).

The metabolic quotient (qCO_2) was calculated as the cumulative C measured after incubation, divided by the MBC (Anderson and Domsch, 1993).

5.2.5. Statistical analyses

Analyses of variance (ANOVA) were performed to evaluate the main effects of land use, depth, and their interactions on the parameters analysed. Data were tested for normality and homoscedasticity with the Kolmogorov-Smirnov and Levene´s statistics respectively. In cases of significant F-statistics, differences between means were tested with the Tukey procedure for multiple comparisons. To simplify interpretation of the results, a principal components analysis (PCA) with quartimax rotation was used, in order to establish the relationships among variables. An ANOVA was applied on the factors identified by PCA to clarify the influence of land use, depth and tree cover on the principal components obtained. All the statistical analyses were performed with the Systat 14.0 Statistical Software Package (SPSS for Windows).

5.3. Results and discussion

5.3.1. Physical and chemical characterization

The main physical and chemical properties are shown in Tables 2 and 3. The soils studied were similar in texture, and classified as sandy-clay-loam, according to the USDA classification, and contained large amount of coarse fragments (mineral fragments > 2 mm).

The soils studied also showed a moderately alkaline soil reaction (7.9-8.2), with an electrical conductivity lower than 5 dS/m and a high content of carbonates (15-39%), which showed a significant tendency ($p< 0.05$) to increase with depth.

The soil N content, organic C, and C/N ratio were higher in the upper soil layers (Table 4), probably because of the higher input of fresh organic matter at the soil surface. Furthermore, the organic matter content and N availability varied with the land use. The cropped land displayed the lowest content of organic C. This is explained by the low input of organic matter and the losses by tillage. Soil tillage induces soil C loss by acceleration of organic C oxidation, which results in the release of large amounts of CO_2 to the atmosphere (La Scala et al., 2008; Prior et al., 2000; Ellert and Janzen, 1999). Another tillage-related factor that contributes to soil C losses is soil aggregate disruption, which exposes once-protected organic matter to decomposition (Grandy and Robertson, 2007; De Gryze et al., 2006).

5.3.2. C pools

MBC and $C\text{-}CO_{2(21d)}$ were considered as labile fractions of organic matter and were tested as sensitive indicators of land use change. All the profiles showed a decrease in labile C pools with increasing depth (Table 5). The soils under tree cover (pine or holm-oak) presented the highest labile C pools and a sharper depth gradient.

MBC ranged from 11 to 59 $\mu g.g^{-1}$, and C_{K2SO4} from 10 to 23 $\mu g.g^{-1}$ (Table 5). These values are low in comparison with those reported by other authors for non-calcareous soils (Lagomarsino et al., 2006), but are consistent with the values reported by other authors for calcareous soils (García et al., 1997).

Many studies of SOC and land use change use measurements of MBC as early indicators of changes in SOM (Powlson et al., 1987; García-Gil et al., 2000; Palma et al., 2000). However, in the calcareous soils studied, measurements of C_{K2SO4} and $C\text{-}CO_{2(21d)}$ appeared to be at least as sensitive as MBC measurements (Table 5).

Table 3. Main physical properties of soils sampled under different land use and at different depths, and the results of the analysis of variance. CL: Cropped land; QFOC: Native *Quercus* forest outside tree cover; QFTC: Native *Quercus* forest under tree cover; PP: *Pinus* plantation. Coarse: mineral fragments >2 mm, H.C.: soil holding, B.D.: Bulk density.

	------Texture------						
	Sand	**Silt**	**Clay**	**Coarse**	**$CaCO_3$**	**H.C.**	**B.D.**
	----------%----------						**g ml^{-1}**
CL							
0-10 cm	52	19	28	45.3 ±5.3	19.9 ±8.8	23	1.25 ±0.17 ab
10-20 cm	51	16	31	38.6 ±13.0	13.9 ±4.8	20	1.62 ±0.03 b
20-30 cm	49	18	32	51.5 ±11.2	28.1 ±14.9	20	1.82 ±0.24 c
QFOC							
0-10 cm	52	25	22	52.7 ±1.7	23.4 ±5.9	21	1.68 ±0.17 b
10-20 cm	50	22	27	59.0 ±7.3	28.4 ±6.4	20	1.68 ±0.13 b
20-30 cm	46	26	27	60.0 ±11.9	38.5 ±6.5	21	1.64 ±0.22 b
QFTC							
0-10 cm	51	27	21	58.3 ±5.4	20.9 ±4.6	26	1.32 ±0.13 ab
10-20 cm	51	23	25	64.9 ±5.4	24.3 ±5.7	24	1.65 ±0.08 b
20-30 cm	44	27	28	58.4 ±16.2	33.8 ±8.5	23	1.73 ±0.29 b
PP							
0-10 cm	57	21	21	48.9 ±18.2	18.4 ±11.8	30	1.06 ±0.20 a
10-20 cm	49	18	22	53.5 ±7.9	23.3 ±11.4	23	1.49 ±0.27 ab
20-30 cm	43	31	25	56.9 ±5.4	29.2 ±12.5	21	1.55 ±0.33 ab
Analysis of variance							
Land use				n.s.	n.s.		n.s.
Depth				n.s.	**		**
Land use*Depth				n.s.	n.s.		**

Means and standard error for n= 4. Values of p>0.05: n.s., p<0.05:*, p<0.01:** and p<0.001:*** are reported. Values with the same letters are not significantly different (p<0.05) for the interaction Land use*Depth.

Table 4. Main chemical properties of soils sampled under different land use and different depths, and the results of the analysis of variance. CL: Cropped land; QFOC: Native *Quercus* forest outside of tree cover; QFTC: Native *Quercus* forest under tree cover; PP: *Pinus* plantation. C.E.: electrical conductivity

	pH (H_2O)	C.E. dS m^{-1}	Total C	Total N	Organic C	C/N
			----------%	----------		
CL						
0-10 cm	8.13 ±0.01	0.27	4.28 ±0.29 bc	0.24 ±0.02 bc	2.51 ± 0.22 cd	10.59 ±0.45 bc
10-20 cm	8.15 ±0.08	0.25	3.74 ±0.35 c	0.17 ±0.02 c	1.95 ±0.27 cd	11.16 ±1.21 bc
20-30 cm	8.18 ±0.03	0.22	3.88 ±0.82 c	0.19 ±0.01 c	1.50 ±0.63 d	9.01 ±3.61 c
QFOC						
0-10 cm	8.21 ±0.07	0.25	6.15 ±0.91 bc	0.26 ±0.01 b	3.34 ±0.41 bc	12.61 ±0.93 ab
10-20 cm	8.19 ±0.02	0.34	5.78 ±1.10 bc	0.20 ±0.01 bc	2.37 ±0.44 cd	11.39 ±1.85 bc
20-30 cm	8.24 ±0.02	0.28	6.56 ±0.97 bc	0.17 ±0.01 c	1.94 ±0.38 cd	11.18 ±2.18 bc
CFTC						
0-10 cm	7.91 ±0.04	0.44	7.50 ±0.23 ab	0.34 ±0.05 a	4.99 ±0.52 ab	14.89 ±2.92 a
10-20 cm	8.06 ±0.03	0.47	5.69 ±0.95 bc	0.20 ±0.01 bc	2.77 ±0.27 cd	13.71 ±0.71 ab
20-30 cm	8.15 ±0.02	0.35	6.17 ±1.33 bc	0.16 ±0.01 c	2.11 ±0.33 cd	12.57 ±0.88 ab
PP						
0-10 cm	7.91 ±0.04	0.36	7.16 ±1.25 a	0.35 ±0.04 a	4.96 ±0.63 ab	14.12 ±1.58 ab
10-20 cm	8.06 ±0.03	0.41	6.05 ±1.79 bc	0.25 ±0.04 b	3.25 ±0.48 bc	12.94 ±1.57 b
20-30 cm	8.15 ±0.02	0.31	6.17 ±1.67 bc	0.23 ±0.03 bc	2.66 ±0.36 cd	11.50 ±0.19 bc
Analysis of variance						
Land use	n.s.		***	*	**	***
Depth	n.s.		n.s.	***	***	*
Land use*Depth	n.s.		**	***	***	**

Means and standard error for n= 4. Values of p>0.05: n.s., p<0.05:*, p<0.01:** and p<0.001:*** are reported. Values with the same letters are not significantly different (p<0.05) for the interaction Land use*Depth.

Table 5. Microbial biomass carbon (MBC), mineralized C accumulated in 21 days of incubation ($C\text{-}CO_{2(21d)}$), K_2SO_4 extractable C (C_{K2SO4}) and metabolic quotient (qCO_2) of the soils sampled under different land use and at different depths, and results of analysis of variance. CL: Cropped land; QFOC: Native *Quercus* forest outside of tree cover; QFTC: Native *Quercus* forest under tree cover; PP: *Pinus* plantation.

	MBC **µg C g^{-1}**	**$C\text{-}CO_{2\ (21d)}$** **mg $C\text{-}CO_2$ g^{-1}**	**C_{K2SO4}** **µg C g^{-1}**	**qCO_2** **mg $C\text{-}CO_2$ g^{-1}C 21days^{-1}**
CL				
0-10 cm	35.6 ±4.1 ab	0.55 ±0.01 bcd	13.7 ±4.4 ab	22.3 ±1.7
10-20 cm	19.8 ±3.4 de	0.34 ±0.06 cde	12.7 ±2.9 ab	21.9 ±4.1
20-30 cm	11.1 ±4.2 e	0.23 ±0.05 e	10.8 ±4.6 b	28.4 ±11.7
QFOC				
0-10 cm	43.3 ±3.5 ab	0.52 ±0.04 bc	16.6 ±1.9 ab	19.1 ±2.5
10-20 cm	17.0 ±2.0 de	0.43 ±0.06 cd	10.8 ±1.3 b	38.9 ±9.7
20-30 cm	12.1 ±3.7 e	0.37 ±0.06 de	9.9 ±0.9 b	44.2 ±13.9
CFTC				
0-10 cm	41.2 ±8.8 ab	1.07 ±0.05 a	23.32 ±2.4 a	39.8±14.1
10-20 cm	24.7 ±4.8 cde	0.71 ±0.05 b	13.21 ±4.2 ab	38.6 ±8.5
20-30 cm	23.7 ±2.6 de	0.45 ±0.02 de	11.46 ±4.6 ab	26.9 ±2.6
PP				
0-10 cm	58.9 ±6.6 a	1.04 ±0.03 a	23.29 ±9.9 a	27.7 ±5.9
10-20 cm	30.4 ±5.8 bcd	0.72 ±0.02 b	16.62 ±4.6 ab	34.2 ±7.2
20-30 cm	15.3 ±5.3 cde	0.43 ±0.05 de	16.90 ±3.9 ab	40.4 ±15.6
Analysis of variance				
Land use	n.s.	*	**	n.s.
Depth	***	***	**	n.s.
Land use*Depth	***	***	***	n.s.

Means and standard error for n= 4. Values of p>0.05: n.s., p<0.05:*, p<0.01:** and p<0.001:*** are reported. Values with the same letters are not significantly different (p<0.05) for the interaction Land use*Depth.

5.3.3. Microbial activity and C mineralization

Respiration processes reflect the functionality of microorganisms and their efficiency in utilizing the available substrates. The first-order equation $C_m=C_o(1- e^{-kt})$ shows that with no additional soil C input, the initial amount of potentially mineralizable C (C_o) should decay exponentially over time, as a function of the mineralization rate (k) (La Scala et al., 2008). This equation provided a good description of the C mineralization kinetics, and the correlation coefficient ranged from 0.96 to 0.98 (Figure 7).

The cumulative mineralized C presented a curvilinear relationship over time, and showed a larger initial release of CO_2 followed -after 21 days incubation- by a slower linear increase throughout the remaining 98 day incubation period (Figure 7).

The microbial activity decreased clearly with increasing depth in all the profiles studied, in response to the decreasing labile C pools (Agnelli et al., 2004). The soil respiration was higher under than outside tree cover (Table 6). Several studies have shown that tree canopy cover reduces oscillations in soil temperature (Kang et al., 2000) and evaporative water losses (Aguilera et al., 1999) and this influences biological processes in the soil, such as the rate of SOM decomposition of SOM (Paul et al., 2004). However, there were no significant differences between pine and holm-oak cover for C mineralization kinetic parameters (C_o, C_ok) (Table 6). Thus, for these calcareous soils, the effect of tree cover on soil mineralization appears to be independent of the tree species (pine or holm-oak).

In the soils studied the C_o ranged from 0.6 to 1.3 mg $C.g^{-1}$ of soil (Table 6), values that are low in comparison with those reported by Goberna et al. (2006) for Calcixerolls in the semiarid Mediterranean region and under different types of land use, or by Fernández et al. (1999), for Cambisols under pine forest located within a humid temperate zone, but consistent with those reported by Baldock and Skjemstad (2000), who revealed the role of carbonates in organic C stabilization against microbial attack.

C mineralization rates (k) ranged from 0.043 to 0.078 (Table 6), but no regular trends were observed as regards these parameters. These values are similar to those reported by Lagomarsino et al. (2006) on loam soils within a Mediterranean region but lower than those reported by Fernandez et al. (1999) for Cambisols within a humid temperate zone. The differences in C mineralization rates among different areas of study may be explained by differences in mean precipitation rates. Turrión et al. (2001) showed that mean annual precipitation affects microbial activity.

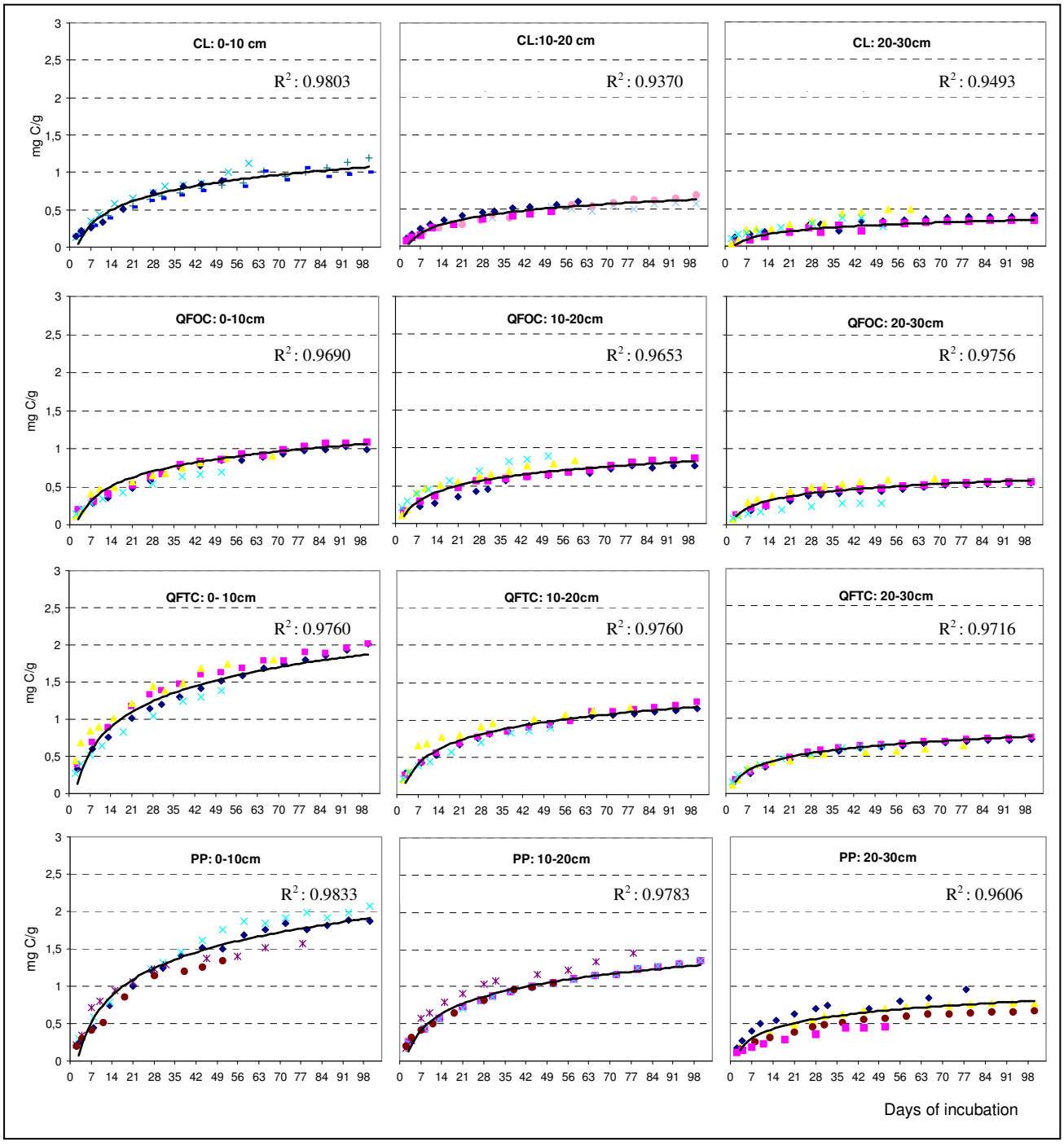

Figure 7

Cumulative curves of the C mineralization during 98 days of incubation and the first-order equation ($C_m=C_o(1- e^{-kt})$) fitted to the experimental curve. CL: Cropped land; QFOC: Native *Quercus* forest outside of tree cover; QFTC: Native *Quercus* forest under tree cover; PP: *Pinus* plantation. The correlation coefficient of the equation $C_m=C_o(1- e^{-kt})$ is shown.

Table 6. Parameters of microbial mineralization activity of soils sampled under different land use and at different depths, estimated according to the first-order equation and analysis of variance. CL: Cropped land; QFOC: Native *Quercus* forest outside of tree cover; QFTC: Native *Quercus* forest under tree cover; PP: *Pinus* plantation. C_o: Potentially mineralizable C, k: mineralization rate, C_ok: initial potential rate of C mineralization, R^2: correlation coefficient.

	$C_m = C_o (1- e^{-kt})$		
	C_o **mg C g^{-1}**	**k** **day^{-1}**	**C_ok** **mg C g^{-1} day^{-1}**
CL			
0-10 cm	0.809 ±0.078 bc	0.053 ±0.004	0.043 ±0.002 bc
10-20 cm	0.405 ±0.280 cd	0.072 ±0.005	0.029 ±0.006 cd
20-30 cm	0.340 ±0.180 d	0.063 ±0.023	0.021 ±0.008 d
QFOC			
0-10 cm	0.824 ±0.031 bc	0.055 ±0.009	0.045 ±0.008 bc
10-20 cm	0.648 ±0.095 cd	0.070 ±0.013	0.046 ±0.015 bc
20-30 cm	0.514 ±0.055 cd	0.067 ±0.009	0.035 ±0.009 cd
QFTC			
0-10 cm	1.597 ±0.021 a	0.054 ±0.003	0.085 ±0.008 a
10-20 cm	0.913 ±0.041 bc	0.071 ±0.005	0.064 ±0.002 ab
20-30 cm	0.634 ±0.025 bcd	0.078 ±0.019	0.049 ±0.010 bc
PP			
0-10 cm	1.538 ±0.041 a	0.043 ±0.007	0.065 ±0.008 ab
10-20 cm	1.060 ±0.005 b	0.059 ±0.002	0.063 ±0.002 ab
20-30 cm	0.591 ±0.125 bcd	0.060 ±0.003	0.035 ±0.005 cd
Analysis of variance			
Land use	**	n.s.	**
Depth	***	**	***
Land use*Depth	***	n.s.	***

Means and standard error for n= 4. Values of p>0.05: n.s., p<0.05:*, p<0.01:** and p<0.001:*** are reported. Values with the same letters are not significantly different (p<0.05) for the interaction Land use*Depth.

C_ok may be a more precise estimate than the individual parameters examined (Saviozzi et al., 1993). A regular depth-related pattern towards less degradable substrate was observed throughout all profiles, as indirectly indicated by the decrease in the initial potential mineralization rate (C_ok) (Table 6). The deeper layers of the soil profile are enriched in progressively more recalcitrant products because microorganisms use easily degradable substrate (Dell´Abate et al., 2002).

5.3.4. Principal Components Analysis

In order to obtain a better understanding of the factors most directly related to the behaviour of the different samples studied, PCA was used to examine the data.

The PCA carried out with 11 selected variables (Co, C-$CO_{2(21d)}$, organic C content, total N content, WHC, percentage of clay, Cok, C_{K2SO4}, C/N ratio, bulk density, qCO_2) identified two components (PC1 and PC2) that accounted for 80.3% of the total variance, with most of the variation explained by PC1 (67.5 %) (Table 7).

PC1 was mainly related to depth and tree cover (Figure 8). The most heavily weighted variables were those related to availability and lability of substrate at its positive extreme, and bulk density and clay content at the negative extreme. Differences in mineralization parameters may be attributed to differences in physical protection of SOM (Hassink, 1997). The quantity of soil clay is believed to be one of the main factors that affect the capacity of soil to stabilize organic carbon. This stabilizing effect has been partly ascribed to adsorption of organic substances onto clays (Golchin et al. 1994).

Table 7. Principal Components Analysis.Variable weightings in two principal components. Co: potentially mineralizable C; $C\text{-}CO_{2(21d)}$: accumulated mineralized C in 21 days; Organic C: organic C content; Total N: total N content; WHC: water holding capacity; Clay: percentage of clay; Cok: initial potential rate of C mineralization; MBC: microbial biomass C; C_{K2SO4}: K_2SO_4 extractable C; C/N: C/N ratio; BD: bulk density; qCO_2: metabolic quotient.

VARIABLES	PC 1	PC 2
Co	0.975	0.132
$C\text{-}CO_{2\ (21d)}$	0.969	0.172
Organic C	0.953	-0.037
Total N	0.896	-0.192
WHC	0.850	-0.044
Clay	-0.845	-0.127
Cok	0.827	0.364
MBC	0.812	-0.481
C_{K2SO4}	0.780	-0.170
C/N	0.760	0.142
BD	-0.734	0.373
qCO2	-0.064	0.935

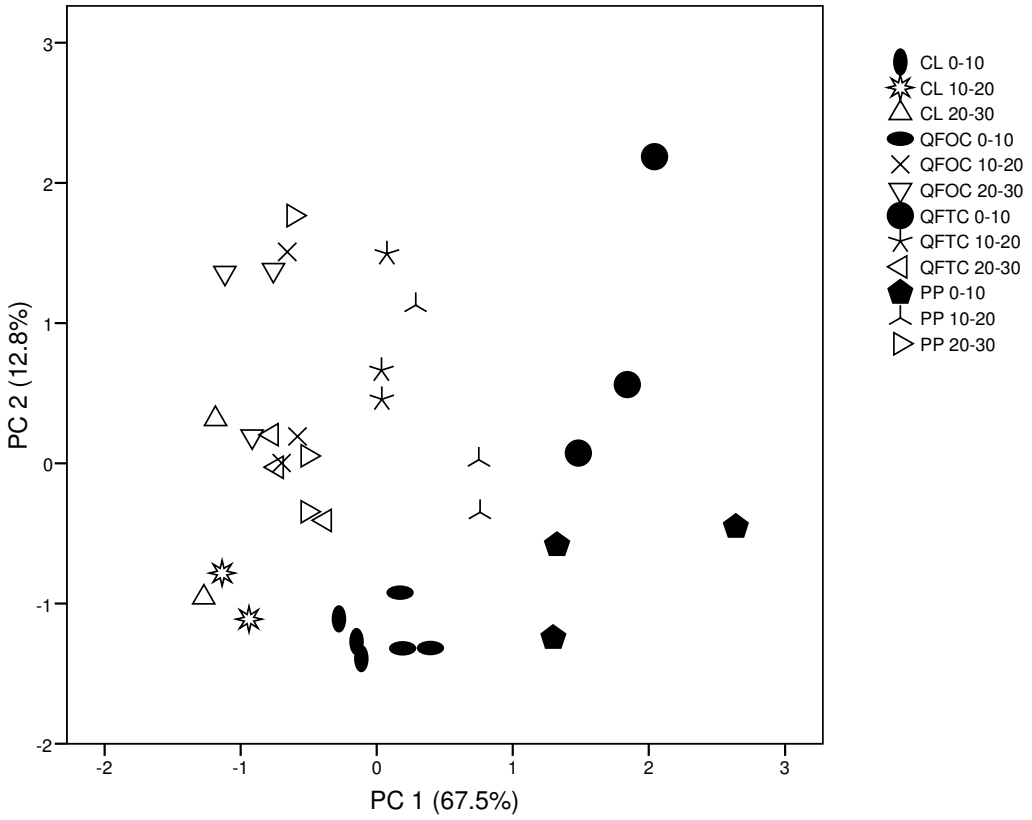

Figure 8

Sample distribution based on PCA factor scores. The variation explained by each PC is given in brackets. CL: Cropped land; QFOC: Native *Quercus* forest outside of tree cover; QFTC: Native *Quercus* forest under tree cover; PP: *Pinus* plantation.

The most heavily weighted variables in PC2 were qCO_2 at the positive extreme, and microbial biomass C at the negative extreme. PC2 was mainly associated with the interaction between land use and depth ($p<0.001$) (Figure 8). The metabolic quotient (qCO_2) is an index of microbial efficiency in the utilization of C resources

(Anderson, 2003; Moscatelli, 2005). Greater efficiency results in a low metabolic quotient (Xu et al., 2006). We found, in accordance with Paul and Clark (1989) and Margalef (1974), that micro-organisms were more efficient at utilizing C resources in soils in which less substrate was available.

5.4. Conclusions

In the calcareous soils under study, microbial biomass and activity clearly declined with increasing depth and showed low values in comparison with non-calcareous soils. The parameters C_{K2SO4}, and $C\text{-}CO_{2\ (21d)}$ appeared to be as sensitive indicators of land use changes as microbial biomass C.

The first-order equation $C_m=C_o(1- e^{-kt})$ provided a good description of the C mineralization kinetics. The kinetic parameter calculated, potentially mineralizable C (C_o), was found to be a sensitive indicator of land use change, and the initial potential mineralization rate (C_ok) appeared to be a good indicator of substrate degradability.

Metabolic quotient (qCO_2) was negatively correlated with microbial biomass and micro-organisms were more efficient at utlizing C resources in soils in which substrate availability was lower, which suggests the involvement of competition for substrate.

The microbiological parameters studied indicated that, for these calcareous soils, tree cover appears to have a greater influence on soil carbon dynamics than the tree species (pine or holm-oak).

5.5. Acknowlegements

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6. STORAGE OF ORGANIC CARBON AND BLACK CARBON IN DENSITY FRACTIONS OF CALCAREOUS SOILS UNDER DIFFERENT LAND USES

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Abstract

The association between soil particles and their spatial arrangement plays a key role in soil organic matter (OM) dynamics. Density fractionation combined with ultrasonic dispersion enables separation and study of soil OM fractions, considered on the basis of the mechanisms of physical protection: non-physically protected OM (FF), OM occluded into aggregates (OF), and OM stabilized in organo-mineral complexes (DF).

In the present study, whole soils and density fractions of calcareous soils under three different management systems - native *Quercus ilex* forest, a *Pinus halepensis* plantation and cropped land- were analyzed for organic C (OC), total N, and Black carbon (BC) content. Black carbon is often considered as a very recalcitrant pool in the soil. However, as well as BC content of soils has seldom been quantified, long-term studies on BC stability are scarce and conclusions about BC stability are not widespread.

About 67% of the total C in the topsoil was lost as a result of converting the natural *Quercus ilex* forest to cropped land, 100 years ago. After crop abandon, the stock of OC in the topsoil greatly increased upon reforestation of the studied plot with *Pinus halepensis*. An average recovery of 71% of the previously lost OC had been recovered, after 40 years of pine plantation. The changes in OC stocks affected mainly the free fraction (FF). Black carbon represented between 1.2 to 2.3% of the TOC of soil with the highest concentrations in OF. The maintenance of BC proportion through land uses suggests equilibrium between inputs and outputs, and leads to the suspicion that BC could be less stable and less resistant to biodegradation than is often taken for granted.

Keywords: Soil organic matter; Density fractionation; Black carbon; Land use change; Calcareous soil.

6.1. Introduction

The reservoir of soil organic matter (SOM) has been proposed as both a significant source and sink of atmospheric CO_2. Because of this, the capacity of soils to accumulate and stabilize organic carbon has received great attention in recent years. In particular, SOM is recognized as an important compartment in C-driven climate change (Sanderman et al., 2003). The long-term storage of C in soil ecosystems is determined by the balance between the rate of incorporation of new organic matter (OM) in soil and the decomposition of SOM (Johnson, 1995). The properties of the soil matrix play an important role in the protection of SOM against biodegradation. It is generally accepted that Black carbon (BC) represents a long-term carbon sink in

soil (Kuhlbusch, 1998; Schmidt and Noack, 2000) and that, therefore, BC plays an important role in the global carbon cycle. The amount of available data about BC content of soils is already considerable, but for Mediterranean ecosystems it is still scarce, particularly in calcareous soils.

Calcareous soils, which represent approximately 12% of the world soil resources (FAO, 1996), are of particular interest because of the high stability of their OM. Some studies of the decomposition dynamics of SOM in calcareous soils (García et al., 1997; Llorente and Turrión, 2009) have shown that the decomposition rates in such soils are lower than those in other soils with similar organic carbon content.

SOM is heterogeneous and it is possible to distinguish OM fractions that vary in their stability and therefore in their intrinsic decay rates, ranging from a few days to hundreds of years (Schimel et al., 1985). The location of SOM within the soil matrix is considered a major factor determining its turnover (Tamon et al., 2006). There are two main mechanisms of SOM physical stabilization based on the location of the SOM within the soil matrix: (1) physical protection by aggregates; and 2) OM stabilization by organo-mineral complex formation. Physical fractionation methods, such as density fractionation (after ultrasonic dispersion or not), enable separation and study of SOM fractions differing in dynamics, structure and function (Golchin et al., 1994a, Six et al., 2001). Several studies have addressed the effect of land use on the size and composition of different SOM fractions (e.g., Preston et al., 1994; Guggenberger et al., 1995; Golchin et al., 1995; Gregorich et al., 1996; Helfrich et al., 2006). Subdividing SOM according to physical properties highlights the observation that physical location within the soil matrix is a key factor determining turnover (Balesdent, 1996).

SOM fractionation by density following ultrasonic dispersion enables separation and study of three different fractions according to the different mechanisms of physical protection (Sohi et al., 2001). Based on these stabilization mechanisms, it is possible to distinguish three SOM fractions: (1) “free” OM (free fraction, FF), isolated before ultrasonic break-down of stable aggregates; (2) OM occluded within aggregates (occluded fraction, OF), isolated after ultrasonic dispersion to break the aggregates; and 3) organo-mineral fraction (dense fraction, DF) recovered as the residual (heavy) material. Many studies (e.g. Golchin et al., 1994b; Six et al., 2002b; John et al., 2005) have shown that the unprotected FF represents a labile SOM pool with a rapid turnover rate. In contrast, OF and DF are more stable pools, with turnover times ranging from decades to centuries. Therefore, density fractions may be affected differently by changes in land use.

In Mediterranean forests, very prone to wildfires, black carbon is expected to be found
in relevant amounts. Thus it is worth to quantify its presence in the overall soil, and also its distribution among the density fractions.

In this study, carried out with soils from a calcareous moor in the region of *Castilla y León* (northwestern Spain), we studied 36 soils, taken from plots under contrasting land uses: native *Quercus ilex* stands, cereal crops, and *Pinus halepensis* plantations. Density separation was carried out in combination (or not) with ultrasonic dispersion in order to obtain SOM fractions according to different mechanisms of physical stabilization. The objectives of the present study were a) to compare the effect of land use on SOM content,

b) to study the effect of land use on the distribution and characteristics of density fractions, and c) to quantify and to compare the effect of land use on BC content and distribution on whole soil and density fractions.

6.2. Materials and methods

6.2.1. Site description

The study was carried out in a calcareous moor in the region of *Castilla y León* (northwestern Spain), UTM: 30T 384465 E 4639001 N. The mean annual rainfall in the region is below 400 mm, under a xeric moisture regime, and the mean annual temperature is approximately 12.3^{o}C. The altitude of the moor is between 800 and 900 m, with low slopes (< 7%). 'The soils (Xerepts, according to USDA, 2006) are quite homogeneous in spite of differences in their land use history. The native vegetation in the studied calcareous soils is Holm-oak wood (*Quercus ilex* subsp *ballota*). In the 19th century, most of the natural forest was converted into agricultural land (cereal crops), but since the 1950s, reforestation with *Pinus halepensis* has been carried out on abandoned agricultural land.

6.2.2. Sampling procedures

A land use map of the calcareous moor of Castilla y León was elaborated with a GIS (ArcGis 9.0 for Windows) (Figure 9). The map was used to select the sampling plots on the basis of the following criteria: a) *Quercus ilex* forest, cropped land, and *Pinus halepensis* plantations in adjacent areas; b) minimum area of each land use, 1 ha; and c) establishment of each land use for at least 40 years.

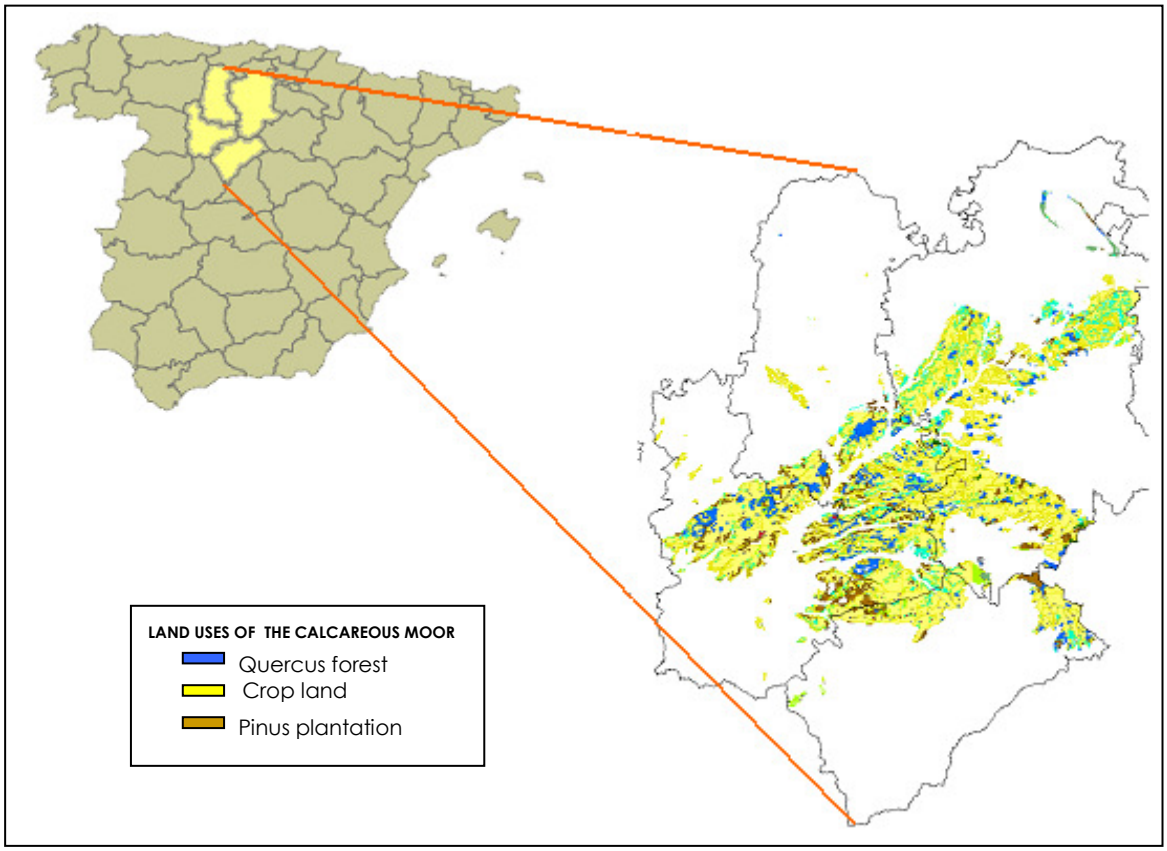

Figure 9

Land use map of the calcareous moor of Castilla y León

For this study, plots of the above-mentioned land uses were selected in three different regions of the calcareous moor, and four representative plots were sampled (0-10 cm depth). A total of 36 topsoils (0-10 cm depth) (3 regions x 3 land uses x 4 plots) were thus sampled. A composite sample, by joining 3 soil subsamples, was obtained for each plot. Visible plant residues and roots were removed; soil was air-dried, sieved (< 2 mm) and stored in plastic bags until analysis.

For soil characterization, % of carbonates, texture, pH, total N, total C, organic C, and C/N were determined. Total concentrations of soil C and N were determined with an automated C/N analyzer (CHN-2000, Leco). Organic carbon was calculated as the difference between total and carbonate carbon. Soil total calcium carbonates were determined by use of 1M HCl titrated with 0.5M NaOH (FAO, 2007).

6.2.3. Ultrasonic equipment

We used a Branson 450 W Sonicator, equipped with a titanium probe. The probe depth was fixed in 15 mm. The sonicator was calibrated by determining the real power output calorimetrically (North, 1976). The probe output energy was calculated from:

$$P = (m_w c_w + C_{cont}) \Delta T\, t^{-1} + Ht,$$

where P is the calculated power (W), m_w is the mass of water (g), c_w is the specific heat of water (4.18 J g^{-1} K^{-1}), C_{cont} is the heat capacity of the container (J K^{-1}), ΔT is the temperature change (K), t is the sonication time (s) and H is the heat loss (J s^{-1}). The heat capacity of the glass beaker (C_{cont}) was determined using the method of mixtures (Morra et al., 1991), according to:

$$C_{cont} = m_1 c_w ((T_1 - T_2) (T_3 - T_2)^{-1}) - m_2 c_w,$$

where C_{cont} and c_w are as above and m_1 is the mass (g) of an amount of water heated to T_1 (K), which is added to the beaker that already contained an amount of water m_2 (g) at room temperature (K). The final equilibrium temperature (K) of the water in the beaker is T_3.

The wanted energy output was 300 J ml^{-1} and the corresponding time was calculated from:

$$t = m_s E P^1,$$

where t is the sonication time (s), m_s is the mass of soil (g), E is the energy (J g^{-1}) and P is the power output (W).

6.2.4. Density fractionation of soil

A density fractionation procedure was applied to the topsoils (0-10 cm depth) of the 36 sampled plots. A flow diagram of the method used is provided in Figure 10. The method follows the concept of Golchin et al. (1994a), who differentiated three degrees of physical protection of OM: FF, non-protected; OF, occluded within aggregates - extractable by sonication; and DF, retained in the dense residual material after sonication.

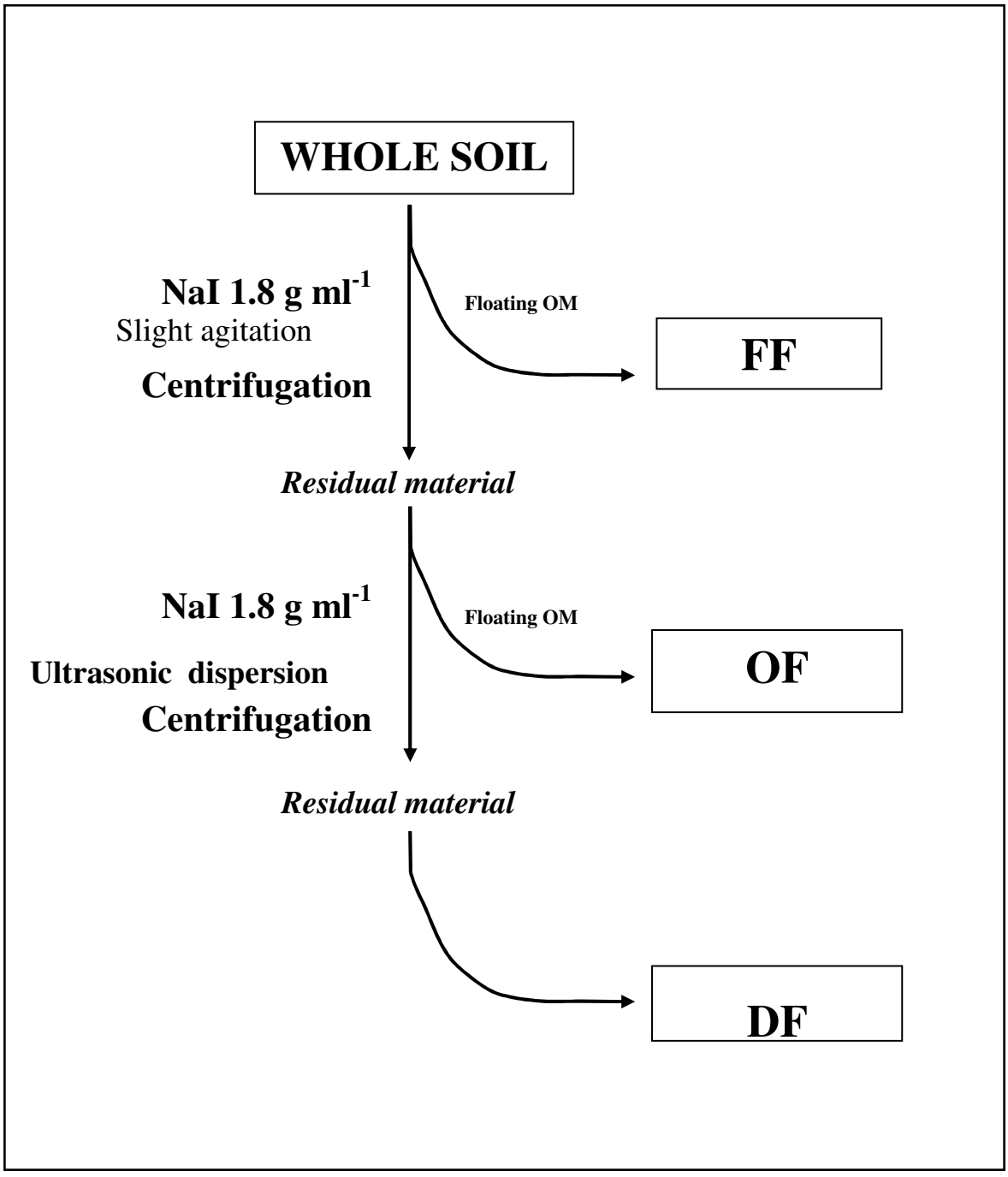

Figure 10

Flow diagram of the density fractionation method

Briefly, 5 g of soil sample were placed in small centrifuge bottles (50 ml capacity), and 35 ml of NaI at 1.8 g ml^{-1} density were added. The bottles were shaken gently and the floating material, considered as the FF, was then recovered by centrifugation at 8000 g for 30 min at 18 ^{o}C, and filtered over a vacuum filter, using a glass fiber filter (Whatman GF/F), by washing with deionized water. The recovered NaI (not mixed with the washing water) was added to the residue remaining in the bottle and the solution was fitted to 1.8 g ml^{-1} density. The bottle was placed in an ice bath and sonicated at 300 J ml^{-1} with a probe-type ultrasonic disintegrator (Branson 450 W).

The floating material, considered as the OF, was recovered by centrifugation and washed in the same way as the FF. The remaining material, considered the DF, was washed with deionized water. All fractions were dried at 40 ^{o}C, weighed, ground in a mortar and pestle, and analyzed for C, N and black carbon contents. Carbonates were analyzed for the whole soils and it was assumed that all carbonates were recovered in the DF. To obtain enough sample to perform analysis, it was necessary to accumulate several replicates of the fractions.

6.2.5. Black carbon analysis

Black carbon was analyzed using benzenepolycarboxylic acids (BPCA) as a molecular marker, following the indications of Glaser et al. (1998). Briefly, 0.5 g of ground sample was digested with 10 ml of 4 mol l^{-1} trifluoroacetic acid (TFA) for 4 hours at 105 °C (modification proposed by Brodowski et al., 2007).
The residue was collected by filtration through a glass fiber filter, rinsed several times with deionized water and dried at 40°C for 2 h. The residue was quantified and transferred to a glass digestion tube and oxidized with 2 ml of 65% HNO_3 for 8 h at 170°C at high pressure. The digestion solution was poured through an ash-free cellulose filter into a 10 ml volumetric flask, and the volume made up with deionized water. An aliquot of 2 ml was diluted with 4 ml of deionized water and 100 µl of the first internal standard containing 100 µg citric acid were added. The solution was cleaned using a cation exchange resin (Dowex 50WX8, 200–400 mesh). The BPCAs were freeze-dried in conical flasks, then re-dissolved in methanol and transferred to reactivials. Then 100 µl of a second internal standard solution containing 100 µg of biphenyl-2,2-dicarboxylic acid were added prior to drying the solvent by evaporation. The BPCAs were derivatized to trimethylsilyl derivatives by adding 125 µl of pyridine and 125 µl of N,O-bis(trimethylsilyl)-trifluoroacetamide and heating to 80 °C for 2 h. Capillary gas chromatography was performed in an HP 6890 instrument equipped with an HP-5 capillary column (30 m x 250 µm x 0.25 µm film thickness) and a flame ionization detector (FID). For correct data acquisition, standard solutions of 20, 50, 100, 250 and 500 µg of BPCAs in methanol were used. The sum of the yields of BPCA was multiplied by a correction factor of 2.27 for BC quantification (Glaser et al., 1998).

6.2.6. Statistical analyses

Analysis of variance (ANOVA) was used: a) to compare the yields, C and N contents, and C/N ratio, of the fractions, overall or for a given land use type, and b) to compare different land use for a given fraction. In case of significant F-statistics ($p > 0.05$), differences between means were tested with the Tukey multiple comparisons procedure. Data were tested for normality and homoscedasticity with the Kolmogorov-Smirnov and Levene statistics, respectively.

The statistical analyses were performed with the Systat 14.0 Statistical Software Package (SPSS for Windows).

6.3. Results

6.3.1. Effect of land use on C storage in whole soils and density fractions

The main characteristics of the samples are shown in Table 8.

Percentage of OC in topsoil (from 0 to10 cm depth), under a land use change from a natural Quercus ilex forest to cropped land (100 years ago) showed an average net drop of 67%. The subsequent reforestation of the studied calcareous soils with Pinus halepensis resulted in a great recovery of OC. An average OC recovery of 71% of the OC (referring to % of OC in soils under Quercus ilex forest) was measured in topsoil (from 0 to10 cm depth) after 40 years of pine plantation.

Table 8. Physicochemical properties of the soil samples

AREA	LAND USE	TEXTURE	$CaCO_3$ (%)	OC (%)	N (%)	OC/N	pH
CERRATO	Cropped land	Clay loam	19.9 ±8.8	2.78 ±0.62	0.25 ±0.12	11.0 ±2.1	8.1 ±0.1
CERRATO	Quercus forest	Clay loam	20.9 ±4.6	5.42 ±0.74	0.41 ±0.19	13.1 ±2.3	7.9 ±0.1
CERRATO	Pinus plantation	Clay loam	18.4 ±1.8	5.45 ±0.71	0.38 ±0.11	14.1 ±2.6	7.9 ±0.1
AMPUDIA	Cropped land	Clay loam	10.1 ±1.0	1.59 ±0.38	0.18 ±0.09	10.6 ±2.1	8.1 ±0.1
AMPUDIA	Quercus forest	Clay loam	13.4 ±1.7	5.87 ±0.82	0.46 ±0.18	12.7 ±2.4	8.1 ±0.1
AMPUDIA	Pinus plantation	Clay loam	22.2 ±3.2	3.47 ±0.51	0.21 ±0.08	21.0 ±3.1	8.2 ±0.1
MONTE VIEJO	Cropped land	Clay loam	59.8 ±9.8	1.56 ±0.29	0.26 ±0.07	10.6 ±1.7	8.3 ±0.1
MONTE VIEJO	Quercus forest	Clay loam	31.1 ±7.3	6.51 ±0.80	0.50 ±0.20	11.9 ±1.9	7.9 ±0.1
MONTE VIEJO	Pinus plantation	Clay loam	42.0 ±6.9	3.70 ±0.67	0.36 ±0.08	13.1 ±1.9	8.1 ±0.1

Table 9. Recoveries of mass total organic C (TOC) and total N (TN) after density fractionation.

	% of soil weight	% of TOC	% of TN
CERRATO			
CL	100.33 ±1.33	99.42 ±3.81	95.20 ±5.12
QF	102.72 ±3.43	99.51 ±2.12	98.90 ±5.93
PP	102.14 ±2.12	100.78 ±1.21	84.54 ±3.16
AMPUDIA			
CL	98.46 ±5.10	98.77 ±4.27	99.51 ±3.12
QF	102.33 ±3.52	107.19 ±9.11	80.95 ±7.74
PP	100.92 ±4.11	102.28 ±2.69	97.94 ±5.60
MONTE VIEJO			
CL	99.88 ±2.14	102.81 ±3.15	98.88 ±2.98
QF	102.63 ±5.04	99.20 ±4.29	90.30 ±9.02
PP	101.63 ±1.44	101.23 ±3.37	89.89 ±7.84

CL: Cropped land; QF: *Quercus* forest; PP: *Pinus* plantation; FF: free fraction; OF: occluded fraction; DF: dense fraction. Means ±standard error for n= 4.

Table 10. Density fraction yields

	FF	OF	DF
		(% of soil weight)	
CERRATO			
	b	*c*	*a*
CL	1.20 ±0.02 C	0.27 ±0.11 B	98.86 ±0.38 A
QF	9.44 ±0.37 A	0.58 ±0.28 A	92.70 ±1.91 B
PP	7.38 ±0.33 B	0.45 ±0.09 AB	94.31 ±3.43 AB
AMPUDIA			
	b	*c*	*a*
CL	1.06 ±0.50 C	0.11 ±0.02 B	97.30 ±2.00 A
QF	9.06 ±0.20 A	0.73 ±0.32 A	92.54 ±3.53 B
PP	4.32 ±1.38 B	0.21 ±0.11 AB	96.39 ±3.52 AB
MONTE VIEJO			
	b	*c*	*a*
CL	1.09 ±0.12 C	0.08 ±0.58 B	98.71 ±0.34 A
QF	10.85 ±1.94 A	0.72 ±0.16 A	91.06 ±1.16 B
PP	7.20 ±0.14 B	0.16 ±0.10 AB	94.27 ±3.65 AB

CL: Cropped land; QF: *Quercus* forest; PP: *Pinus* plantation; FF: free fraction; OF: occluded fraction; DF: dense fraction. Fractions indicated with the same italic letter are not significantly different ($p<0.05$). Land uses indicated with the same capital letter are not significantly different ($p<0.05$) for a given fraction. Means ±standard error for n= 4.

The free and intra-aggregate fractions obtained by density fractionation were visually distinct. The FF comprised recognizable plant material, whereas the intra-aggregate was amorphous dark material. The recovery of fractions with respect to the initial soil weight varied between 98 and 103% (Table 9). Recoveries higher than 100% may be due to incomplete removal of NaI.

As expected, most of the fine soil mass was located in the DF (Table 10), and OF represented a minor part of the soil mass. The yields of all the density fractions were significantly different among land uses, with a significantly greater amount of FF in soils under tree cover than in the soils under agricultural land use, due to greater incorporation of organic matter.

Comparison of OC concentration (g C g^{-1} fraction) among density fractions revealed the lowest values in DF, as the latter was dominated by minerals, and the highest values in OF (Table 11). Cropped land presented the lowest OC concentration overall fractions. As regards the % of OC in soil that is represented by each fraction, the major portion of OC was associated with DF (Table 11). The DF accounted for about 83% of TOC under cropped land, but for about 53% of TOC in topsoils (0-10 cm depth) under tree cover, with no-significant differences among soils under *Pinus* or *Quercus* vegetation.

The recovery of organic C after fractionation ranged from 97% to 107%, with an average of 101% (Table 9). These recoveries are too much high, taking into account that the repeated soil washing and decanting is expected to result in losses of soluble organic matter, especially if an ultrasonic treatment is applied. Such high recoveries may be due to organic impurities contained in the NaI solution, and/or to the possible presence in OF and/or FF of small amounts of carbonates, whose carbon could have been taken as organic C. Owing to all these constraints, the data for C can not be taken as precise estimations.

Table 11. Organic carbon (OC), %OC of total OC of the soil in density fractions.

	OC (mg C g^{-1}fraction)			%OC (of total OC of soil)		
	FF	OF	DF	FF	OF	DF
CERRATO						
	b	*a*	*c*	*b*	*c*	*a*
CL	227 ±29 B	360 ±11 B	21 ±1 B	10.9 ±3.9 B	3.8 ±0.9 A	84.7 ±3.4 A
QF	276 ±22 AB	386 ±7 A	25 ±2 AB	50.6 ±3.2 A	4.3 ±1.4 A	44.6 ±2.6 B
PP	296 ±11 A	383 ±10 A	27 ±4 A	44.1 ±4.3 A	3.5 ±1.7 A	53.2 ±3.8 B
AMPUDIA						
	b	*a*	*c*	*b*	*c*	*a*
CL	229 ±27 B	331 ±18 B	14 ±4 B	14.3 ±2.4 B	2.1 ±2.5 A	82.4 ±5.3 A
QF	266 ±24 AB	364 ±16 AB	28 ±1 A	47.1 ±3.2 A	5.2 ±1.3 A	54.9 ±3.7 B
PP	319 ±31 A	391 ±13 A	26 ±3 A	37.6 ±5.1 A	2.1 ±1.7 A	62.5 ±4.2 B
MONTE VIEJO						
	b	*a*	*c*	*b*	*c*	*a*
CL	227 ±21 B	343 ±11 B	12 ±2 B	17.9 ±4.8 B	2.1 ±2.4 A	82.9 ±6.3 A
QF	259 ±16 AB	376 ±9 A	33 ±6 A	43.2 ±3.7 A	4.2 ±1.8 A	50.9 ±3.2 B
PP	295 ±22 A	389 ±14 A	20 ±7 AB	48.7 ±1.2 A	2.3 ±0.7 A	50.2 ±1.7 B

CL: Cropped land; QF: *Quercus* forest; PP: *Pinus* plantation; FF: free fraction; OF: occluded fraction; DF: dense fraction. Fractions indicated with the same italic letter are not significantly different ($p<0.05$). Land uses indicated with the same capital letter are not significantly different ($p<0.05$) for a given fraction. Means ±standard error for n= 4.

6.3.2. Effect of land use on N storage and C/N ratio in whole soils and density fractions

As regards N content, FF was the most sensitive fraction to land use change.

The highest concentration of N was found in the OF. However this fraction represented the lowest contribution to total N (always <3% of total N). In contrast, the DF shows the lowest N concentration but contributed the most to total N (Table 12).

Table 12. Nitrogen (N), %N of total N of the soil and OC/N in density fractions.

	N (mg N g^{-1}fraction)			%N (of total N of soil)			OC/N		
	FF	OF	DF	FF	OF	DF	FF	OF	DF
CERRATO									
							a	*ab*	*b*
CL	15.1 ±0.1 A	26.7 ±4.1 A	2.0 ±0.2 A	7.6 ±3.2 B	3.0 ±0.3 A	84.5 ±6.1 A	15.1 ±3.3 B	13.4 ±3.2 A	10.6 ±0.7 A
QF	14.6 ±0.3 B	18.7 ±3.2 A	2.0 ±0.1 A	40.3 ±5.9 A	3.2 ±0.5 A	55.4 ±2.1 B	18.9 ±0.5 B	20.6 ±6.1 A	12.1 ±2.3 A
PP	13.4 ±0.8 B	18.8 ±3.8 A	1.9 ±0.3 A	28.3 ±7.1 A	2.4 ±0.2 A	53.8 ±4.8 B	22.2 ±0.9 A	20.4 ±4.7 A	14.1 ±3.1 A
AMPUDIA									
							a	*ab*	*b*
CL	15.3 ±2.3 AB	35.3 ±10.2 A	0.9 ±0.5 A	14.7 ±3.5 B	3.4 ±0.3 A	81.4 ±6.4 A	15.0 ±2.9 B	9.4 ±7.1 A	15.7 ±0.4 A
QF	16.5 ±0.6 A	17.6 ±7.3 A	1.9 ±0.7 A	34.7 ±5.2 A	3.0 ±0.2 A	43.3 ±6.6 B	16.1 ±0.8 B	20.7 ±5.2 A	15.1 ±1.2 A
PP	11.9 ±1.3 B	31.1 ±8.1 A	1.6 ±0.3 A	21.8 ±7.7 A	2.7 ±0.5 A	60.2 ±10.2 B	26.9 ±1.4 A	12.6 ±3.3 A	16.1 ±2.1 A
MONTE VIEJO									
							a	*ab*	*b*
CL	14.3 ±0.9 AB	23.0 ±2.1 A	1.2 ±0.7 A	12.0 ±2.1 B	1.5 ±1.1 A	85.4 ±4.0 A	15.9 ±1.1 B	14.9 ±3.5 A	10.0 ±3.7 A
QF	14.9 ±0.7 A	20.3 ±5.3 A	2.3 ±0.5 A	27.9 ±4.2 A	2.5 ±0.4 A	49.9 ±6.3 B	17.0 ±0.4 B	18.6 ±5.2 A	14.6 ±2.0 A
PP	12.4 ±1.1 B	23.0 ±3.2 A	1.5 ±0.6 A	26.9 ±3.9 A	1.8 ±0.3 A	61.1 ±4.9 B	23.9 ±2.7 A	16.9 ±3.8 A	13.0 ±0.9 A

CL: Cropped land; QF: *Quercus* forest; PP: *Pinus* plantation; FF: free fraction; OF: occluded fraction; DF: dense fraction. Fractions indicated with the same italic letter are not significantly different ($p<0.05$). Land uses indicated with the same capital letter are not significantly different ($p<0.05$) for a given fraction. Means ±standard error for n= 4.

The OC/N ratio was significantly higher ($p < 0.05$) in FF than in the DF. Comparison of the OC/N ratio in FF among the different types of land use, revealed significantly higher values ($p < 0.01$) in soils under pine forest (Table 12).

The recovery of N after fractionation ranged from 81% to 99% (Table 9), expectable recoveries because of the extraction of soluble N from repeated washing during the fractionation process.

6.3.3. Black carbon content in whole soils and density fractions

Black carbon represents a small proportion of the whole soil (from 0.4 to 3.1 mg g^{-1} of soil) and from 1.2 to 2.3% of the soil TOC (Table 13).

Table 13. Black carbon (BC) content in whole soils.

	BC (mg C g^{-1} soil)	***BC (mg C g^{-1} TOC)***
CERRATO		
CL	1.19 ±0.91	20.83 ±5.11
QF	2.93 ±1.08	19.34 ±3.95
PP	1.94 ±0.86	17.21 ±2.74
AMPUDIA		
CL	0.89 ±1.17	23.09 ±4.19
QF	1.76 ±0.79	15.17 ±2.06
PP	0.59 ±1.35	10.64 ±4.83
MONTE VIEJO		
CL	0.38 ±1.42	12.18 ±3.17
QF	3.07 ±1.30	20.79 ±2.26
PP	1.48 ±0.93	17.59 ±1.99

CL: Cropped land; QF: *Quercus* forest; PP: *Pinus* plantation. Means ±standard error for n= 4.

A large part of BC was not associated with the mineral phase. As shown in Table 14, there were differences among fractions, both in BC concentration and in % of BC with respect to TOC, in the following order: DF < FF < OF. Between 0.9 and 2.1% of the OC of FF corresponded to BC, and between 1.2 and 3.4% of the OC in OF. Only BC content in OF was sensitive to changes in land uses, showing higher concentration in cropped land than in soils under tree cover.

Table 14. Black carbon content of the different density fractions

	BC (mg C g^{-1} fraction)			BC (mg C g^{-1} OC fraction)		
	FF	OF	DF	FF	OF	DF
CERRATO						
	b	**a**	**c**	**b**	**a**	**c**
CL	10.6 ±1.0 A	25.6 ±1.3 A	0.4 ±0.1 A	20.6 ±3.9 A	34.0 ±4.1 A	7.4 ±2.1 A
QF	8.7 ±0.8 A	17.2 ±0.9 B	0.2 ±0.2 A	13.9 ±2.7 A	19.7 ±1.8 B	2.6 ±2.7 A
PP	8.8 ±0.9 A	18.8 ±1.1 B	ND	13.1 ±2.1 A	21.7 ±2.1 B	ND
AMPUDIA						
	b	**a**	**c**	**b**	**a**	**c**
CL	7.4 ±0.8 A	25.2 ±0.9 A	0.1 ±0.1 A	14.2 ±1.7 A	33.4 ±2.8 A	2.1 ±3.1 A
QF	10.1 ±1.0 A	14.5 ±1.4 B	0.4 ±0.2 A	16.7 ±1.9 A	17.5 ±3.1 B	5.1 ±2.0 A
PP	8.7 ±0.9 A	21.2 ±1.1 B	0.4 ±0.2 A	12.0 ±3.1 A	23.9 ±4.2 B	6.5 ±2.3 A
MONTE VIEJO						
	b	**a**	**c**	**b**	**a**	**c**
CL	4.6 ±1.2 A	23.4 ±1.2 A	0.4 ±0.1 A	8.8 ±4.1 A	32.4 ±3.7 A	11.8 ±1.8 A
QF	7.7 ±0.8 A	13.5 ±1.5 B	0.9 ±0.3 A	14.1 ±3.3 A	15.8 ±2.2 B	9.1 ±2.3 A
PP	11.6 ±1.1 A	10.3 ±0.8 B	0.4 ±0.2 A	17.3 ±2.8 A	11.6 ±3.4 B	9.3 ±1.1 A

CL: Cropped land; QF: *Quercus* forest; PP: *Pinus* plantation; FF: free fraction; OF: occluded fraction; DF: dense fraction. ND: No determination. Fractions indicated with the same italic letter are not significantly different ($p<0.05$). Land uses indicated with the same capital letter are not significantly different ($p<0.05$) for a given fraction. Means ±standard error for n= 4.

6.4. Discussion and conclusions

6.4.1. Effect of land use on C storage in whole soils and density fractions

Land use change from a natural *Quercus ilex* forest to cropped land (100 years ago) has resulted in an average net drop of 67% in the % of OC in the topsoil (from 0 to 10 cm depth). These results are consistent with results reported by Llorente et al. (2009) for the same region. Such loss is also consistent with the findings of Burke et al. (1989), who reported 50% of SOC loss for land use transformation from grassland to crop land, and the findings of other studies (Prior et al., 2000) showing that cultivation generally decreases the amount of organic matter. Soil tillage induces soil C loss by acceleration of organic C oxidation, which results in the release of large amounts of CO_2 to the atmosphere (La Scala et al., 2008). Another tillage-related factor that contributes to soil C losses is disruption of the soil aggregates, which exposes once-protected organic matter to decomposition (De Gryze et al., 2006; Grandy and Robertson, 2007).

Reforestation of the studied calcareous soils with *Pinus halepensis* resulted in a great recovery of OC. An average recovery of 71% of the OC (referring to % of OC in soils under native *Quercus ilex* forest) was measured in topsoil (from 0 to10 cm depth) after 40 years of pine plantation.

Comparison of OC concentration (g C g^{-1} fraction) among density fractions revealed the lowest values in DF, as the latter was dominated by minerals, as suggested by Golchin et al. (1994b; 1995), and the highest values of OC concentration were found in OF. It has been suggested that such high concentration is due to the physical protection of OM by aggregates attributed to compartmentalization of substrate and microbial mass (Killham et al., 1993; Six et al., 2002a).

As regards the % OC in soil that is represented by each fraction, the major portion of OC was associated with DF (Table 10). The DF accounted for about 83% of TOC under cropped land, but for about 53% of TOC in topsoil under tree cover, with no significant differences among soils under *Pinus* or *Quercus* vegetation. These values are consistent with the findings of

John et al. (2005), who reported that 86-91% of total SOC was associated with the mineral-associated SOM fraction at grassland, maize and wheat sites in silty soils; in contrast, the free and occluded fraction accounted for 52% of total SOM in a spruce stand on similar soil.

6.4.2. Effect of land use on N storage and C/N ratio in whole soils and density fractions

The highest concentration of N was found in OF. However, this fraction represented the lowest contribution to total N (always <3% of the total N). In contrast, DF showed the lowest N concentration but contributed the most to total N (Table 11).

OC/N ratio was significantly higher ($p < 0.05$) in FF than in DF. The OC/N ratio value was significantly lower in DF than in OF (Table 11), in contrast to the findings of Rovira and Vallejo (2003) who reported significantly higher OC/N in DF in soils over calcareous material and under *Quercus rotundifolia*, but in agreement with the data of Roscoe and Buurman (2003) and Golchin et al. (1994b) who observed somewhat

higher C/N ratios for FF and also according to those of Kölbl and Kögel-Knabner (2004), who found no differences between FF and OF.

Differences among land uses were only significant in FF. The higher values of OC/N ratio for the soils under pine forest were quite expectable, in aggrement with references such as Duchaufour, 2001. Results are also consistent, with the findings of different studies on litter composition, such as that by Traversa et al. (2008), who compared the C/N ratio of the litter under *Pinus halepensis* and *Quercus ilex*.

6.4.3. Effect of soil management on Black carbon content in whole soils and

In studied calcareous soils, black carbon concentration ranged from 0.38 to 3.07 mg BC g^{-1} of whole soil and the proportion of the TOC represented by BC, ranged from 12.18 to 23.09 mg BC g^{-1} of TOC. These concentration are lower than those found in the soils analyzed by Dai et al. (2005) or in the soils studied by Glaser and Amelung (2003), using the same method. However, our values are only slightly lower than those reported by Rovira and Vallejo (2007) for surface horizons in Mediterranean soils. In any case, our results concur with the fact that BC may be a relevant pool, but it is not a dominant one.

In whole soils, no differences in BC content among land uses were found for the calcareous soils studied. This supports the findings of Rodionov et al. (2006) who did not observe any changes in BC content related to long-term arable cropping of steppe soils of Russia. Likewise, Brodowski et al. (2007) found that land use changes from grassland to cropland decreased C content, but did not affect BC proportion. The maintenance of BC proportion through land uses is relevant because it sugges an equilibrium between inputs and outputs, and leads to the suspicion that BC could be less stable and less resistant to biodegradation than is often taken for granted (e.g., Kuhlbusch, 1998; Schmidt and Noack, 2000). In addition, BC soil content has seldom been quantified, and long-term studies on BC stability are scarce and conclusions about BC stability are not widespread (Dai et al., 2005; Rovira et al., 2009). Contradictory experimental results reported both rapid (Bird et al., 1999) and slow (Shindo, 1991) decomposition of biomass-derived BC in soils. Hockaday et al. (2006) reported an association between soil charcoal fragments and filamentous microbiota that resemble saprophytic fungi; this provides grounds for suspecting that these organisms play a role in fate of soil BC. An additional question about BC stability was introduced by Rovira et al. (2009), who reported an overall decrease in BC content with wildfires; this maybe because the wildfire itself destroys part of the pre-existing BC by combustion.

These facts suggest that, leaving aside specific cases such as “Terra Preta” soils (Glaser et al., 2001), black carbon could be not so stable and relevant pool for C accumulation in soils, as suggested by some studies (Bird et al., 1999; Hockaday et al., 2006).

6.4.4 *Black carbon content in density fractions and effect of soil management on Black carbon contents.*

The highest BC concentration and the highest contribution to the overall BC amount in soil were found in OF, followed by FF. The BC contents in density fractions in this study are consistent with the findings of Rumpel et al. (2006), who reported that a major part of the BC was not associated with the mineral phase, as

well as, with the findings of Skjemstad et al. (1990), who reported that BC may be a major component of both FF and OF. The high proportion of BC indicates a great chemical recalcitrance on the part of the OC in FF and OF, which may explain the low decomposition rate found in these soils (Llorente and Turrión, 2009). A significant portion of BC was localized in FF, the pool commonly assigned to labile humus. Hence, the question arises as to whether BC content also depends on different management practices. The only fraction sensitive to land use change was OF, with higher concentration both respect to the total mass and to the organic C in the fraction for cropped soils than in soils under tree cover.

6.4.5. Conclusions

Historical transformation of *Quercus* forest to cropped lands in calcareous soils in this area has resulted in a major loss of OC, as was expected. However, subsequent reforestation with *Pinus* throughout the past 40 years has resulted in good recovery of the SOC.

SOM fractionation by density and ultrasonic dispersion enables separation of fractions corresponding to different mechanisms of physical protection and determination of the effect of land use on the amount and spatial arrangement of OM in soils. Therefore, study of the density fractions and their BC content enables better understanding of the high stability of OM in calcareous soils.

Despite the different OC content of soils under different land use - higher under tree cover and lower under cultivation - the OM stabilization mechanisms were not significantly different. OM was mainly located in the organo-mineral complex, resulting in physicochemical stabilization against further decomposition.

In our study, Black carbon always represents <3% of the TOC, far lower than the maximums reported in the literature.Furthermore, a significant proportion (between 0.9 and 3.5%) of the free and occluded OM corresponds to black carbon.

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7. ANTHROPOGENIC DISTURBANCE OF NATURAL FOREST VEGETATION ON CALCAREOUS SOILS ALTERS SOIL ORGANIC MATTER COMPOSITION AND NATURAL ABUNDANCE OF ^{13}C AND ^{15}N IN DENSITY FRACTIONS

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Abstract

In the last century, many calcareous soils in Castilla León (northwestern Spain) have been transformed from natural *Quercus ilex* forest to cropped land. Reforestation with *Pinus halepensis* has been taking place during the past 40 years.

In order to obtain a better understanding of how these disturbances affect ecosystem functioning, we studied the quantity and quality of soil organic matter (SOM) in natural forest ecosystems, cropland and Pinus plantations. Density fractionation combined with ultrasonic dispersion enables separation and study of SOM fractions: free organic matter (OM), OM occluded into aggregates, and OM stabilized in organo-mineral complexes, considered on the basis of the type of physical protection provided. We separated SOM density fractions and determined the concentrations of C and N, C:N ratios and the natural isotopic abundance ($\delta^{13}C$ and $\delta^{15}N$ values).

Transformation of Quercus forest to cropland resulted in major losses of SOC and N, as expected. However, subsequent reforestation with Pinus resulted in good recovery of the original SOC and soil N pools. This indicates the potential for enhanced C storage in agricultural soils by their reversion to a forested state.

Study of the density fractions and their ^{13}C and ^{15}N signatures enabled better understanding of the high stability of OM in calcareous soils, and analysis of $\delta^{13}C$ variations throughout the profile also enabled identification of past C3/C4 vegetation change. Despite the different OC contents of soils under different land use, OM stabilization mechanisms were not significantly different. In calcareous soils, accumulation of SOC and N is mainly due to organo-mineral associations, resulting in physicochemical stabilization against further decomposition.

Keywords: Land Use Change; Density Fractions; $\delta^{13}C$; $\delta^{15}N$.

7.1. Introduction

The increase in the concentration of atmospheric carbon dioxide during the past century has drawn attention to the relation between the capacity of ecosystems to act as C sinks, changes in land use and climate change (Briones et al. 2006). Soil organic matter (SOM) is recognised as an important factor in C -driven climate change (Sanderman et al. 2003). Because of this, SOM dynamics and the capacity of soils to accumulate and stabilize organic carbon in response to different land use changes have received much attention in recent years (Rovira and Vallejo 2003; Llorente and Turrión 2010; Wick et al. 2009).

Anthropogenic disturbance of ecosystems leads to changes in the quantity and quality of SOM (Hobbs 1999). Comparisons between cultivated and uncultivated soils have demonstrated a reduction in SOM content with cultivation (Mann 1986). However, when arable land is converted to permanent vegetation, the SOM content increases gradually because of greater inputs of organic matter (Haynes and Beare 1996).

Studies of the effects of land use change on the SOM in calcareous soils are scarce. However, calcareous soils are of particular interest because of the high stability of their organic matter (García et al. 1997), and because they represent approximately 12% of the world soil resources (FAO 1996).

SOM is a complex mixture of material from various sources and may exist in the soil profile as decomposed or stabilized matter, or in any form intermediate between these two extremes. Physical fractionation methods such as density fractionation have been proposed for the study of SOM dynamics (Christensen 1992), because this method yields distinct organic matter (OM) fractions that differ in dynamics, structure and function (Golchin et al. 1994; Six et al. 2001). Many authors have used density fractionation of SOM to represent different pools of SOM with different turnover rates (e.g. Swanston et al. 2002; Helfrich et al. 2006; Llorente and Turrión 2010), and to identify mechanisms that control changes in C and N pools (Jolivet et al. 2003). Density fractionation enables us to identify labile, active fractions of SOM, which may respond much faster to management changes than the total SOM content, and also passive fractions, which are more closely associated with long-term SOM dynamics (Barrios et al. 1996). SOM density fractionation following ultrasonic dispersion enables separation and study of three different pools according to the different degree of physical protection provided (Sohi et al. 2001). The main fractions are: (1) “free” particulate OM (FPF), isolated before ultrasonic break-down of stable aggregates, (2) OM occluded within aggregates (OF), isolated after breakdown of aggregates by ultrasonic dispersion, and 3) the organo-mineral fraction (OMF) recovered as the residual (heavy) material.

A relatively inexpensive analytical method such as stable isotopic analysis is useful for assessing chemical shifts in SOM (Glaser 2005). The principle of using the stable isotope techniques is based on the fact that the abundance of heavy and light isotopes (e.g. ^{13}C and ^{12}C, respectively) varies due to isotopic discrimination of the heavy isotope in SOM compounds during biological and/or physical processes (Andreux et al. 1990). Krull and Skjemstad (2003) demonstrated that isotope fractionation is highly influenced by soil chemistry, mineralogy and type of organic matter input. The application of stable carbon isotope ($\delta^{13}C$) analysis (and to a lesser degree $\delta^{15}N$) to SOM studies has increasingly been used to estimate soil C and N turnover (e.g.

Balesdent and Mariotti 1988; Bernoux et al. 1998; Glaser 2005; Sevink et al. 2005), and to assess the degree of SOM decomposition (e.g. Wedin et al. 1995; Connin et al. 2001). Although many studies have addressed the isotopic composition of density fractions (Shang and Tiessen 2000; Roscoe et al. 2001; Crow et al. 2006), the mechanisms of N fractionation associated with organic matter decomposition are still unclear (Liao et al. 2006). Stable isotope data are useful for assessing C and N turnover in SOM density fractions. Because the light fraction from density fractionation primarily represents labile material of relatively recent origin, we would expect this fraction to reflect ^{13}C and ^{15}N values that are closer to current vegetation (Compton and Boone 2000; Accoe et al. 2002). The heavy fraction, representing older, more humified and amorphous organic compounds, displays relatively enriched $\delta^{13}C$ values (Ehleringer et al. 2000).

The objective of the present study was to investigate organic matter dynamics in calcareous moor soils under different types of land use -native forest (*Quercus ilex*), forest plantation *(Pinus halepensis)* and agricultural land (cropped with cereals) using density fractionation of SOM and stable C and N isotopic approach.

More specifically, the aims of the study were: a) to assess the effect of land use and depth on OM abundance and on the ^{13}C and ^{15}N isotope composition of calcareous soils; b) to study the roles of different density fractions in C and N stabilization in calcareous soils; c) to determine the ^{13}C and ^{15}N isotope composition of density fractions.

7.2. Materials and methods

7.2.1. Site description

The study was carried out in the region of Castilla y León (northwestern Spain), UTM: 30T 384465 E 4639001 N. The mean annual rainfall in the region is below 420 mm, under a xeric moisture regime, and the mean annual temperature is 12.3 ^{o}C. The altitude of the moor is between 800 and 900 m, with gentle slopes (< 7%). The soils are Xerepts, which are quite similar by their physicochemical parameters but differ in their land use history. The native vegetation in the area is holm-oak wood (*Quercus ilex* subsp *ballota*). In the 19th century, most of the forest was converted into agricultural land, but since the 1950s, reforestation with *Pinus halepensis* has been carried out on abandoned agricultural land. Agricultural land is currently cropped with cereals (usually barley). The average basal area of the *Quercus ilex* forest is 13 m^2 ha^{-1} with an average density of 3200 trees ha^{-1}. The average basal area of the Pinus plantation is 50 m^2 ha^{-1}, with an average density of 1184 trees ha^{-1}.

General characteristics of the samples are shown in Table 15

Table 15. Basic properties of the soils under study (0-30 cm).

AREA	LAND USE	TREE COVER	TEXTURE	$CaCO_3$ (%)	SOC (%)	N (%)	C/N	pH
CERRATO	Cropped land	No	Clay loam	19.9	2.78	0.25	11.0	8.1
CERRATO	Quercus forest	No	Clay loam	23.4	2.98	0.29	10.4	8.2
CERRATO	Quercus forest	Yes	Clay loam	20.9	5.42	0.41	13.1	7.9
CERRATO	Pinus plantation	Yes	Clay loam	18.4	5.45	0.38	14.1	7.9
AMPUDIA	Cropped land	No	Clay loam	10.1	1.59	0.18	8.6	8.1
AMPUDIA	Quercus forest	No	Clay loam	15.4	2.54	0.26	9.8	8.1
AMPUDIA	Quercus forest	Yes	Clay loam	13.4	5.87	0.46	12.7	8.1
AMPUDIA	Pinus plantation	Yes	Clay loam	22.2	3.47	0.21	21.0	8.2
MONTE VIEJO	Cropped land	No	Clay loam	59.8	1.56	0.26	10.6	8.3
MONTE VIEJO	Quercus forest	No	Clay loam	26.5	3.75	0.34	10.1	8.2
MONTE VIEJO	Quercus forest	Yes	Clay loam	31.1	6.51	0.50	11.9	7.9
MONTE VIEJO	Pinus plantation	Yes	Clay loam	42.0	3.70	0.36	10.9	8.1

Mean values for 4 samples.

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7.2.2. Sampling procedures

A land use map of the calcareous moor of Castilla y León was elaborated with a GIS (ArcGis 9.0 for Windows) The map was used to select the sampling plots on the basis of the following criteria: a) *Quercus ilex* forest (QF), cropped land (CL), and *Pinus halepensis* plantations (PP) in adjacent areas; b) minimal area of each type of land use, 1 ha, and c) establishment of each type of land use during at least 40 years. Three plots were selected for each type of land use, with four representative profiles for each type of land use. A total of 48 profiles were thus sampled at 0 – 10 cm, 10 – 20 cm, and 20 – 30 cm depth intervals. Visible plant residues and roots were removed, and soil was air-dried, sieved (< 2 mm) and stored at room temperature until analysis.

Litter from soil under each land use type was sampled, oven dried at 50 ^{o}C and ground.

7.2.3. Ultrasonic equipment

We used a Branson 450 W Sonicator, equipped with a titanium probe. The probe depth was fixed in 15 mm. The sonicator was calibrated by determining the real power output calorimetrically (North 1976). The probe output energy was calculated from:

$P = (m_w c_w + C_{cont}) \Delta T t^{-1} + Ht$,

where P is the calculated power (W), m_w is the mass of water (g), c_w is the specific heat of water (4.18 J g^{-1} K^{-1}), C_{cont} is the heat capacity of the container (J K^{-1}), ΔT is the temperature change (K), t is the sonication time (s) and H is the heat loss (J s^{-1}). The heat capacity of the glass beaker (C_{cont}) was determined using the method of mixtures (Morra et al. 1991), according to:

$C_{cont} = m_1 c_w ((T_1 - T_2) (T_3 - T_2)^{-1}) - m_2 c_w$,

where C_{cont} and c_w are as above, m_1 is the mass (g) of an amount of water heated to T_1 (K), which is added to the beaker that already contained an amount of water m_2 (g) at room temperature (K). The final equilibrium temperature (K) of the water in the beaker is T_3.

The wanted energy output was 300 J mL^{-1} and the corresponding time was calculated from:

$t = m_s E P^1$,

where t is the sonication time (s), m_s is the mass of soil (g), E is the energy (J g^{-1}), P is the power output (W).

7.2.4. Density fractionation of soil

A density fractionation procedure was applied to the topsoils of the 48 profiles. The method follows the concepts of Golchin et al. (1994) who differentiated three degrees of physical protection of OM: FPF, non-protected; OF, occluded within aggregates - extractable by sonication (protected); and OMF retained in the dense residue after sonication (the most protected).

Briefly, 5 g of soil sample were placed in small centrifuge bottles (50 mL capacity), and 35 mL of NaI 1.8 g mL^{-1} were added. The bottles were shaken gently and the floating material, considered as the FPF, was then recovered by centrifugation at 8000 g for 30 min at 18 ^oC, and filtered over a vacuum filter by washing with deionised water. The recovered NaI was added to the residue remaining in the bottle. The bottle was placed in an ice bath and sonicated at 300 J mL^{-1} with a probe-type ultrasonic disintegrator (Bronson 450 W). The floating material, considered as the OF was recovered by centrifugation and washed in the same way as the FPF. The remaining material, considered as the OMF, was washed with deionised water. All fractions were dried at 40^oC and ground in a mortar.

7.2.5. Physical and chemical analyses

For soil characterization, texture, pH, total N, total C, carbonate and organic C, and C/N were determined. Total concentrations of soil C and N were determined in an automated C/N analyser (CHN-2000, Leco).

Organic carbon was calculated as the difference between total and carbonate carbon. Soil total calcium carbonates were determined by use of 1M HCl titrated with 0.5M NaOH (FAO 2007). Dried soil samples were crushed and treated with 1 N HCl to remove any inorganic carbonate (acid pre-treatment has no effect on δ13C of soil organic matter (Nordt et al. 1994).

Natural abundances of ^{13}C and ^{15}N values in soils, plant material, and SOM density fractions were measured in dried (at 45°C for one week), ground and weighed into tin capsules for continuous flow isotope ratio mass spectrometry (BayCEER - Labor für Isotopen-Biogeochemie, Bayreuth, Germany).

Isotope ratios were determined with an elemental analyser coupled to an isotope ratio mass spectrometer (Carlo Erba CN 2500, Italy coupled with DeltaPLUS Isotope MS via Conflo III Interface, Thermo Finnigan, Bremen, Germany). Standard gases were calibrated in relation to international standards (CO_2 in PeeDee belemnite) by use of reference substances (NBS 16 to 20) for carbon isotope ratios, supplied by the International Atomic Energy Agency (IAEA), Vienna.

Natural abundances of ^{13}C and ^{15}N were expressed in δ units, by reference to the international standards, according to equations (1) and (2), respectively:

$$\delta^{13}C‰ = 10^3 \times \frac{(^{13}C/^{12}C)_{samp.} - (^{13}C/^{12}C)_{stand.}}{(^{13}C/^{12}C)_{stand.}} ; \quad (1)$$

$$\delta^{15}N‰ = 10^3 \times \frac{(^{15}N/^{16}N)_{samp.} - (^{15}N/^{16}N)_{stand.}}{(^{15}N/^{16}N)_{stand.}} ; \quad (2)$$

Analysis of variance (ANOVA) was used: (i) to compare SOC and N among land use types and depths; (ii) to compare the proportion of the SOC and total N that is represented by each density fraction, the C concentration in each density fraction, and C/N ratio, overall or for a given land use type; (iii) to compare the effect of land use and depth on $\delta^{13}C$ and $\delta^{15}N$ in whole soil, and (iv) to compare the isotope composition of density fractions, overall for given land uses. In cases of significant F-statistics ($P < 0.05$), differences between means were tested with the Tukey procedure for multiple comparisons. Residuals were tested for homocedasticity and normality with Levene´s and Kolmogorov-Smirnov tests, respectively. All statistical analyses were performed with the Systat 14.0 Statistical Software Package (SPSS for Windows).

7.3. Results

7.3.1. C and N content of whole soil

Land use change from a natural *Quercus ilex* forest to cropped land (100 years ago) resulted in a significant ($P < 0.05$) net loss of 47% of SOC and 41% of total N in the upper 30 cm of soil. The subsequent reforestation of the studied calcareous soils with *Pinus halepensis* has resulted in recovery of 90% of the lost

SOC and 70% of total N 40 years after establishment of the plantation (Figure 11). This is probably due to the higher input of fresh organic matter at the soil surface under forest. Total N and SOC contents were not significantly different throughout the soil profile in cropped soils (Figure 11). This is explained by the low input of organic matter and the effect of tillage to 30 cm depth, which homogenizes soil properties throughout the profile. Furthermore, the SOC and total N content varied significantly among land uses, in the order: Quercus forest> Pinus plantation > cropped land. The organic C and total N contents were the lowest in the cropped land (Figure 11).

The C/N ratio varied between 8.6 and 21.0 and did not differ significantly among different types of land use or among depths (data not shown).

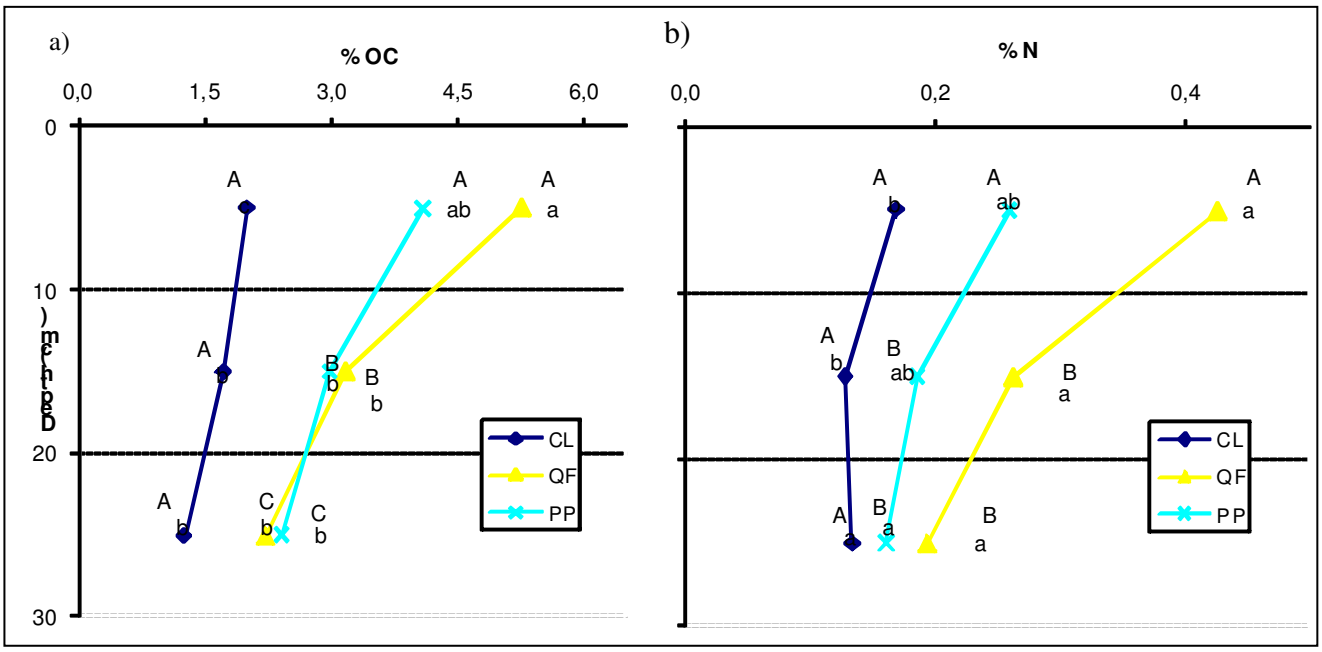

Figure 11

Average contents of OC and N in soils under different types of land use and at different depth. Differences between means were tested by the Tukey procedure for multiple comparisons. Depth indicated with the same capital letter are not significantly different ($p<0.05$) for a given land use (*Depth*land use interaction*). Land uses indicated with the same lower case letter are not significantly different ($p<0.05$) for a given depth (*Land use*depth interaction*).

CL: Cropped land; QF: Native *Quercus* forest; PP: *Pinus* plantation.

7.3.2. C and N distribution in SOM fractions

The recovery of organic C after fractionation ranged from 96% to 105% (average, 100%). Recoveries higher than 100% may be due to incomplete removal of NaI. Losses of OC during fractionation occurred due to loss of material during manipulation of the sample at the various stages. The recovery of N ranged from 70% to 101% (mean, 86%). The greater loss of N than of OC may be due to leaching of soluble N-rich compounds.

The FPF and OF obtained by density fractionation were visually distinct. The FPF comprises recognizable plant material, whereas the intra-aggregate was amorphous dark material.

The concentrations of N for any given fraction were similar for all land use types. The concentration of OC was significantly higher in OF and FPF under Pinus plantation than in cropped land (Figure 12, Figure 13).

Comparison of OC and total concentration of N among density fractions revealed significantly lower values in OMF, as the latter was dominated by minerals. The highest total concentrations of N and OC were observed in the OF. With regard to the contribution of organic C of individual SOM fractions to TOC, most of the OC was associated with OMF, which accounted for 43% to 85% of the TOC, and the lowest portion was represented by OF, which ranged from 2% to 6% (Figure 12).

The relative distribution of total N among the fractions showed that most of the N was located in the OMF, which accounted for 43% to 88% of total N, and the lowest portion was represented by OF, which ranged from 1% to 3% of total N. Comparison of the N contribution of each fraction among different types of land use revealed that FPF and OMF were sensitive to land use change (Figure 13, %N/TN soil).

The OC/N ratio, for any given type of land use, was always lower in the OMF than in OF and FPF, but was only significant for soils under forest cover (Figure 12). There were no clear differences among types of land use, although for any given fraction the C/N ratio in soils under pine forest appeared to be higher than in the other soils.

7.3.3. Natural abundance of ^{13}C and ^{15}N in whole soils

Progressive enrichment of $\delta^{13}C$ was observed with depth in the soil profile for all types of land use, with increases ranging from 2‰ to 4‰ from topsoil to 30 cm depth (Table 16). The same pattern of $\delta^{15}N$ enrichment of SOM with depth in the soil profile was observed; increases in $\delta^{15}N$ ranged from 1.1‰ to 4.4‰ from topsoil to 30 cm depth (Table 16).

Comparison of $\delta^{13}C$ and $\delta^{15}N$ values among different types of land use revealed significant differences ($P < 0.05$) only for the topsoil. Topsoils under *Quercus ilex* cover displayed the lowest $\delta^{13}C$ and $\delta^{15}N$ values (Table 16, Figure 14), associated with the lower isotope signature of the corresponding litter (Table 17).

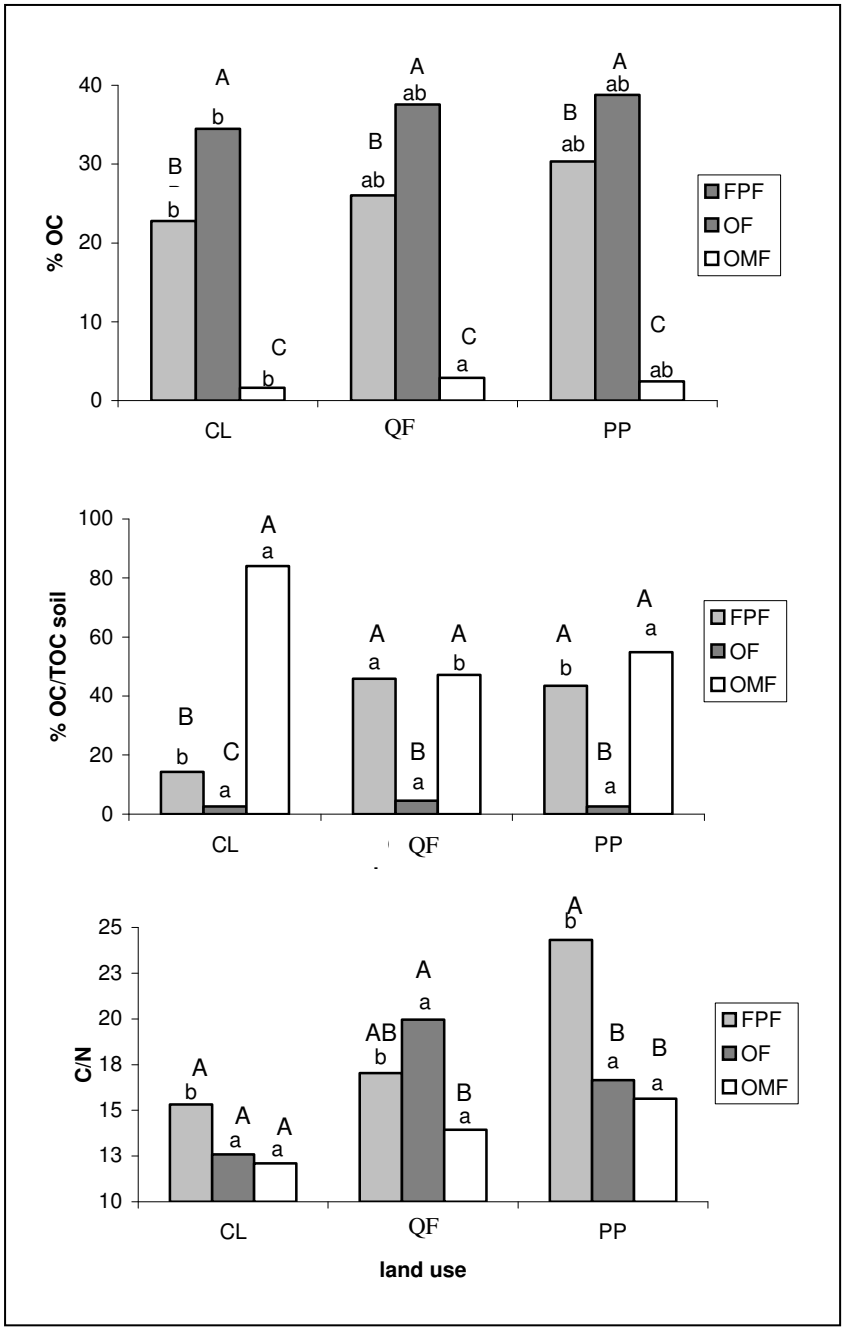

Figure 12

Average contents of OC, OC/ TOC in soil, and C/N ratio in density fractions for the different types of land use compared. Differences between means were tested by the Tukey procedure for multiple comparisons. Fractions indicated with the same capital letter are not significantly different ($p<0.05$) for a given land use (*Fractions* use interaction*). Land uses indicated with the same lower case letter are not significantly different ($p<0.05$) for a given fraction (*Use*fraction interaction*).

CL: Cropped land; QF: Native *Quercus* forest; PP: *Pinus* plantation; FPF: free particulate fraction; OF: occluded fraction; OMF: organo-mineral fraction.

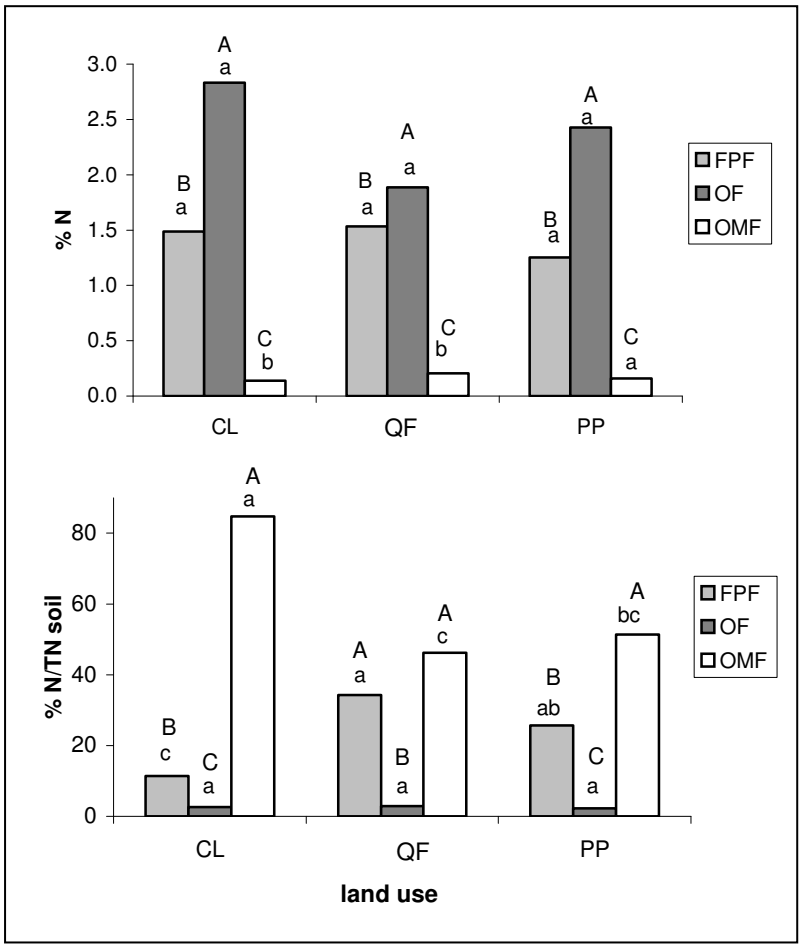

Figure 13

Average content of total N, and % N/TN in soil in density fractions of soils for the different types of land use compared. Differences between means were tested by the Tukey procedure for multiple comparisons. Fractions indicated with the same capital letter are not significantly different ($p<0.05$) for a given land use (*Fractions* use interaction*). Land uses indicated with the same lower case letter are not significantly different ($p<0.05$) for a given fraction (*Use*fraction interaction*).

CL: Cropped land; QF: Native *Quercus* forest; PP: *Pinus* plantation; FPF: free particulate fraction; OF: occluded fraction; OMF: organo-mineral fraction.

Table 16. $\delta^{13}C$ and $\delta^{15}N$ values in whole soil samples

	$\delta^{13}C$ (X ± s.d.)			$\delta^{15}N$ (X ± s.d.)		
Depth (cm)	0-10	10-20	20-30	0-10	10-20	20-30
CERRATO						
CL	-17.36 ±1.54	-17.59 ±1.35	-14.37 ±2.31	3.88 ±0.19	5.13 ±0.82	5.14 ±1.27
QF	-20.97 ±1.44	-16.39 ±1.12	-17.72 ±6.13	0.53 ±0.57	4.35 ±0.10	5.47 ±0.15
PP	-20.04 ±2.71	-18.17 ±1.91	-19.03 ±4.77	1.97 ±1.07	5.09 ±0.19	5.10 ±1.51
AMPUDIA						
CL	-17.23 ±1.58	-16.80 ±1.67	-12.56 ±0.34	4.60 ±0.22	5.13 ±0.29	6.55 ±0.13
QF	-25.71 ±0.75	-21.87 ±0.97	-18.12 ±0.94	2.84 ±0.32	5.65 ±0.36	6.63 ±0.21
PP	-16.19 ±1.92	-13.94 ±2.34	-12.32 ±2.14	3.18 ±0.92	5.75 ±0.57	6.01 ±0.84
MONTE VIEJO						
CL	-11.80 ±0.62	-13.86 ±4.65	-11.90 ±1.15	5.10 ±0.29	5.52 ±0.27	5.86 ±0.27
QF	-19.97 ±3.88	-17.19 ±2.79	-16.29 ±2.79	2.40 ±1.13	4.87 ±1.78	6.21 ±0.94
PP	-16.60 ±1.49	-14.53 ±0.62	-16.41 ±6.14	2.59 ±1.74	5.01 ±0.70	5.38 ±0.41
Analysis of variance						
Use		***			n.s.	
Depth		*			***	
Use*Depth		**			***	

X ± s.d.: Mean value and standard deviation for 4 samples.

CL: Cropped land; QF: Native *Quercus* forest; PP: *Pinus* plantation.

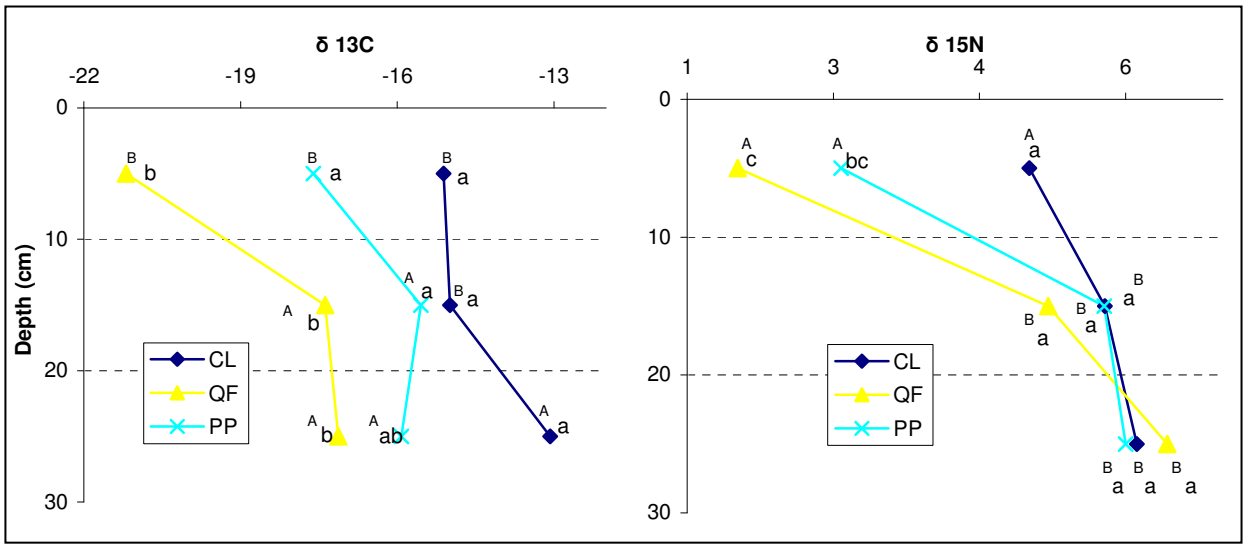

Figure 14

Average $\delta^{13}C$ and $\delta^{15}N$ in soils sampled under different types of land use and at different depths. Differences between means were tested by the Tukey procedure for multiple comparisons. Depth indicated with the same capital letter are not significantly different ($p<0.05$) for a given land use (*Depth*land use interaction*). Land uses indicated with the same lower case letter are not significantly different ($p<0.05$) for a given depth (*Land use*depth interaction*).

CL: Cropped land; QF: Native *Quercus* forest; PP: *Pinus* plantation.

The $\delta^{13}C$ values of *Quercus ilex* forest plant materials ranged from -26.2 to -26.8‰, i.e. slightly lower than the $\delta^{13}C$ values in *Pinus halepensis* plantation plant materials (-25.9‰) and cropped land plant materials (-4.6‰). The $\delta^{15}N$ values for plant material increased in the order Pinus (-4.3‰) < Quercus (-3.9‰ to –3.4‰) < crops (1.5‰) (Table 17).

Table 17. $\delta^{13}C$ and $\delta^{15}N$ values in plant material samples.

	$\delta^{13}C$ (X ± s.d.)	**$\delta^{15}N$ (X ± s.d.)**
CL	-24.56 ±1.47	1.50 ±1.09
QF	-26.78 ±0.55	-3.92 ±0.12
PP	-25.89 ±0.58	-4.28 ±1.01

X ± s.d.: Mean value and standard deviation for 4 samples.

CL: Cropped land; QF: Native *Quercus* forest ; PP: *Pinus* plantation.

7.3.4. Natural abundance of ^{13}C and ^{15}N in SOM fractions

The $\delta^{13}C$ values varied by up to 13‰ among density fractions. The OMF was enriched relative to OF and FPF. Significant differences ($P < 0.05$) were found in $\delta^{13}C$ values in the OMF, in the order: PP > CL > QF (Figure 15). The $\delta^{15}N$ values also displayed enrichment, of up to 4.9‰ among density fractions, of the heavy fraction in relation to OF and FPF (Figure 15). The $\delta^{15}N$ values in the FPF fraction differed significantly ($P<=0.05$) in the order: CL > QF > PP (Figure 15).

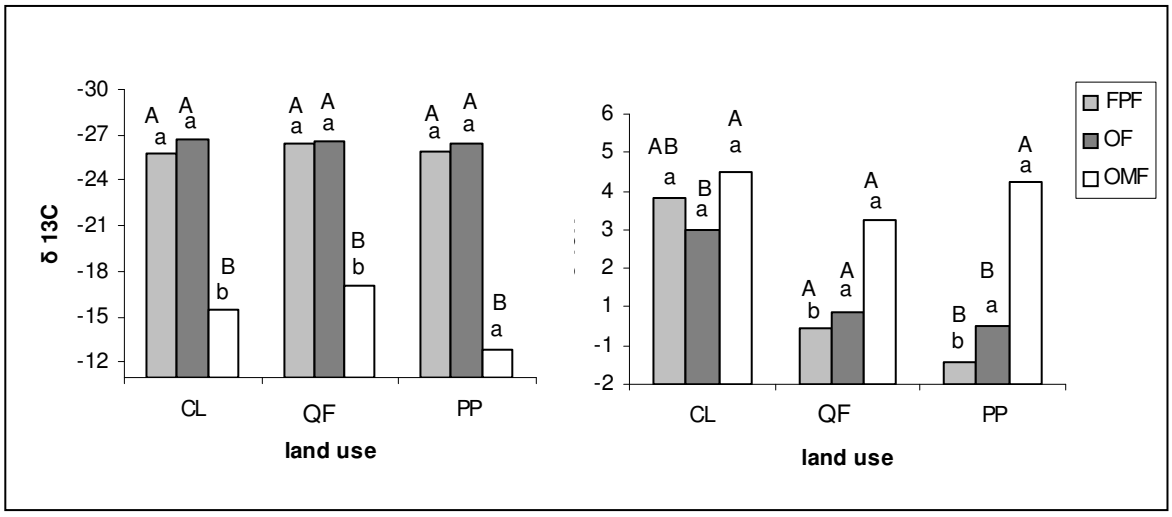

Figure 15

Average $\delta^{13}C$ and $\delta^{15}N$ in density fractions for the different types of land use compared. Differences between means were tested by the Tukey procedure for multiple comparisons. Fractions indicated with the same capital letter are not significantly different ($p<0.05$) for a given land use (*Fractions* use interaction*). Land uses indicated with the same lower case letter are not significantly different ($p<0.05$) for a given fraction (*Use*fraction interaction*).

CL: Cropped land; QF: Native *Quercus* forest; PP: *Pinus* plantation; FPF: free particulate fraction; OF: occluded fraction; OMF: organo-mineral fraction.

7.4. Discussion

7.4.1. C and N contents in whole soil

Land use change from a natural *Quercus ilex* forest to cropped land in the past 100 years, resulted in an average net loss of 47% of SOC and 41% of total N. This loss is consistent with the findings of Burke et al. (1989) who reported 50% SOC loss for land use transformation from grassland to cropped land in US soils, and those of other authors who reported that cultivation generally leads to decreases in SOM contents (e.g. Glaser et al. 2000; La Scala et al. 2008). Soil tillage induces soil C loss by acceleration of organic C oxidation, which results in the release of large amounts of CO_2 to the atmosphere (Ellert and Janzen 1999; Prior et al. 2000). Another tillage-related factor that contributes to soil C losses is soil aggregate disruption, which exposes once-protected organic matter to decomposition (De Gryze et al. 2006; Grandy and Robertson 2007).

The subsequent reforestation of the studied calcareous soils with *Pinus halepensis* results in recovery of 90% of the lost SOC and 70% of total N after 40 years of the plantation. This is probably due to the higher input of fresh organic matter at the soil surface under forest land and indicates potential storage of OC in the reforested soils.

7. 4.2. C and N distribution in SOM fractions

Comparison of OC and N concentration among density fractions revealed significantly lower values in OMF than in the other fractions, as the latter was dominated by minerals (Christensen 1992; Golchin et al. 1995). The highest concentrations of N and OC corresponded to the OF, in accordance with studies documenting a positive influence of aggregation on the accumulation of N and OC (e.g. Six et al. 2000). This accumulation is due to the physical protection of OM by aggregates attributed to compartmentalization of substrate and microbial mass (Six et al. 2002).

Regarding the contribution of different SOM fractions to soil TOC content, most of the OC was associated with OMF. The present results are similar to those reported by Baisden et al. (2002), who found about 69 – 86% of TOC in the mineral-associated fraction in Californian soils, and also similar to those reported by Golchin et al. (1995), who found that the heavy fraction accounted for about 30 – 90% of TOC in soils from temperate climates. OM was mainly located in the organo-mineral complex, resulting in physicochemical stabilization against further decomposition.

However, comparison of OC concentration (g C g^{-1} fraction) among density fractions revealed the lowest values in OMF, as the latter was dominated by minerals, as suggested by Golchin et al. (1995), and the highest values of OC concentration were found in OF. This high concentration has been suggested to be due to the physical protection of OM by aggregates attributed to compartmentalization of substrate and microbial mass (Killham et al. 1993).

Most of the N was located in the OMF, in accordance with the results reported by Billings (2006).

The OC/N ratio, for any given type of land use, was always lower in the OMF. These results, which indicate a higher degree of decomposition in mineral-associated SOM, are consistent with those reported by Rovira and Vallejo (2003) for soils over calcareous material and under *Quercus rotundifolia*. The higher values of the OC/N ratio for the soils under pine forest are consistent with the findings of different studies of litter composition, such as that by Traversa et al. (2008), who compared the C/N ratio of the litter under *Pinus halepensis* and *Quercus ilex.*

7.4.3. Natural abundance of ^{13}C and ^{15}N in whole soils

The utility of $\delta^{13}C$ of SOM is based on the systematic isotopic variation between C3 (trees, shrubs and cool season grasses) and C4 plants (warm season grasses). The $\delta^{13}C$ value of SOM in the upper soil profile (0-10 cm) for a given site is similar to that of the vegetation at the site (Bekele and Hudnall, 2003). Isotopic signals

from antecedent vegetation persist deeper in the soil profile (Boutton, 1996). Thus, changes in the relative proportions of C3 and C4 plants can be detected by a measured difference between the isotopic composition of the current plant community and that of the SOM at various depths. The studied calcareous soils are commonly mixed with gypsy outcrops that supports a shrub steppe vegetation dominated by *Salsola vermiculata*, *Kochia postrata* and *Camphorosma monspeliaca*, all of them Chenopodiaceae and, therefore, C4 plants. Over those calcareous soils plants of Crassulaceae family, C4 plants, are also presents nowadays (Jiménez et al. 2006) and probably were abundant in the past.

Progressive enrichment of $\delta^{13}C$ was observed with depth in the soil profile for all types of land use, with increases ranging from 2‰ to 4‰ from topsoil to 30 cm depth. As decomposition of fresh plant litter progresses and the decomposition products become incorporated into the soil profile, the ^{13}C isotopic signature normally increases, by about 2 – 4‰, with increasing soil depth (Nadelhoffer and Fry 1988, Handley and Scrimgeour 1997; Boutton et al. 1998; Buchmann et al. 1998; Glaser 2005); this - was also the case in the present study.

Hypotheses proposed to explain this pattern include: (1) changes in the abundance of atmospheric $\delta^{13}CO_2$ since the Industrial Revolution, (2) preferential feeding by microbes on isotopically light material, (3) metabolic fractionation during decomposition, (4) long-term changes in plant water-use efficiency, amongst others (Balesdent et al. 1993; Ehleringer et al. 2000).

The abundances of $\delta^{13}C$ and $\delta^{15}N$ in fresh plant litter were lower than in the bulk soil. These results are consistent with those reported by other authors, e.g. Nadelhoffer and Fry (1988), Accoe et al. (2003), and Bird et al. (2003). The results of a litter bag experiment led Connin et al. (2001) to suggest that mechanisms responsible for isotopic discrimination are, at least partly, characteristic of earlier decay stages. Recent studies suggest that soil microorganisms may alter the isotopic signature of SOM during decomposition, through mechanisms such as metabolic discrimination, selective consumption of substrates, and preferential use of intramolecular position within substrates (Schmidt and Gleixner 1998; Hobbie and Werner 2004). As decomposition of fresh plant litter progresses and the decomposition products become incorporated into the soil profile, the ^{13}C isotopic signature normally increases, by about 2 – 4‰, with increasing soil depth (Handley and Scrimgeour 1997; Boutton et al. 1998; Buchmann et al. 1998; Glaser 2005); this was also the case in the present study.

The same pattern of $\delta^{15}N$ enrichment of SOM with depth in the soil profile was observed. In whole soil, $\delta^{15}N$ is related to the degree of decomposition (Koba et al. 1998). The $\delta^{15}N$ profiles may reflect ^{15}N fractionation by microbial decomposers (Silfer et al. 1992) and increasing substrate age (Tiessen et al. 1984). However, patterns of $\delta^{15}N$ enrichment in soil profiles are not consistent across locations. Shearer et al. (1978) reported higher mean $\delta^{15}N$ values in soil samples collected near the surface than in those from underlying horizons, across a range of cultivated and undisturbed sites. Similar trends have been reported by Riga et al. (1970) for agricultural soils. In other cases, little variation in $\delta^{15}N$ with depth has been observed (Rennie et al. 1975).

7.4.4. Natural abundance of ^{13}C and ^{15}N in SOM fractions

It is important to note that any technique that requires repeated soil washing and decanting, such as the density fractionation method may result in loss of organic compounds (Gavinelli et al. 1995), with resulting impacts on the $\delta^{13}C$ and $\delta^{15}N$ signatures of the fractions. However, use of stable isotopes yielded some insight into the nature of density fractions.

The light fraction obtained by density fractionation primarily represents labile material of relatively recent origin, and we would expect this fraction to reflect ^{13}C and ^{15}N values similar to current vegetation (Compton and Boone 2000; Accoe et al. 2002).

The OMF was enriched in ^{13}C relative to OF and FPF in accordance with the findings of other authors (e.g. Six et al. 2001; Fernández et al. 2003; Crow et al. 2006). Average variations in $\delta^{13}C$ values of 10‰ between OMF and the other fractions were observed (Figure 15). These variations in $\delta^{13}C$ values may be explained by past input of plant material form different types of plant (i.e. C3 compared with C4). Generally, the heavy fraction, which represents older, more humified and amorphous organic compounds, is relatively enriched in $\delta^{13}C$ (Ehleringer et al. 2000). More labile C pools are isotopically lighter than less labile pools and are utilized first by the microbial community (Crow et al. 2006). However, microbial enrichment of ^{13}C never exceeds 4‰, so that a different origin of plant material is more likely for these very large differences, which suggests that C4 plant material predominated in the past throughout the study region.

Relative enrichment of $\delta^{15}N$ was also observed in the heavy fraction in relation to OF and FPF (Figure 15). The relative enrichment by $\delta^{15}N$ in the heavy fraction is consistent with the generally elevated $\delta^{15}N$ signatures observed in older SOM, which has been subjected to significant microbial processing (Billings et al. 2002). Liao et al. (2006) suggested that greater abundance of $\delta^{15}N$ in a fraction reflect a greater degree of humification. Kramer et al. (2003) found that $\delta^{15}N$ values increased in separated density fractions with increasing aliphaticity, which reflects microbial processing. Other researchers have shown that microbial processing results in accumulation of ^{15}N (e.g. Piccolo et al. 1996).

7.5. Conclusions

Historical transformation of Quercus forest to cropped lands in calcareous soils resulted in major losses of OC and N, as expected. However, subsequent reforestation with Pinus throughout the past 40 years has resulted in good recovery of the original SOC and soil N. This shows a major potential for enhanced soil C storage in agricultural soils by their reversion to a forested state. The present results further reveal that in calcareous soils, accumulation of OC and N is mainly due to organo-mineral associations, resulting in physicochemical stabilization against further decomposition, which guarantees long-term C sequestration. The calcareous soils under study showed typical enrichment in ^{13}C and ^{15}N with depth, which is explained by isotopic fractionation during decomposition in relation to the increasing age of the SOM with depth. The

same applies to the enrichment of ^{13}C and ^{15}N in OMF, which corresponds to a more humified OM. However, the results of the study revealed the possible past presence of C4 type vegetation in the study area.

7.6.Acknowledgements

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8. EFFECT OF LAND USE CHANGE ON CONTENTS AND DISTRIBUTION OF CARBOHYDRATES WHITIN DENSITY FRACTIONS OF CALCAREOUS SOIL

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Enviado a Science of the Total Environment

Abstract

In the last century, many calcareous soils in Castilla León (northwestern Spain) have been transformed from natural *Quercus ilex* forest to cropped land. During the past 40 years, after crop abandon, reforestation with *Pinus halepensis* has been taking place on this soils. In order to evaluate the effect of those land use changes in the soil organic matter content and properties, we analyzed and compared soil organic C content (OC), carbohydrate content, monosaccharide composition and microbial biomass C in whole soil and density fractions. About 67% of the total C in the topsoil was lost as a result of converting the natural *Quercus ilex* forest to cropped land, 100 years ago. After crop abandon, the stock of OC in the topsoil greatly increased upon reforestation of the studied plot with *Pinus halepensis*. An average recovery of 71% of the previously lost OC had been recovered, after 40 years of pine plantation. Soil carbohydrates accounted for 6 to 10 % of the total organic C of soils. Cultivation also affected the proportion of soil C present as carbohydrates. Monosaccharide analysis revealed significant differences in carbohydrate composition between land uses. Significant differences in monosaccharide composition were also found among density fractions however, the proportion of the organic C represented by monosaccharide C among fractions was constant. Whatever the fraction and land use considered, glucose was the dominant sugar monomer, followed by mannose and xylose. Stands out the positive and significant correlation between monosaccharide C content in samples and OC, C/N ratio, microbial biomass C, and microbial biomass C/N ratio.

Keywords: land use change; soil density fractions; neutral sugars

8.1. Introduction

Soil organic matter is recognised as an important factor in C driven climate change (Sanderman et al. 2003). Because of this, the capacity of soils to accumulate organic C has received great attention in recent years. Land use and management influence the amount and the dynamics of SOM (Zhang et al., 1999). Numerous studies have shown that conversion of native ecosystems to agriculture disturbs the ecological balance, disrupt the C cycle and generally results in net loss of soil C (e.g., Paustian et al., 1997; Llorente and Turrión, 2010). However, when arable land is converted to permanent vegetation the soil organic carbon (OC)

increases gradually (Haynes and Beare, 1996). Studies of the effects of land use change on soil OC in calcareous soils are scarce. However, calcareous soils are of particular interest because they represent approximately 12% of the world soil resources (FAO 1996). Some studies of the decomposition dynamics of SOM in calcareous soils (García et al., 1997; Llorente et al., 2010; Llorente and Turrión, 2010) have shown that the decomposition rates in such soils are lower than those in other soils with similar organic carbon content.

In the last century, many calcareous soils in Castilla y León (northwestern Spain) were transformed from natural *Quercus ilex* forest (QF) to cropped land (CL). Latter, reforestation to *Pinus halepensis* plantation (PP) has been taking place during the past 40 years.

Changes in management not only influence SOC quantity but may also affect its quality (Feller and Beare, 1997; Glaser et al., 2000; Turrión et al., 2000). Little is know for calcareous soil about the changes in SOM quality with increasing and decreasing organic C levels.

Of the various components of the soil organic matter, carbohydrates have an important role on nutrient cycling (Cheshire, 1979; Hu et al., 1997). Carbohydrates represent from 5 to 25% of the SOM (Lowe, 1978) and occur in a variety of forms, as undecomposed plant debris and faunal remains or as products of chemical and biochemical decomposition and synthesis.

Carbohydrates in soil are subject to have a rapid turnover and are involved in biochemical and organo-mineral reactions. They represent a main source of nutrients and energy for soil micro-organisms and contribute to microbial activity and, therefore, soil carbohydrates may be a sensitive indicator of several biological properties. Carbohydrates and monosaccharide (MS) also play a role in enhancing soil structural stability because carbohydrates are thought to be of particular importance in promoting aggregate stability (Feller and Beare, 1997).

Physical fractionation methods enable the study of SOM fractions differing in dynamics, structure and function (e.g., Six et al., 2001; Sohi et al., 2001). Soil organic matter density fractionation following ultrasonic dispersion enables separation and study of three different fractions according to the different mechanisms of physical protection (Sohi et al., 2001). Based on these stabilization mechanisms, it is possible to distinguish three SOM fractions: (1) “free” OM (free fraction, FF), isolated before ultrasonic break-down of stable aggregates; (2) OM occluded within aggregates (occluded fraction, OF), isolated after ultrasonic dispersion to break the aggregates; and 3) organo-mineral fraction (dense fraction, DF) recovered as the residual (heavy) material. Many studies (e.g. Golchin et al., 1994b; Six et al., 2002; John et al., 2005) have shown that the unprotected FF represents a labile SOM pool with a rapid turnover rate. In contrast OF and DF are more stable pools, with turnover times ranging from decades to centuries. Therefore, density fractions may be affected differently by changes in land use.

Density fractionation technique, applied to monosaccharide measurements, may provide a useful tool to investigate early changes in SOM quality caused by management practices.

In this study, carried out with soils from a calcareous moor in the region of *Castilla y León* (northwestern Spain), we studied 36 soils, taken from plots under contrasting land uses: native *Quercus ilex* stands, cereal

crops, and *Pinus halepensis* plantations. Density separation was carried out in combination (or not) with ultrasonic dispersion in order to obtain SOM fractions according to different mechanisms of physical stabilization.

The objectives of the present study were to assess and compare land use effects on (i) soil C content (ii) distribution of density fraction associated C; (iii) amount and nature of monosaccharide in soils; (iv) the amounts and nature of monosaccharides in the density fractions; (v) the relationship among soil organic matter, microbial biomass, and monosaccharide content in soils.

8.2. Material and methods

8.2.1. Site description

The study was carried out in a calcareous moor in the region of *Castilla y León* (northwestern Spain), UTM: 30T 384465 E 4639001 N. The mean annual rainfall in the region is around 400 mm, under a xeric moisture regime, and the mean annual temperature is approximately 12.3^{o}C. The altitude of the moor is between 800 and 900 m above sea level, with low slopes (< 7%). The soils (Xerepts, according to Soil Survey Staff, 2006) are quite homogeneous in spite of differences in their land use history. The native vegetation in the studied calcareous soils is Holm-oak wood (*Quercus ilex* subsp *ballota*). In the 19th century, most of the natural forest was converted into agricultural land (cereal crops), but since the 1950s, reforestation with *Pinus halepensis* has been carried out on abandoned agricultural land.

The main characteristics of the samples are shown in Table 18.

Table 18. Physicochemical properties of the soil samples

CODE	LAND USE	T.C.	TEXTURE	B.D. (g ml^{-1})	$CaCO_3$ (%)	pH
CL	Cropped land	No	Clay loam	1.25 ±0.17	19.9 ±8.8	8.13 ±0.01
QF	*Quercusilex* forest	Yes	Clay loam	1.32 ±0.13	20.9 ±4.6	7.91 ±0.04
PP	*Pinus halepesis* plantation	Yes	Clay loam	1.06 ±0.20	18.4 ±1.8	7.91 ±0.04

T.C.: Tree cover; B.D.: bulk density; Means and standard error for n= 3.

8.2.2. Sampling procedures

A land use map of the calcareous moor of Castilla y León was elaborated with a GIS (ArcGis 9.0 for Windows) (Figure 9). The map was used to select the sampling plots on the basis of the following criteria: a) *Quercus ilex* forest, cropped land, and *Pinus halepensis* plantations in adjacent areas; b) minimum area of each land use, 1 ha; and c) establishment of each land use for at least 40 years. For this study, plots of the above-mentioned land uses were selected in three different regions of the calcareous moor, and four representative plots were sampled (0-10 cm depth). A total of 36 topsoils (0-10 cm depth) (3 regions x 3 land uses x 4 plots) were thus sampled. A composite sample, by joining 3 soil subsamples, was obtained for each plot. Visible plant residues and roots were removed; soil was air-dried, sieved (< 2 mm) and stored in plastic bags until analysis.

8.2.3. Density fractionation of soil

A density fractionation procedure was applied to the topsoils (0-10 cm depth) of the 36 sampled plots. A flow diagram of the method used is provided in Figure 10. The method follows the concept of Golchin et al. (1994a), who differentiated three degrees of physical protection of OM: FF, non-protected; OF, occluded within aggregates - extractable by sonication; and DF, retained in the dense residual material after sonication.

Briefly, 5 g of soil sample were placed in small centrifuge bottles (50 ml capacity), and 35 ml of NaI at 1.8 g ml^{-1} density were added. The bottles were shaken gently and the floating material, considered as the FF, was then recovered by centrifugation at 8000 g for 30 min at 18 ^{o}C, and filtered over a vacuum filter, using a glass fiber filter (Whatman GF/F), by washing with deionized water. The recovered NaI (not mixed with the washing water) was added to the residue remaining in the bottle and the solution was fitted to 1.8 g ml^{-1} density. The bottle was placed in an ice bath and sonicated at 300 J ml^{-1} with a probe-type ultrasonic disintegrator (Branson 450 W).

The floating material, considered as the OF, was recovered by centrifugation and washed in the same way as the FF. The remaining material, considered the DF, was washed with deionized water. All fractions were dried at 40 ^{o}C, weighed, ground in a mortar and pestle, and analyzed for C and N. Carbonates were analyzed for the whole soils and it was assumed that all carbonates were recovered in the DF. To obtain enough sample to perform analysis, it was necessary to accumulate several replicates of the fractions.

8.2.4. Carbon and nitrogen determination

For soil characterization, % of carbonates, texture, pH, total N, total C, organic C, and C/N were determined. Total concentrations of soil C and N were determined with an automated C/N analyzer (CHN-2000, Leco). Organic carbon was calculated as the difference between total and carbonate carbon. Soil total calcium carbonates were determined by use of 1M HCl titrated with 0.5M NaOH (FAO, 2007).

8.2.5. Microbial biomass C and N determination

For microbial biomass C and N (MBC and MBN, respectively) determination chloroform fumigation-extraction method was used (Brookes et al., 1985).

Previous to the extraction of the microbial biomass, each air dried soil (< 2 mm) was incubated at 70% field capacity at room temperature (~ 25^{o}C) for 1 week. After incubation, triplicate subsamples of each soil were fumigated for 24 h in a vacuum dessicator with ethanol-free $CHCl_3$ in the dark. Following fumigation, each soil (triplicate subsamples) was extracted by shaking 15 g with 60 ml of 0.5 M K_2SO_4 for 30 min on a rotary shaker and filtered through Whatman no. 42 filter paper. As well, triplicate subsamples of unfumigated control soils were extracted in the same way. Organic C and total N were measured in aliquots of the K_2SO_4 extracts of fumigated and unfumigated subsamples by a Skalar Formacs TOC/TN combustion analyzer for liquid samples.

The MBC was calculated using the equation: Biomass C = K_{Ec}*E_c, where E_c is the difference between organic C from fumigated soil and organic C from unfumigated soil (Vance et al., 1987) and K_{Ec} is the extractable part of microbial biomass C after fumigation. We applied a factor 2.64 as recommended by Joergensen (1996).

The MBN was calculated using the equation: Biomass N= K_{EN}*E_N, where E_N is the difference between total N from fumigated soil and total N from unfumigated soil and K_{EN} is the extractable part of microbial biomass N after fumigation. We applied a factor 1.85 as is recommended by Brookes et al. (1985).

8.2.6. Monosaccharide determinations

Carbohydrate concentration and composition was determined by the analysis of sugar monomers released by acid hydrolysis. Samples of 500 mg of soil, spiked with 100 µl internal standard solution (containing 80 µl myo-inositol) (Neeser and Schweizer, 1984), were hydrolysed with 10 ml of 4 M trifluoroacetic acid in closed 25 ml hydrolysis flasks at 105 ^{o}C for 4 h (Guggenberger et al., 1994). Following filtration through glass fibre filters (GF 6, Whatman) the samples from hydrolyses were dried using a rotatory evaporator. Saccharides were re-dissolved using 5 x 5 ml deionised water and dissolved humic-like substances were removed passing the solution through XAD-7 adsorption resin (conditioned and purified with 1 M NaOH, deionised water, isopropanol, deionised water, 0.1 M NaOH, deionised water, 0.1 M HCl, deionised water). A second purification step was performed by dropping the solution through a column (1 x 20 cm) filled with 4 g of cation exchange resin Dower 50W X 8 (conditioned and purified with 10 ml 2 M NaOH, deionised water, 10 ml 2 M HCl, deionised water until pH approached neutrality).

After freeze dried, we re-dissolved samples with 200 µl of a solution containing 100µg ml-1 3-O-methylglucose (as second internal standard) in N-methyl-pyrrolidone, then added 200µl O-methylhydroxylaminehydrochloride. The solution was heated to 75 ^{o}C for 30 min and subsequent cooled at

room temperature. Final derivatisation was accomplished with 400 µl bis-(trimethylsilyl)-trifluoroacetamide at 75 ^{o}C for 5 min.

Gas chromatography was performed on a Hewlett Packard 6890 gas chromatograph equipped with a flame ionisation detector. A capillary column, HP-5 (30 m x 0.32 mm x 0.25 µm film) was used with nitrogen as the carrier gas. The operating conditions were: injection temperature 300 ^{o}C, detection temperature 300 ^{o}C column temperature programmed from 100 ^{o}C to 250 ^{o}C at a rate of 20 ^{o}C min^{-1}and from 250 ^{o}C to 300 ^{o}C at a rate of 10 ^{o}C min^{-1}. The split ratio was 50:1.

The identity of each sugar peak in the chromatograms was determined by comparing the retention times observed for standard solutions with that of the peaks observed in the chromatograms. The relative concentration of the sugar was calculated by means of response factors relative to that of a myo-inositol internal standard added to each sample before derivatization.

The carbon content of each monosaccharide was calculated from its chemical formula and ranged from 37.1% to 43.9%.

Cellular plant polysacharides are characterized by high proportions of the pentose sugars - arabinose, ribosa and xylose-, whereas microbial populations synthesize dominantly the hexoses - galactose, glucose, fructose, and mannose- (Moers et al., 1990). The relative proportion of carbohydrates from plant and microbially-derived carbohydrates can be estimated by calculation of simple molar ratios of the sugars monomers. Oades (1984) stated that the ratio of galactose + mannose / arabinose + xylose [(G + M)/(A + X)] would be low (< 0.5) for plant-derived carbohydrates and high (> 2.0) for microbial polysaccharides.

8.2.7. Statistical analyses

Analysis of variance (ANOVA) was used: a) to compare the yields, C and N contents, and C/N ratio, of the fractions, overall or for a given land use type, and b) to compare different land use for a given fraction. In case of significant F-statistics ($p > 0.05$), differences between means were tested with the Tukey procedure for multiple comparisons. Data were tested for normality and homoscedasticity with the Kolmogorov-Smirnov and Levene´s statistics respectively.

The statistical analyses were performed with the Systat 14.0 Statistical Software Package (SPSS for Windows).

8.3. Results

8.3.1. Effect of land use on C storage, OC/N ratio, microbial biomass C and microbial biomass C/N ratio in whole soils

The organic C varied with the land use. The cropped land displayed the lowest content of organic C (Table 19). Percentage of OC in topsoil (from 0 to10 cm depth), under a land use change from a natural Quercus ilex

forest to cropped land (100 years ago) showed an average net drop of 67%. The subsequent reforestation of the studied calcareous soils with Pinus halepensis resulted in a great recovery of OC. An average OC recovery of 71% of the OC (referring to % of OC in soils under Quercus ilex forest) was measured in topsoil (from 0 to10 cm depth) after 40 years of pine plantation.

The sugar-C constituted from 6.6 to 10.2% of total organic C content of soils with the highest values in soils under the *Quercus ilex* forest (Table 20), however no significant differences among land use types were found for this variable.

As regards to[(G + M)/(A + X)] ratio, significant differences ($p < 0.05$) were found among land use types the lowest values in soils under *Quercus ilex* forest (Table 20).

Table 19. OC, OC/N ratio, microbial biomass C (MBC), and MBC/MBN ratio in whole topsoils (0-10 cm).

	MSC	MSC/OC	(G+M) / (A+X)
	g C kg^{-1}	%	
CL	1.34	6.58	1.21
QF	5.70	10.25	1.08
PP	3.63	8.63	1.42
Analysis of variance			
	**	n.s.	*

CL: Cropped land; QF: Native *Quercusilex* forest; PP: *Pinushalepensis* plantation. For ANOVA analysis, values of p>0.05: n.s., p<0.05:*, p<0.01:** and p<0.001:*** are reported.

Table 20. Monosaccharide C (MSC), % of OC represented by monosaccharide C (MSC/OC), and the ratio of galactose + mannose / arabinose + xylose (G+M) / (A+X) in whole topsoils (0-10 cm).

	OC	OC/N	MBC	MBC/MBN
	%		μg C g^{-1}	
CL	1.87	11.73	603.42	94.00
QF	5.60	12.42	1182.00	103.56
PP	4.18	14.30	642.09	77.21
Analysis of variance				
	**	n.s.	**	**

CL: Cropped land; QF: Native *Quercus ilex* forest; PP: *Pinushalepensis* plantation. For ANOVA analysis, values of p>0.05: n.s., p<0.05:*, p<0.01:** and p<0.001:*** are reported.

8.3.2. Effect of land use on monosaccharide C content and carbohydrate nature in whole soils

Monosaccharide (MS) content in whole soils varied from 1.3 to 5.7 g C kg^{-1} (Table 20). Cultivated soil showed lower MS contents than forested soils. Significant differences ($p < 0.01$) were found among soils under different land use type in the following order: QF >PP> CL.

8.3.3. Monosaccharide composition of whole soils

Results of the analyses of neutral sugars in whole soils are listed in Table 21.

Among the monosaccharides that were identified in the whole soils, glucose was always dominant. For any given sugar, except for glucuronic acid, differences in abundance among land use types were significant, with higher contents in soils under *Quercus ilex* forest and lower in cultivated soils.

Table 21. Monosaccharide composition of whole soils

	Xylosa	Arabinose	Ribose	Rhamnose	Fucose	Fructose	Mannose	Galactose	Glucose	Glucuronic acid	Galacturonic acid
						mg C kg^{-1}					
CL	172	193	15	73	35	6	227	214	568	5	21
QF	498	535	123	272	117	27	569	547	2752	12	213
PP	265	353	62	186	101	19	470	426	1656	15	79
Analysis of variance											
Use	**	**	*	*	*	**	*	*	**	n.s.	**

CL: Cropped land; QF: Native *Quercusilex* forest; PP: *Pinushalepensis* plantation. For ANOVA analysis, values of p>0.05: n.s., p<0.05:*, p<0.01:** and p<0.001:*** are reported.

As regard to relative abundance of each sugar to total sugar contents, glucose was also dominant represents from 42.1 to 48.3% of the total sugar (Figure 16).

Only xylose, galactose and manose shown significant differences ($p < 0.05$) among land use types. Relative contribution of xylose was significantly ($p < 0.05$) higher in cultivated soils and lower in soils under *Quercus ilex* (CL > PP> QF). In contrast, relative contribution of hexoses (galactose and manose) to total sugar contents was lower for soils under *Quecus ilex* cover than in soils under other land use type.

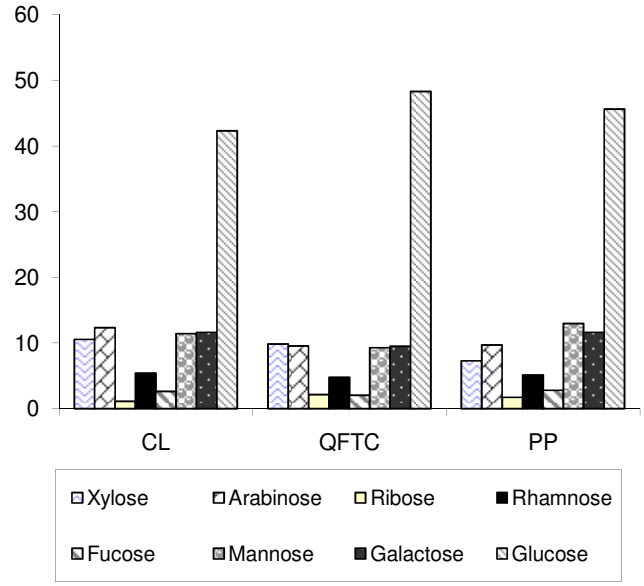

Figure 16

Monosaccharides relative contribution to total sugar content in whole soils. CL: Cropped land; QF: Native *Quercus ilex* forest; PP: *Pinus halepensis* plantation

8.3.4. Land use effect on organic carbon content and OC/N ratio in density fractions

The free and intra-aggregate fractions obtained by density fractionation were visually distinct. The FF comprised recognizable plant material, whereas the intra-aggregate was amorphous dark material. The recovery of fractions with respect to the initial soil weight varied between 98 and 103% (Table 19). Recoveries higher than 100% may be due to incomplete removal of NaI.

As expected, most of the fine soil mass was located in the DF (Table 20), and OF represented a minor part of the soil mass. The yields of all the density fractions were significantly different among land uses, with a significantly greater amount of FF in soils under tree cover than in the soils under agricultural land use, due to greater incorporation of organic matter.

Comparison of OC concentration (g C g^{-1} fraction) among density fractions revealed the lowest values in DF, as the latter was dominated by minerals, and the highest values in OF (Table 21). Cropped land presented the lowest OC concentration overall fractions. As regards the % of OC in soil that is represented by each fraction, the major portion of OC was associated with DF (Table 21). The DF accounted for about 83% of TOC under

cropped land, but for about 53% of TOC in topsoils (0-10 cm depth) under tree cover, with no-significant differences among soils under *Pinus halepensis* or *Quercus ilex* vegetation.

The recovery of organic C after fractionation ranged from 97% to 107%, with an average of 101% (Table 19). These recoveries are too much high, taking into account that the repeated soil washing and decanting is expected to result in losses of soluble organic matter, especially if an ultrasonic treatment is applied. Such high recoveries may be due to organic impurities contained in the NaI solution, and/or to the possible presence in OF and/or FF of small amounts of carbonates, whose carbon could have been taken as organic C. Owing to all these constraints, the data for C cannot be taken as precise estimations. (Table 22).

The OC/N ratio was significantly higher ($p < 0.05$) in FF than in the DF. Comparison of the OC/N ratio in FF among the different types of land use, revealed significantly higher values ($p < 0.01$) in soils under pine forest (Table 22).

Table 22. OC, % OC of total OC of the soil, and OC/N ratio in density fractions

Land use	OC (g C g^{-1}fraction)			%OC (of total OC of soil)				OC/N		
	FF	OF	DF	FF	OF	DF	Rec.(%)	FF	OF	DF
CL	0.23	0.34	0.02	14.34	2.65	83.34	100.33	15.32	12.59	12.09
QF	0.27	0.37	0.03	46.94	4.56	50.13	101.97	17.36	19.96	13.95
PP	0.30	0.39	0.02	43.46	2.62	55.31	101.43	24.32	16.65	14.41
Analysis of variance										
Use		n.s.			n.s.				n.s.	
Fraction		***			***				*	
Use*fraction		***			***				*	

CL: Cropped land; QF: Native *Quercus ilex forest*; PP: *Pinus halepensis* plantation; FF: free fraction; OF: occluded fraction; DF: organo-mineral fraction; Rec.: recovery. For ANOVA analysis, values of $p>0.05$: n.s., $p<0.05$:*, $p<0.01$:** and $p<0.001$:*** are reported

8.3.5. Land use effect on the monosaccharide C content and on the C/N ratio in density fractions

Monosaccharide C (MSC) associated with DF was generally depleted in MS, while MSC associated with FF and OF contained high concentrations of MS without significant differences ($p < 0.05$) between FF and OF (Table 23).

For FF and OF the effect of land use change in MS concentration was significant ($p < 0.001$). In these fractions, cultivated soils were depleted in MS respect to forested soils.

However, the proportion of the OC represented by MSC was no significantly different ($p < 0.05$) among fractions and neither among land uses.

Table 23. Monosaccharide C content (MSC) and % of OC represented by MSC in density fractions

	MSC			MSC/OC		
	---------- (g C kg^{-1}) ----------			----------(%) ----------		
	FF	OF	DF	FF	OF	DF
CL	6.12	6.04	0.61	2.69	3.09	3.22
QF	11.72	11.58	1.90	4.38	3.08	3.17
PP	18.09	13.79	1.43	5.67	3.53	5.89
Analysis of variance						
Use		n.s.			n.s.	
Fraction		***			n.s.	
Use*fraction		***			n.s.	

CL: Cropped land; QF: Native *Quercus ilex* forest; PP: *Pinus halepensis* plantation; FF: free fraction; OF: occluded fraction; DF: organo-mineral fraction. For ANOVA analysis, values of $p>0.05$: n.s., $p<0.05$:*, $p<0.01$:** and $p<0.001$:*** are reported.

8.3.6. Monosaccharide composition and carbohydrate nature of density fractions

Results of the analyses of neutral sugars in density fractions are listed in Table 24.

Although for all density fractions glucose was the most abundant MS, sugar composition differed significantly between density fractions. For any given sugar concentration was significantly lower in DF, without significant differences detected between FF and OF.

Comparing the effect of land use type in MS composition of each density fraction, greater differences were found in FF. In soils under cultivation FF was depleted in arabinose, fructose and galactose. In soils under pinus plantation FF was enriched in fructose and glucose. Occluded fraction appears not to be affected for land use change in abundance or relative contribution of sugar.

As regards to DF, in cultivated soils xylose appeared to have higher relative contribution to total sugar than in other land uses types.

The relative abundance of glucose show the highest variations in the FF. Glucose varied from 29.9 to 48.1% of the total sugar content of FF (Figure 17), with the highest values in soils under *Pinus* plantations. In comparison to the other density fraction, in FF underlines the high relative contribution of mannose, which represents from 17.7 to 27.1% of the total sugars content of the fraction. The free fraction presents significant less relative abundance of rhamnose than the other fractions.

In OF, glucose represented from 42.8 to 56.1% of the total sugar content, also with the higher values in soils under *Pinus halepensis* plantation.

Table 24. Monosaccharide composition of fractions

		Xylose	Arabinose	Ribose	Rhamnose	Fucose	Fructose	Mannose	Galactose	Glucose	Glucuronic acid	Galacturonic acid
		(mg C kg^{-1})										
CL	FF	609	287	23	203	37	44	1226	610	2385	551	104
	OF	802	407	58	73	197	37	1320	626	2406	63	51
	DF	40	105	12	33	19	8	86	92	212	0	0
QF	FF	1547	1249	174	225	666	89	3578	1004	3362	137	148
	OF	1107	1053	11	578	154	84	1084	1107	6083	40	274
	DF	34	81	10	29	16	7	70	65	205	0	12
PP	FF	717	1984	151	283	156	107	2056	1199	5915	236	365
	OF	1017	834	20	729	200	131	1955	1466	8323	11	156
	DF	63	189	14	81	42	20	182	176	585	2	13
Analysis of variance												
Use		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Fraction		***	***	***	***	n.s.	***	**	***	***	*	**
Fraction*land use		*	**	***	n.s.	n.s.	***	*	**	**	n.s.	**

CL: Cropped land; QF: Native *Quercus ilex* forest; PP: *Pinushalepensis* plantation; FF: free fraction; OF: occluded fraction; DF: organo-mineral fraction. For ANOVA analysis, values of $p>0.05$: n.s., $p<0.05$:*, $p<0.01$:** and $p<0.001$:*** are reported

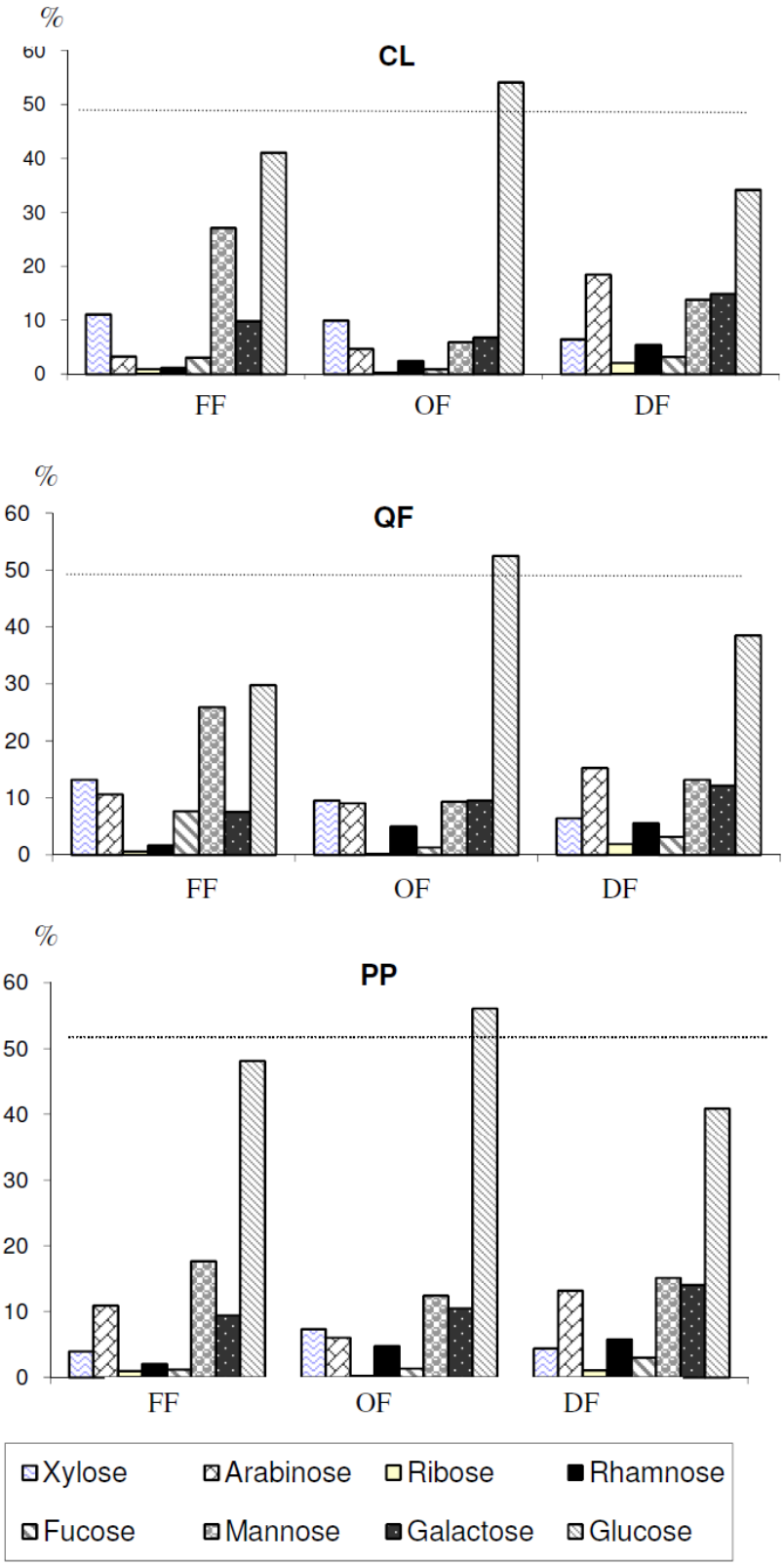

Figure 17

Monosaccharides relative contribution to total sugar content in density fractions. CL: Cropped land; QF: Native *Quercus ilex* forest; PP: *Pinus halepensis* plantation; WS: whole soil; FF: free fraction; OF: occluded fraction; DF: organo-mineral fraction

DF showed values of glucose from 34.2 to 44.2% of the total sugar content. Compared to the other density fractions, DF was significantly ($p < 0.05$) enriched in arabinose and galactose and depleted in the relative contribution of xylose.

As regards to [(G + M)/ (A + X)] sugar ratio, not significant differences ($p < 0.05$) were found among density fractions. However, significant differences were detected among land uses with higher values in all the fractions corresponding with soils under *Pinus halepensis* plantation (Table 25).

Correlation coefficients of the leading variables are reported in Table 26.

Stands out the positive and significant correlation between MSC content in samples and OC, C/N ratio, MBC, and MB C/N ratio. Also stands out the absence of significant correlation of [(G + M)/(A + X)] sugar ratio and the others variables.

Table 25. Galactose plus mannose to arabinose plus xylose ratio in density fractions

	(G+M) / (A+X)		
	FF	OF	DF
CL	1.25	1.61	1.14
QF	1.63	1.01	1.17
PP	1.25	1.85	1.22
Analysis of variance			
Use		**	
Fraction		n.s.	
Use*fraction		*	

CL: Cropped land; QF: Native *Quercus ilex* forest; PP: *Pinus halepensis* plantation; FF: free fraction; OF: occluded fraction; DF: organo-mineral fraction. For ANOVA analysis, values of $p>0.05$: n.s., $p<0.05$:*, $p<0.01$:** and $p<0.001$:*** are reported

Table 26. Correlation coefficients between OC, C/N ratio, microbial biomass C (MBC), microbial biomass C/N ratio (MBC/MBN), monosaccharide C, and (G+M)/(A+X)

Variables	OC	C/N	MBC	MBC/MBN	MS C
C/N	0.543***				
MBC	0.718**	-0.249			
MBC/MBN	0.745**	-0.069	0.696**		
MS C	0.732**	0.395*	0.681**	0.789**	
G+M/A+X	-0.105	0.400	-0.276	-0.440	0.235

The asterisks *, **, and *** indicate $P < 0.05$, 0.01, and 0.001, respectively

8.4. Discussion

8.4.1. Effect of land use on C storage, C/N ratio, microbial biomass C and microbial biomass C/N ratio in whole soils

Land use change from a natural *Quercus ilex* forest to cropped land (100 years ago) has resulted in an average net drop of 67% in the % of OC in the topsoil (from 0 to 10 cm depth). These results are consistent with results reported by Llorente et al. (2010) for those calcareous soils. Such loss is also consistent with the findings of Burke et al. (1989), who reported 50% of SOC loss for land use transformation from grassland to crop land, and the findings of other studies (Prior et al., 2000) showing that cultivation generally decreases the amount of organic matter. Soil tillage induces soil C loss by acceleration of organic C oxidation, which results in the release of large amounts of CO_2 to the atmosphere (La Scala et al., 2008). Another tillage-related factor that contributes to soil C losses is disruption of the soil aggregates, which exposes once-protected organic matter to decomposition (De Gryze et al., 2006; Grandy and Robertson, 2007).

The soils under Quercus forest presented the highest MBC and MBC/MBN values. This is according to many studies of SOC and land use change that use measurements of MBC as early indicator of changes in SOM (e.g., Powlson and Brookes 1987; García-Gil et al. 2000; Palma et al. 2000). Microbial biomass usually declines when soils under forest and grassland vegetation are brought under cultivation (Srivatava and Singh 1991).

8.4.2. Effect of land use on monosaccharide C content and carbohydrate nature in whole soils

Soils under tree forest (*Quercus* or *Pinus*) showed significantly higher monosaccharide concentration than soils under cultivation. Several studies documented the impact of land-use changes on soil carbohydrates (e.g., Sanger et al., 1997; Trouvé et al., 1996; Turrión et al., 2002). Soil carbohydrate levels appear to depend largely on OC content of soils (r^2: 0.732, Table 26), hence those factor which influence inputs of organic matter and rates of decomposition, will also be those controlling total carbohydrate levels in soil. Decreasing amounts of labile substances are usually observed after cultivation of native soils with little return of plant debris to soil (Christensen, 1992).

The % of OC represented by MS presented the highest values in soils under *Quercus ilex* forest. Forest clearing and cultivation induced a rapid decrease in total soil organic C, carbohydrate content and sugar-C to total-C ratio (Hu et al., 1997).

The studied soils showed (G+M) / (A+X) ratio from 0.97 to 1.41. Polysaccharides of microbial origin normally have a (G+M) / (A+X) ratio of > 2.0, while plant polyssacharides typically have a low ratio (< 0.5) (Oades, 1984). The studied soils showed the lowest values of (G+M) / (A+X) ratio under *Quercus* forest and the highest ones in soils under the *Pinus* forest.

Low [(G + M)/(A + X)] ratio for the soils under *Quercus* forest indicated the dominating contribution of plant derived carbohydrates. In contrast higher ratios found in soils under *Pinus* suggested an increasing proportion of microbially-synthesized carbohydrates

8.4.3. Monosaccharide composition of whole soils

Among the monosaccharides that were identified in the whole soils, glucose was always dominant. Glucose is the most abundant monosaccharide encountered both in plant-derived and microbial-derived soil materials.

For any given sugar, except for glucuronic acid, differences in abundance among land use types were significant, with higher contents in soils under *Quercus ilex* forest and lower in cultivated soils. Most of the existing differences in relative amounts of the monosaccharides appeared to be associated with xylose, arabinose, glucose, and fructose. Cellular plant polysaccharides are characterised by the high proportion of pentoses whereas microbial polysaccharides are enriched in hexoses (Cheshire, 1977).

8.4.4. Land use effect on organic carbon content and OC/N ratio in density fractions

Comparison of OC concentration (g C g^{-1} fraction) among density fractions revealed the lowest values in DF, as the latter was dominated by minerals, as suggested by Golchin et al. (1994b; 1995), and the highest values of OC concentration were found in OF. It has been suggested that such high concentration is due to the physical protection of OM by aggregates attributed to compartmentalization of substrate and microbial mass (Killham et al., 1993; Six et al., 2002).

As regards the % OC in soil that is represented by each fraction, the major portion of OC was associated with DF (Table 20). The DF accounted for about 83% of OC under cropped land, but for about 53% of OC in topsoil under tree cover, with no significant differences among soils under *Pinus* or *Quercus* vegetation. These values are consistent with the findings of

John et al. (2005), who reported that 86-91% of soil OC was associated with the mineral-associated SOM fraction at grassland, maize and wheat sites in silty soils; in contrast, the free and occluded fraction accounted for 52% of total SOM in a spruce stand on similar soil.

OC/N ratio was significantly higher ($p < 0.05$) in FF than in DF. The OC/N ratio value was significantly lower in DF than in OF (Table 21), in contrast to the findings of Rovira and Vallejo (2003) who reported significantly higher OC/N in DF in soils over calcareous material and under *Quercus rotundifolia*, but in agreement with Golchin et al. (1994b) who observed somewhat higher C/N ratios for FF.

Differences among land uses were only significant in FF. The higher values of OC/N ratio for the soils under pine forest were quite expectable. Results are also consistent, with the findings of different studies on litter composition, such as that by Traversa et al. (2008), who compared the C/N ratio of the litter under *Pinus halepensis* and *Quercus ilex*.

8.4.5. Land use effect on monosaccharide C content in density fractions

The monosaccharide C concentration was significantly different among fractions. The highest concentration was found in FF and the lowest was detected in the DF. This is in agreement with the generally held opinion that carbohydrates are labile compounds which are degraded rapidly (e.g., Hatcher et al., 1981; Benner et al., 1984).

However the proportion of the total soil OC represented by monosaccharide C was not significantly different in any fraction for any land use, according to Preston et al. (1994) who found that the proportion of soil organic present as carbohydrates remains markedly constant irrespective of the land use, and in spite of absolute changes in both C and carbohydrates.

8.4.6. Monosaccharide composition and carbohydrate nature of density fractions

The study of the sugar composition of fractions permitted to precise the origin, microbial or vegetal, of the organic matter stored in the different density fractions.

Glucose was also the predominant sugar in all the fractions. Hexoses represented most of the saccharides from the soils. According with the observations of Puget et al. (1999), we found a decrease of glucose associated with more stable fraction (DF).

Non-cellulosic saccharides are easily-available C and energy sources for microorganisms (Martin and Haider, 1986). Plant material contains large proportions of pentose sugars (mainly xylose and arabinose). The soil microbial population, in contrast, synthesizes dominantly galactose, glucose, mannose and little, if any, arabinose and xylose (Oades, 1984).

The ratio of mannose+galactose/arabinose+xylose is widely used to estimate the decomposition of plant residues and the stock of compounds synthesized (Oades, 1984). In the studied soils, [(G + M)/ (A + X)] ratio shown significant differences among land uses with higher values in all the fractions corresponding with soils under *Pinus halepensis* plantation (Table 25).

Oades et al. (1987) found a stabilization effect of microbial carbohydrates by close association with inorganic components. However, we do not found significant differences among density fractions.

Stands out the positive and significant correlation between MSC content in samples and OC, OC/N ratio, MBC, and MBC/MBN ratio. Carbohydrates represent a main source of nutrients and energy for soil micro-organisms and contribute to microbial activity (Jolivet et al., 2006).

8.5. Conclusions

Historical transformation of *Quercus ilex* forest to cropped lands in calcareous soils in this area has resulted in a major loss of OC, as was expected. However, subsequent reforestation with *Pinus halepensis* throughout the past 40 years has resulted in good recovery of the SOC. Despite the different OC content of soils under different land use the SOM stabilization mechanisms were not significantly different. Soil organic matter was mainly located in the organo-mineral complex, resulting in physicochemical stabilization against further decomposition.

Soil carbohydrates accounted for 6 to 10 % of the total organic C of soils. Cultivation also affected the proportion of soil C present as carbohydrates. Monosaccharide analysis revealed significant differences in carbohydrate composition between land uses. Significant differences in monosaccharide composition were also found among density fractions however, the proportion of the organic C represented by monosaccharide C among fractions was constant. Whatever the fraction and land use considered, glucose was the dominant sugar monomer, followed by mannose and xylose.

Stands out the positive and significant correlation between monosaccharide C content in samples and OC, OC/N ratio, MBC, and MBC/MBN ratio.

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9. ESTIMATION OF MICROBIAL BIOMASS CARBON AND NITROGEN IN CALCAREOUS SOILS BY UV-ABSORBANCE

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Abstract

The ability to estimate soil microbial biomass C (SMB C) and nitrogen (SMB N) is of fundamental importance for studying a range of soil processes. The widely used method for microbial biomass estimation is the fumigation-extraction method, been complemented by the analyses of the C and N released. The present study, carried out on calcareous soils of north-western Spain under different types of land use, assesses the relationship between SMB C and SMB N measured by conventional fumigation-extraction method and the estimates obtained by increases in UV absorbance at 224, 260, 280, and 340 nm of 0.5M K_2SO_4 extracts of fumigated soils.

Increases in absorbance at 260 and 280 nm were strongly correlated with SMB C ($r^2 = 0.82$, $p < 0.001$; $r^2 = 0.83$, $p < 0.001$, respectively) and SMB N ($r^2 = 0.69$, $p < 0.001$; $r^2 = 0.66$, $p < 0.001$, respectively) also for calcareous soils. However, the strongest correlation was found with the increase in absorbance of extracts after fumigation at 340 nm ($r^2 = 0.85$, $p < 0.001$; $r^2 = 0.72$, $p < 0.001$, respectively) although measurements at that wavelength, related to microbial activity, were not used before as SMB C or SMB N estimator.

Values of increase in absorbance at 224 nm were the highest and shown also a positive, correlation with SMB C and SMB N ($r^2 = 0.68$, $p < 0.001$; $r^2 = 0.46$, $p < 0.05$, respectively).

Keywords**:** *Soil microbial biomass; ultraviolet absorbance; land uses; calcareous soil.*

9.1. Intoduction

Often, the size of soil microbial community is expressed as the overall biomass. A merit of the biomass approach is that cheap and well-standardised methods can be used. Moreover, it represents a holistic approach to the soil ecosystem (Hofman and Dušek 2003). A potentially more accurate method of enumerating all the microbes contained in a soil sample is suggested by calculations based on biomass-C (Watson et al. 1977).

The ability to estimate soil microbial biomass carbon (SMB C) and soil microbial biomass nitrogen (SMB N) is of fundamental importance for studying a range of soil processes, including those involved in organic

matter decomposition, nutrient cycling and soil quality (Karlen et al. 1997). Also, SMB responds more quickly than soil organic matter (SOM) as a whole to changes in management of soil (Powlson and Brookes 1987; Patra et al. 1990). Therefore, SMB has appeared to be early sensitive indicator of land use changes (Lagomarsino et al. 2006; Llorente and Turrión 2009).

Several methods have been applied to estimate SMB (Alef and Nannipieri 1995). The widely used method is the chloroform fumigation-extraction method (Brookes et al. 1985) because this method has several advantages in comparison with others, such as time and resource intensity of the analysis (Joergensen 1996). This method is based on the lyses of microbial cells by exposure to chloroform. Microbial constituents, especially cytoplasm, are degraded by enzymatic autolysis and transformed into extractable components (Glaser et al. 2004). Its utility for estimating SMB is clearly demonstrated in the literature (e.g., Smith et al. 1995). The SMB C and SMB N are calculated from the comparison of C (Vance et al. 1987) and N amounts (Brookes et al. 1985) in chemically analyzed extracts of fumigated and unfumigated soils. However, this method is labour intensive and time consuming and, therefore, it is not suitable for routine estimation of biomass. Automated procedures for measuring biomass C and N, on the other hand, require the use of expensive equipment which is not widely available.

The use of near ultraviolet (UV) absorbance measurement of compounds released from soil during chloroform fumigation as an estimate of microbial biomass represents a simple and inexpensive adaptation of the fumigation-extraction method. This adaptation is based on the fact that certain released molecules absorb in the near UV region (Nunan et al. 1998). Different studies have reported a correlation between SMB, as measured by fumigation-extraction method and UV absorbance at different wavelengths of extracts of fumigated soils (Nunam et al. 1998; Turner et al. 2001; Ladd and Amato 1989).

Merckx and Martin (1987) observed that extracts of fumigated soils contained UV absorbing compounds, predominantly nucleotides. It has been shown that nucleotides absorb maximally at 260 nm (Beaven et al, 1955). Based on that, Ladd and Amato (1989) found that UV-absorbance at 260 nm was correlated with SMB C measurements. However, absorbance at 260 nm includes not only nucleic acids but aromatic amino acids and plant phenolics and for this reason the correlation was poor (Turner et al. 2001).

Based on the fact that tyrosine and tryptophane have absorbance maxima near 280 nm (Plummer 1987), Nunan et al. (1998) found a strong linear correlation between SMB C (r^2 = 0.94) and SMB N (r^2 = 0.92) measured by fumigation-extraction and UV-absorbance at 280 nm. At this wavelength, absorption due to aromatic amino acids is near the maximum (260-280 nm), but absorbance by plant phenolics is reduced (mostly 250-270 nm). Turner et al. (2001) confirmed that this correlation between SMB C measured by fumigation-extraction and UV-absorbance at 280 nm was strong in soils of a wide range of organic matter and clay contents. Also they found a strong correlation between SMB N and UV-absorbance at 280 nm. However, the technique has currently never been applied to calcareous soils.

In other hand, Taylor et al. (2002) found a very strong correlation between dehydrogenase activity and microbial biomass. Dehydrogenase is an enzymatic complex of intracellular nature (Nannipieri et al. 1990)

which is present in all microorganisms (Von Mersi and Schinner 1991; Dick 1997). Therefore, dehydrogenase activity assays are considered to be an accurate measure of the microbial oxidative activity of the soil and should have a direct relationship to total viable microorganisms (Dick 1997). Dehydrogenase enzymes are redox enzymes which transfer hydrogen atoms and electrons from a substrate to an electron acceptor (Nannipieri et al. 1990) . The electron acceptor is the co-enzyme nicotinamide adenine dinucleotide (NAD) (Simon and Bartlett 2003). NAD^+ and NADH, which represents the oxidized and reduced forms of β-nicotinamide adenine dinucleotide, have a maximum of absorbance at 340 nm (Zhang and Stanton 2000). Therefore, by analogy with the approach of Ladd and Amato (1989) and Nunan et al. (1998), it is possible that UV measurements at 340 nm could also provide a reliable measure of SMB C and SMB N.

Also, benzene and benzene derivatives, normally related to degradation products of pesticides and petroleum pollutants, have a maximum of absorbance at 224 nm (Sandrin et al. 2008). Based on that, also 224 nm absorbance of the extracts was measured.

The aims of our study were: 1) to investigate the validity of the 260 and 280 nm UV absorbance technique for estimating SMB C and SMB N in soils with high carbonate contents; 2) to investigate whether the increase in absorbance at 340 nm of K_2SO_4 extracts of fumigated soils could be used as an estimate of SMB C and SMB N; 3) to test 224 nm absorbance of K_2SO_4 extracts of soils as a possible indicator of pesticide/herbicide applications

9.2. Materials and methods

9.2.1.Soils

The study was carried out in calcareous soils in the region of *Castilla y León* (north-western Spain), UTM: 30T 384465 E 4639001 N. The mean annual rainfall in the region is below 400 mm under a xeric moisture regime, and the mean annual temperature is approximately 12.3^{o}C. The altitude of the moor varies between 800 and 900 m, with low slopes (< 7%).

Calcareous soils from 7 profiles were sampled at depths of 0-10, 10-20 and 20-30 cm. Visible plant residues and roots were removed, and soil samples were air dried and sieved (< 2 mm). Soil textures were classified as sandy-clay-loam, according to the USDA classification, and contained large amount of coarse fragments (mineral fragments > 2 mm). The soils studied also showed a alkaline soil reaction (7.9-8.4) and a high content of carbonates (10-60%), The organic carbon content ranged from 1.0 to 6.5%.

9.2.2. Physical and chemical characterization

Water holding capacity (WHC), % of carbonates, texture, pH, electrical conductivity (EC), total N, total C, organic C and C/N were determined.

The WHC was determined gravimetrically. Particle-size distribution was determined by the International Pipette Method (USDA, 1972). pH (soil:water, 1:2.5) was measured with a Crison pH-meter. Total concentrations of soil C and N were determined with an automated C/N analyser (CHN-2000, Leco). Organic

carbon was calculated as the difference between total and carbonate carbon. Soil total calcium carbonates were determined by use of 1M HCl titrated with 0.5M NaOH (FAO, 2007).

9.2.3. Chloroform fumigation extraction

Previous to the extraction of the microbial biomass, each air dried soil (< 2 mm) was incubated at 70% field capacity at room temperature (~ 25°C) for 1 week. After incubation, triplicate subsamples of each soil were fumigated for 24 h in a vacuum dessicator with ethanol-free $CHCl_3$ in the dark. Following fumigation, each soil (triplicate subsamples) was extracted by shaking 15 g with 60 ml of 0.5 M K_2SO_4 for 30 min on a rotary shaker and filtered through Whatman no. 42 filter paper. As well, triplicate subsamples of unfumigated control soils were extracted in the same way. Organic C and total N were measured in aliquots of the K_2SO_4 extracts of fumigated and unfumigated subsamples by a Skalar Formacs TOC/TN combustion analyzer for liquid samples.

The SMB C was calculated using the equation: Biomass C = K_{Ec}*E_c, where E_c is the difference between organic C from fumigated soil and organic C from unfumigated soil (Vance et al., 1987) and K_{Ec} is the extractable part of microbial biomass C after fumigation. We applied a factor 2.64 as recommended by Joergensen (1996).

The SMB N was calculated using the equation: Biomass N= K_{EN}*E_N, where E_N is the difference between total N from fumigated soil and total N from unfumigated soil and K_{EN} is the extractable part of microbial biomass N after fumigation. We applied a factor 1.85 as is recommended by Brookes et al. (1985).

9.2.4. UV-spectroscopy

UV absorbance of the K_2SO_4 extracts from fumigated and unfumigated soils was measured at 224, 260, 280, and 340 nm using a U-2001 Hitachi Spectrophotometer. Extracts were analyzed as soon as possible after filtration to avoid the white insoluble precipitate that can be formed in some K_2SO_4 extracts, which can interfere with absorbance measurements.

Results for UV absorbance are expressed as the increase in absorbance after fumigation on a dry soil basis.

9.2.5. Data analysis

SMB and absorbance data were expressed on an oven-dry soil basis. The means and standard errors are of triplicate soil extracts. Analyses of variance (ANOVA) were performed to evaluate the main effects of land use, depth, and their interactions on the parameters analysed. Data were tested for normality and homoscedasticity with the Kolmogorov-Smirnov and Levene´s statistics respectively. In cases of significant F-statistics, differences between means were tested with the Tukey procedure for multiple comparisons.

Correlations between datasets were estimated by Pearson procedure. Statistical analyses were performed with the Systat 14.0 Statistical Software Package (SPSS for Windows).

9.3. Results

Physico-chemical characteristics of the samples are shown in Table 27.

The soil N content, organic C, and C/N ratio were higher in the upper soil layers. Furthermore, the organic matter content and N availability varied with land use. Carbonates content ranged from 10 to 60% with a tendency to increase with depth.

A wide range of SMB C and SMB N contents were found amongst the 20 soils examined using the conventional fumigation-extraction method (Table 28). SMB C ranged from 228 to 1461 μg C g^{-1} dry soil and SMB N ranged from 13 to 164 μg N g^{-1} dry soil. Both SMB C and SMB N, for all the studied profiles, were significantly ($p < 0.05$) higher in the upper soil layers and higher contents were found in soils under tree cover (both pinus and quercus) than in cropped soils (Figure 18).

The average of increases in absorbance at 224, 260, 280 and 340 nm in extracts of soils after fumigation are shown in Table 3. For all the K_2SO_4 extracts, the maximum increases were found at 224 nm and in decreasing order at 260, 280, and 340 nm.

Table 27. Physicochemical properties of the samples

Soil No	Location	Land Use	Depth (cm)	pH	$CaCO_3$ (%)	OC (%)	C/N
1	Ampudia	CL	0-10	8.13	10.1	1.70	15.57
2		CL	10-20	8.37	12.41	1.33	11.11
3		CL	20-30	8.43	25.31	1.00	10.03
4		QF TC	0-10	8.12	14.08	2.82	14.63
5		QFTC	10-20	8.28	16.03	1.14	8.87
6		QFOC	0-10	8.03	14.92	5.12	17.00
7		QFOC	10-20	8.21	15.58	3.48	16.56
8		QFOC	20-30	8.26	29.5	1.72	9.38
9	Monte Viejo	CL	0-10	8.29	59.84	1.39	10.97
10		CL	10-20	8.37	59.51	1.46	12.80
11		CL	20-30	8.43	60.20	1.56	14.79
12		QFTC	0-10	8.19	26.48	3.45	10.74
13		QFTC	10-20	8.26	33.37	2.80	12.57
14		QFTC	20-30	8.37	44.90	2.21	14.18
15		QFOC	0-10	7.94	31.14	6.51	11.27
16		QFOC	10-20	8.22	39.32	4.44	12.28
17		QFOC	20-30	8.29	47.39	3.16	13.53
18		PP	0-10	8.13	42.04	3.70	13.07
19		PP	10-20	8.37	44.49	2.79	15.40
20		PP	20-30	8.43	45.14	2.25	14.47

CL: Crop Land; QFTC: soils under Quercus tree cover; OC: soils outside of Quercus tree cover; PP: Pinus Plantation.

Table 28. Values for soil microbial biomass C and N in samples as determined by conventional fumigation-extraction procedures.

	SMB C	SMB N
	µg C g^{-1} dry soil	µg N g^{-1} dry soil
Soil No	Average ± S.E.	
1	647.10 ± 81.71	118.19 ± 0.65
2	416.40 ± 42.06	70.27 ± 8.54
3	278.82 ± 63.22	50.79 ± 11.06
4	902.40 ± 88.25	92.15 ± 11.89
5	466.92 ± 224.63	110.73 ± 8.59
6	966.74 ± 40.50	122.58 ± 10.14
7	358.98 ± 68.99	45.28 ± 0.30
8	228.24 ± 67.57	28.89 ± 7.26
9	559.76 ± 80.21	69.81 ± 9.46
10	304.98 ± 79.17	31.76 ± 8.54
11	273.24 ± 13.2	47.38 ± 20.75
12	1461.60 ± 175.48	123.02 ± 12.34
13	1617.6 ± 41.58	164.39 ± 1.55
14	893.24 ± 126.95	60.99 ± 9.23
15	743.98 ± 78.96	70.03 ± 17.27
16	392.34 ± 68.80	27.24 ± 7.06
17	257.32 ± 31.72	18.58 ± 5.15
18	638.11 ± 133.64	65.77 ± 23.40
19	478.51 ± 163.07	34.21 ± 12.42
20	270.81 ± 69.76	23.88 ± 9.10
Analysis of variance		
Use	***	*
Depth	***	***
Use*Depth	***	***

For ANOVA analysis, values of p>0.05: n.s., p<0.05:*, p<0.01:** and p<0.001:*** are reported

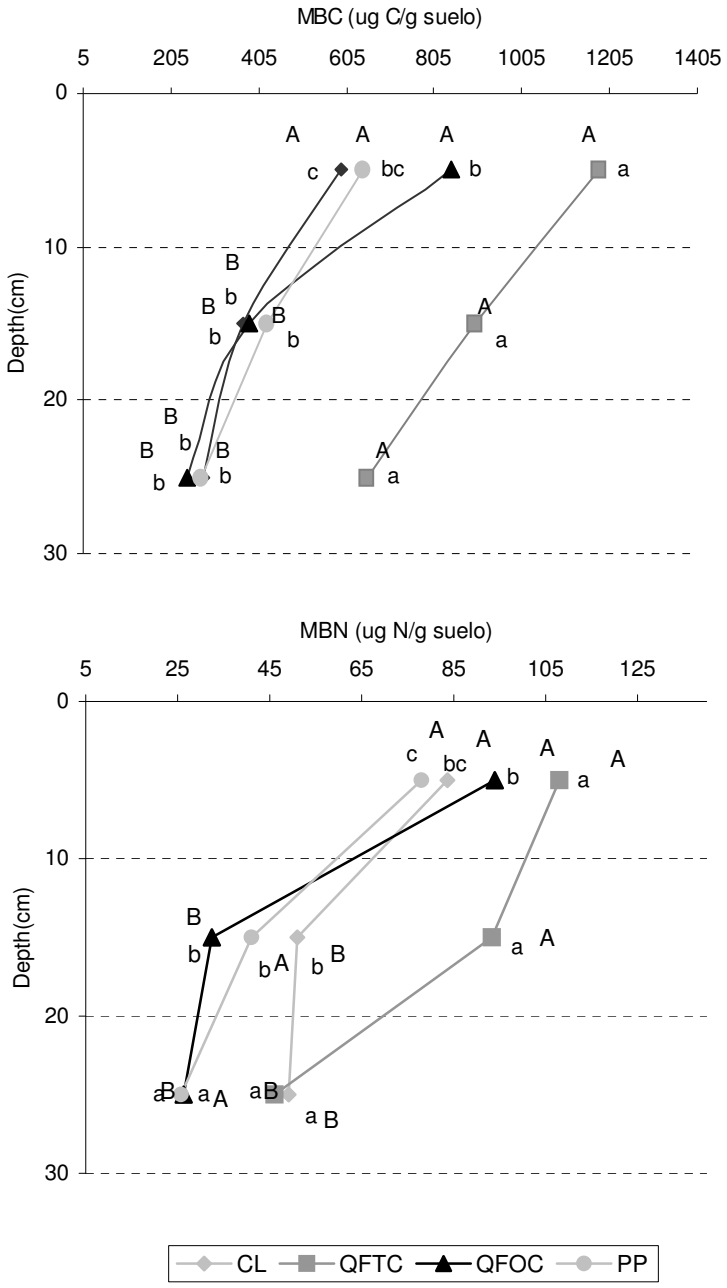

Figure 18

SMB C and SMB N in soils under different types of land use and at different depths. Differences between means were tested by the Tukey procedure for multiple comparisons. Depths indicated with the same capital letter are not significantly different ($p<0.05$) for a given land use. Land uses indicated with the same lower case letter are not significantly different ($p<0.05$) for a given depth. CL: Cropped land; QFOC: Native *Quercus ilex* forest outside tree cover; QFTC: Native *Quercus ilex* forest under tree cover; PP: *Pinus halepensis* plantation.

Table 29. Increase in UV absorbance at 224, 260, 280, and 340 nm of 0.5 M K_2SO_4 extracts of fumigated soils.

	Incr UV 224 nm	Incr UV 260 nm	Incr UV 280 nm	Incr UV 340 nm
Soil No	Average ± S.E. (abs g^{-1} dry soil)*10			
1	18.36 ± 0.31	n.d.	n.d.	n.d.
2	n.d.	8.50 ± 0.24	6.09 ± 0.10	1.04 ± 0.26
3	23.38 ± 0.12	4.23 ± 0.28	n.d.	n.d.
4	34.09 ± 0.09	33.82 ± 0.11	28.00 ± 0.14	n.d.
5	19.67 ± 0.43	16.23 ± 0.53	8.99 ± 0.32	2.35 ± 0.12
6	33.45 ± 0.27	31.42 ± 0.18	19.05 ± 0.12	5.03 ± 0.13
7	12.27 ± 0.09	11.23 ± 0.25	5.73 ± 0.21	1.45 ± 0.07
8	11.04 ± 0.13	8.18 ± 0.12	4.36 ± 0.17	1.02 ± 0.06
9	10.63 ± 0.31	9.52 ± 0.43	5.39 ± 0.34	1.72 ± 0.11
10	6.77 ± 0.05	3.71 ± 0.21	1.86 ± 0.13	0.12 ± 0.11
11	6.00 ± 0.06	5.91 ± 0.12	3.54 ± 0.28	0.62 ± 0.12
12	n.d.	n.d.	n.d.	n.d.
13	30.07 ± 0.12	14.90 ± 0.23	12.77 ± 1.95	2.64 ± 0.65
14	30.75 ± 0.61	11.55 ± 0.38	7.63 ± 0.52	1.52 ± 0.09
15	29.91 ± 0.58	27.72 ± 0.54	20.18 ± 0.21	6.34 ± 0.06
16	24.05 ± 0.46	13.86 ± 0.43	12.77 ± 0.66	2.63 ± 0.21
17	12.41 ± 0.65	9.33 ± 0.09	2.14 ± 0.13	n.d.
18	25.16 ± 0.38	18.91 ± 0.48	8.36 ± 0.17	2.02 ± 0.04
19	20.73 ± 0.51	15.57 ± 0.57	9.53 ± 0.35	1.07 ± 0.07
20	16.08 ± 0.59	10.84 ± 0.47	6.59 ± 0.44	1.70 ± 0.11
Analysis of variance				
Use	**	**	**	*
Depth	n.s.	***	**	n.s.
Use*Depth	**	***	***	n.s.

For ANOVA analysis, values of p>0.05: n.s., p<0.05:*, p<0.01:** and p<0.001:*** are reported. n.d.: not determined

The correlation coefficients between increases in absorbance in extracts of soils after fumigation and SMB C and SMB N estimated using the conventional fumigation-extraction method are shown in Table 30. Strong and significant ($p < 0.001$) correlations were found at all the tested wavelengths and SMB C and SMB N. Only the correlation between increase in absorbance at 224 nm and SMB N was less significant ($r^2 = 0.461$, $p < 0.05$). However, for all the tested wavelengths, correlations were stronger between those and SMB C than between those and SMB N. For both, SMB C and SMB N, the higher correlation was found in absorbance at 340 nm ($r^2 = 0.854$, $p < 0.001$, and $r^2 = 0.719$, $p < 0.001$ respectively).

As could be expected a positive linear relationship was found between SMB C and SMB N ($r^2 = 0.834$, Table 30).

Table 30. Correlation coefficients for relationships between SMB C and SMB N, and the increase in UV absorbance at 224, 260, 280, and 340 nm of 0.5 M K_2SO_4 extracts of fumigated soils.

	Correlation (r^2)	**p-value**
SMB C		
x Incr UV_{224}	0.677	< 0.001
x Incr UV_{260}	0.823	< 0.001
x Incr UV_{280}	0.834	< 0.001
x Incr UV_{340}	**0.854**	< 0.001
SMB N		
x Incr UV_{224}	0.461	< 0.05
x Incr UV_{260}	0.686	< 0.001
x Incr UV_{280}	0.660	< 0.001
x Incr UV_{340}	**0.719**	< 0.001
SMB C x SMB N	0.834	< 0.001

9.4. Discussion

9.4.1. Soil microbial biomass estimation

All the studied profiles showed a decrease in SMB C and SMB N with increasing depth (Figure 18). The soils under tree cover (pinus or quercus) presented the highest SMB C values and a sharper depth gradient. This is according to many studies of SOC and land use change that use measurements of SMB C as early indicator of changes in SOM (e.g., Powlson and Brookes 1987; García-Gil et al. 2000; Palma et al. 2000). Microbial biomass usually declines when soils under forest and grassland vegetation are brought under cultivation (Srivatava and Singh 1991).

SMB C in cropped soils ranged from 273 to 647 μg C g^{-1} dry soil. These values were slightly higher than values reported by Weigand et al (1995) in 32 agricultural soils which ranged from 84 to 548 μg C g^{-1} dry soil and close to those reported by Álvarez et al (1995) for tillage systems from the Argentine Rolling Pampa (from 207 to 378 μg C g^{-1} dry soil). In forest soils SMB C ranged widely from 228 to 1461 μg C g^{-1} dry soil. SMB C values reported in the literature are large, for example Vázquez-Murrieta et al. (2007) reported values ranging from 161 to 2195 μg C g^{-1} dry soil in soils under different land uses of the central highlands of Mexico. SMB N in cropped soils ranged from 31 to 118 μg N g^{-1} dry soil, which can be considered high values if comparing with other studied soils like those reported by Bending et al. (2004) in different agricultural soils (ranging from 13 to 25 μg N g^{-1} dry soil). SMB N in our soils under forest ranged from 12 to 164 μg N g^{-1} dry soil closer to values reported in the literature. For example, Reyes-Reyes et al. (2007) reported values from 35 to 107 μg N g^{-1} dry soil in soils under canopy of different tree species.

As could be expected a high correlation between SMB C and SMB N ($r^2 = 0.83$, $p < 0.001$) was found other authors have reported similar correlation values (Joergensen and Muller 1996).

The K_2SO_4 extracts of controls (unfumigated) soils absorbed UV radiation. However, the extracts following fumigation absorbed more, indicating a release of UV sensitive material, almost certainly of biomass origin, as a result of chloroform fumigation. Estimations of SMB C and N by the increase in UV absorbance at different wavelengths from 224 to 340 nm due to chloroform fumigation was found to correlate well with results obtained using the conventional fumigation extraction method (Table 30).

Correlations between absorbance at 280 nm and SMB C and SMB N were positive and significant as was expected ($r^2 = 0.86$; $r^2 = 0.64$ respectively), however these correlation coefficients were lower than those found by Turner et al. (2001) who studied 29 soils under grassland ($r^2 = 0.92$; $r^2 = 0.90$, respectively), or those found by Nunan et al. (1998) in 17 soils of Ireland ($r^2 = 0.94$; $r^2 = 0.92$, respectively).

Correlations between SMB C and increases in absorbance at 260 nm were also positive and significant ($r^2 = 0.82$) as was expected following Ladd and Amato (1989) study. In our calcareous soils not really better correlation was found between SMB C and increases in absorbance at 280 nm than at 260 nm. As well as, UV

absorbance measurement at 260 nm confirms the validity as a simple way of estimating SMB C also in calcareous soils, a positive correlation between that absorbance and SMB N was also found ($r^2 = 0.69$).

It is possible that correlations between the conventional techniques and the UV absorbance technique were not stronger than the one found by Nunan et al. (1998) because of the error involved in both techniques. Much of the error is probably introduced for the use of a single correction factor (k) when correcting for unrecovered microbial biomass. In fact, K_E factor (proportion of C and N extracted from the fumigated soil) have been found to ranges from 0.2 to 0.48 (Martens 1995; Zagal 1993; Sparling and West 1988). Variations in this values is due to differences in the recovery of the different types of organisms and variations on soil pH (Tate et al. 1988).

Both SMB C and SMB N showed the stronger correlation with increases in absorbance at 340 nm. So good correlation could be explained because co-enzymes of dehydrogenase have a maximum of absorbance at that wavelength. Dehydrogenase activity assays are considered to be an accurate measure of the microbial activity of the soil and should have a direct relationship to total viable microorganisms. Taylor et al. (2002) found a strong correlation between dehydrogenase and biomass C ($r^2 > 0.95$) in different agricultural soils from USA. However, not all the studies are agree with that, like the one of Bastida et al. (2007), who found no significant correlation between deshydrogenase and biomass C. Whatever, for the use of deshydrogenase as indicator of microbial biomass is important to measure the absorbance of extracts just in a fix time because co-enzymes have significant changes in molecular characteristics over time (Scott et al. 2001).

Values of increases in absorbance at 224 nm were the highest, however the correlation between these increases and SMB C or SMB N were the lowest although significant ($r^2 = 0.68$; $r^2 = 0.46$ respectively). Benzene and benzene derivatives have a maximum of absorbance at 224 nm (Sandrin et al. 2008). Benzene derivatives can enter the environment from natural sources or anthropogenic activity, however many times are related to pollutants as pesticide degradation products or petroleum derivatives. Kuppithayanant et al. (2003) found a specific relation between 224 nm absorbance and some polycyclic aromatic hydrocarbons (PAHs) as naphthalene and acenaphthylene. Therefore, it could be really interesting to investigate whether 224 nm absorbance of extracts of soil could be useful for soil pollutant detection and monitoring. However, in our study appear not to be a relationship between pesticide presence and absorbance at 224 nm since values of absorbance are higher in soils under forest than in cropped soils.

9.4.2. Conclusions

The UV technique described here provides and simple a adequate way of SMB C and SMB N estimations in calcareous soils, being the increase in absorbance at 260 nm the best estimator of SMB C and SMB N in the studied calcareous soils. This will have applications as a simple and inexpensive method of monitoring soil microbial biomass. That has many potential applications in the field of environmental monitoring and turnover of C in soil.

9.5. Acknowledgements

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10. CONCLUSIONES GENERALES

a) La transformación histórica (hace unos 100 años) del bosque original de encina (*Quercus ilex*) en tierras de cultivo en los suelos del páramo calizo castellanoleonés, supuso una gran pérdida de MO en estos suelos y consecuentemente del C y el N almacenado en los mismos. La posterior reforestación, hace 40 años, con pino (*Pinus halepensis*) ha supuesto una significativa recuperación de la MOS perdida, lo cual muestra el potencial para aumentar la cantidad de C almacenada en el suelo que supone la devolución de tierras agrícolas a su estado forestal.

b) La biomasa y la actividad microbianas que, como cabía esperar, muestra valores decrecientes según profundizamos en el perfil del suelo, presentan valores inferiores a los descritos por la bibliografía para suelos no calcáreos bajo usos de suelo similares.Aunque el C de la biomasa microbiana del suelo suele describirse como un indicador temprano de los cambios de uso del suelo, los parámetros C extraíble con K_2SO_4 (C_{K2SO4}) y C respirado tras 21 días de incubación ($C\text{-}CO_{2\ (21d)}$ se mostraron como indicadores al menos tan sensibles como la biomasa microbiana ante los cambios de uso del suelo. La ecuación de primer orden $C_m=C_o(1- e^{-kt})$ supuso un buen descriptor de la cinética de la mineralización del C en el suelo. El parámetro cinético calculado a partir de dicha ecuación "C potencialmente mineralizable" (C_o) también se mostró como un buen indicador de cambio de uso del suelo. El parámetro "tasa inicial de mineralización potencial" (C_ok) se mostró como buen indicador de degradabilidad del sustrato. Los parámetros microbiológicos estudiados indicaron que, para estos suelos calcareos, la cobertura arbórea tiee una influecia mayor sobre la dinámica del C edáfico que la especie forestal concreta (*Pinus halepensis* o *Quercus ilex*).

c) El cociente metabólico (qCO_2) se mostró negativamente correlacionado con la biomasa microbiana siendo los microorganismos más eficientes en el uso del C cuando la disponibilidad de sustrato es menos lo que probablemente significa que esta correlación negativa tiene relación con un fenómeno de competencia por el sustrato.

d) Mediante el análisis de las fracciones de densidad se pudo observar que, bajo los distintos usos del suelo estudiados, a pesar de las diferencias globales en los contenidos de MO, los mecanismos de estabilización no son significativamente diferentes, siendo la formación de complejos órgano-minerales el mecanismo que estabiliza la mayor parte de la MO, lo que supone una fuerte estabilización físico-química de la MO frente a su descomposición garantizando una estabilización del C de largo plazo.

e) El contenido de Black Carbon representó siempre <3% del total de C orgánico del suelo, representando una proporción significativa tanto de la fracción lábil como de la fracción ocluida (entre el 0.9 and 3.5%).

f) Los suelos calcáreos estudiados mostraron un enriquecimiento isotópico en ^{13}C y ^{15}N a medida que profundizamos en el perfil del suelo lo cual puede explicarse debido al fraccionamiento isotópico que sucede durante la descomposición de la MOen relación al envejecimiento de la MOS al aumentar la profundidad. El mismo proceso explica el enriquecimiento en ^{13}C y ^{15}N que presenta la MO contenida en la fracción órgano-mineral del suelo ya que esta fracción corresponde a una MO con mayor grado de humificación. Además, los resultados arrojados por los análisis isotópicos hacen sospechar de una posible presencia en el pasado de vegetación de metabolismo C4 asentada en el área de estudio.

g) El uso del suelo influyó tanto en la proporción de C monosacárido presente en el suelo respecto del C orgánico total como en su composición en monosacáridos. También se encontraron diferencias en la composición de azúcares de las distintas fracciones de densidad. Sin embargo, para cualquiera de las fracciones de densidad y de los usos del suelo, la glucose siempre es el monómero de azúcar más abundante, seguida por la manosa y la xilosa.

h) Existe una correlación positive y significativa entre el contenido de C representado por monosacáridos y el C orgánico, la relación C/N, el C microbiano y la relación C/N en la biomasa microbiana.

i) En cuanto a la técnica aplicada de medición de la UV absorbancia de los extractos de suelo a distintas longitudes de onda como método para estimar C y N microbianos, las mediciones a 260 nm arrojaron las mejores estimaciones. Este método supone una mayor simplicidad y un menor presupuesto para la estima del C y N microbianos respecto a otros métodos.

10´. **GENERAL CONCLUSIONS**

a) Historical transformation of *Quercus ilex* forest (around 100 years ago) to cropped lands in calcareous soils in this area has resulted in a major loss of OC, as was expected. However, subsequent reforestation with Pinus (*Pinus halepensis*) throughout the past 40 years has resulted in good recovery of the SOC. This shows a major potential for enhanced soil C storage in agricultural soils by their reversion to a forested state.

b) Microbial biomass and activity clearly declined with increasing depth and showed low values in comparison with non-calcareous soils. The parameters CK2SO4 and C-CO2(21d) appeared to be as sensitive indicators of land use changes as microbial biomass C.
The first-order equation Cm = Co(1 - e-kt) provided a good description of the C mineralization kinetics. The kinetic parameter calculated, potentially mineralizable C (Co), was found to be a sensitive indicator of land use change, and the initial potential mineralization rate (Cok) appeared to be a good indicator of substrate degradability. The microbiological parameters studied indicated that, for these calcareous soils, tree cover appears to have a greater influence on soil carbon dynamics than the tree species (pine or holm-oak).

c) Metabolic quotient (qCO2) was negatively correlated with microbial biomass and micro-organisms were more efficient at utilizing C resources in soils in which substrate availability was lower, which suggests the involvement of competition for substrate.

d) Despite the different OC content of soils under different land use — higher under tree cover and lower under cultivation — the OM stabilization mechanisms were not significantly different. OM was mainly located in the organo-mineral complex, resulting in physicochemical stabilization against further decomposition.

e) In our study, Black carbon always represents b3% of the TOC, far lower than the maximums reported in the literature.Furthermore, a significant proportion (between 0.9 and 3.5%) of the free and occluded OM corresponds to Black carbon.

f) The calcareous soils under study showed typical enrichment in 13C and 15N with depth, which is explained by isotopic fractionation during decomposition in relation to the increasing age of the SOM with depth. The same applies to the enrichment of 13C and 15N in OMF, which corresponds to a more humified OM. However, the results of the study revealed the possible past presence of C4 type vegetation in the study area.

g) Monosaccharide analysis revealed significant differences in carbohydrate composition between land uses. Significant differences in monosaccharide composition were also found among density fractions however, the proportion of the organic C represented by monosaccharide C among fractions was constant. Whatever the fraction and land use considered, glucose was the dominant sugar monomer, followed by mannose and xylose.

h) Stands out the positive and significant correlation between monosaccharide C content in samples and OC, OC/N ratio, MBC, and MBC/MBN ratio.

i) The UV technique described here provides and simple a adequate way of SMB C and SMB N estimations in calcareous soils, being the increase in absorbance at 260 nm the best estimator of SMB C and SMB N in the studied calcareous soils. This will have applications as a simple and inexpensive method of monitoring soil microbial biomass.

11. BIBLIOGRAFÍA GENERAL

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