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DOCTORAL THESIS:

Soil chemical and microbiological properties affected by land use, management, and time since deforestations and crop establishment. The case of wheat (not irrigated) and cotton (irrigated) fields in Filyria, Kilkis, Greece.

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To Artemis…

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This big journey finally came to an end, and there are quite a few people who have made it possible. I recall (with a big smile) during the sampling process when, either with my father or his older brother Yannis, we travelled across the land with a tractor in order to collect the soil samples.

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LIST OF ABBREVIATIONS

CT	:	Conventional tillage
FS	:	Fine sand
IR	:	Irrigated (plots)
MB	:	Microbial biomass
MBC	:	Microbial biomass carbon
MBC/SOC	:	Microbial biomass carbon/ Soil organic carbon
NIR	:	Not irrigated (plots)
ns	:	not significant (for statistical tables)
NT	:	No tillage
OM	:	Organic matter
POM	:	Particulate organic matter
RT	:	Reduced tillage
SMB	:	Soil microbial biomass
SOC	:	Soil organic carbon
SOM	:	Soil organic matter
SQ	:	Soil quality
SR	:	Soil respiration
TN	:	Total nitrogen

ABSTRACT

ABSTRACT

Deforestation is a common practice in many countries worldwide since many years, in order to gain land for agricultural purposes, and is one of the main reasons for the global net release of CO_2 from soil to atmosphere. The conversion from natural vegetation to cropland often leads to a depletion of the soil organic carbon (SOC) stock due to reduced input of biomass and enhanced decomposition after physical disturbance. The effect of forest clearing for other land uses is often a low level of soil microbial population.

In a part of Northern Greece, Filyria, which belongs to prefecture of Kilkis, three deforestations took place in 1933, 1971 and 1980, in order to give land for agriculture to refugees and residents with no property. The natural vegetation consists mainly of *Quercus pubescens* stands. Each family was given 2-9 hectares of deforested land. The agricultural fields are cultivated with wheat, cotton and tobacco, and cherry trees are established in several plots. The soils of the study area have developed from limestone, and are classified as Xeralfs according to Soil Taxonomy. The plots of the study are cultivated with cotton and wheat. Wheat fields are not irrigated and their management is considered as reduced tillage, whereas cotton plots are irrigated by sprinklers, considered as conventional tillage and are altered with wheat every 2 years (two consecutive years of cotton crops, one year of wheat). The big amount of time transcurred since the first deforestation, the availability of three different deforestation dates and the remaining undisturbed forest, offer an interesting opportunity of studying these changes, along with assessing factors such as land use (forestry vs agriculture) and land management types (wheat fields vs cotton fields) correlating with the amount of time since deforestation (26, 35 and 73 years).

Soil microbial and biochemical status in both natural and agricultural ecosystems have been used as bioindicators of soil stress or benefits from reclamation efforts. In addition to chemical and physical properties, soil microbial properties may indicate suitable management and restoration practices towards sustainability. Since microorganisms respond rapidly to changing environmental conditions they are considered as sensitive indicators of soil health and could be therefore used for soil status monitoring. Organic carbon and nitrogen in the soils, have different behaviour in the different soil fractions, where clay, mainly, and silt seem to preserve better the contents of the two elements. The aims of this thesis are to determine the changes in terms of soil microbiological properties that resulted from 3 stages of deforestation (1933, 1971, 1980), by measuring and assessing microbial biomass carbon (MBC), SOC, the MBC/SOC ratio, soil respiration (SR), the metabolic quotient (qCO_2), and the relationship between SOC, N and the soil fractions. Land use (forestry vs agriculture), type of crop management (wheat vs cotton) and the amount of time since deforestation (26, 35 and 73 years since deforestations) are the factors discussed.

Three sampling categories referring to deforestation year (1933, 1971, and 1980) in the cultivated fields and one category referring to the natural remaining forest were considered. For each deforestation year category, two crop subcategories were considered (wheat and cotton), and from each crop subcategory, 12 plots were sampled. In adjacent areas, twelve plots of remaining undisturbed forest (*Quercus pubescens*) were selected. A composite sample was taken in each plot (72 cultivated plots and 12 forest plots). The composite sample was obtained by mixing fifteen random subsamples from the 0-15 cm mineral layer. Soil samples were collected at the end of November 2005, after harvest.

Microbiological and chemical properties at whole soil samples, and chemical properties at soil particle-size fractions were studied. Soil organic carbon was calculated from the total carbon measurement subtracting C from carbonates. Soil Microbial Carbon was determined by the fumigation-incubation method and posterior C in extracts was determined by wet oxidation with dichromate. The microbial quotient (MBC/SOC) represented the fraction of MBC with respect to the SOC. Potential Soil Respiration was determined in closed jars and under laboratory-controlled conditions. The metabolic quotient (qCO_2) represents the potential soil respiration per unit microbial biomass, and was calculated as SR/MBC.

Particle-size fractionation was carried out through ultra sonification. Particulate organic matter (POM) was separated in the coarse sand fraction, and the fractions of fine sand (FS), silt and clay were analyzed for C (as previously SOC), N (as total nitrogen at a LECO C/N/H analyzer) and the ratio C/N.

The results showed significant differences for the values of SOC, MBC, MBC/SOC, SR, qCO_2, between forest and agricultural plots. Higher values for forests were detected for SOC, MBC and SR (these three properties are highly and positively correlated to each other). This was expected according to the findings of many researchers, as tillage promotes soil organic matter (SOM) decomposition, decreasing the sources of energy and the available substrates for the microorganisms, leading to decreases in MBC, and SR which is highly dependent by the availability of SOM. Factors that were significant for these three properties were changes in land use and the amount of years since deforestation. For SOC and MBC, management as a factor was also significant. Forest values for the two microbiological ratios (MBC/SOC and qCO_2) had significantly lower values, as reported by several researchers for MBC/SOC and by many researchers for qCO_2. These lower values for the forest soils of the ratios are indicators of less stressed conditions of the microbial community, due to better preserve of SOC and higher accumulation of microbial biomass. For these ratios, land use was always a significant factor. For the MBC/SOC ratio the amount of years since deforestation was also significant, whereas for qCO_2 the type of crop (management factor) was significant. The newest deforestation (1980) holded the biggest values for every property, except for the ratios. The amount of time since deforestation was adversely associated with SOC contents. Continuous soil tillage induces C loss and releases large amounts of CO_2 to the atmosphere. After many years systems reach or are near an equilibrium, leading to lesser values of qCO_2. Wheat plots had always significantly higher values than cotton plots, when referring to SOC and MBC, whereas for qCO2 the reverse trend was observed. The reduced tillage practices (including N fertilization which accumulates higher N contents in wheat fields compared to cotton fields) of the wheat crops have improved conditions of the soil to protect SOC, compared to the conventional tillage practices of the cotton plots. For the other two properties management was not a significant factor. The quantity and the amount of years since land use change are more important in the SOM breakdown functions than the type and intensity of cultivation. The interaction between the amount of years and management was not significant for any property.

Nitrogen and the C to N ratio in the whole soil, and particulate organic matter (POM) followed the same trends as SOC. Always significantly higher in forest (referring to land use), in the most recent deforestation (1980) (referring to the amount of years since deforestation), and in the wheat than in cotton plots (but not significantly, and not for the C/N ratio, referring to management as a factor). These 3 factors were significant for N, but only the first two (land use and the amount of years since deforestation) for the C/N ratio and POM. The amounts of N and POM were highly correlated with the contents of OM of a soil, and to each other. The ratio in forests refers to more fresh (less humified) material hence in a higher value. In the forest soils most of SOC and N were present in the fine sand fraction, which is expected (fresh organic material), with significantly higher values than silt and clay (except for the N contents in forest fine sand and clay where there are no significant differences). The reverse trend was observed for the agricultural soils, where clay holded the (significantly) greater values, due to protection of SOM. The C/N ratio holds its (significantly) greatest values for forest in the fine sand fraction, whereas for the agricultural plots in the silt fraction. Land use was a significant factor for all the properties in the studied fractions. Forest soils holded the greater values for all the properties in all the fractions compared to the agricultural plots, and except the N contents in clay, the differences were significant. Cultivation results in the breaking up of the aggregates, leading to protected C losses from silt and clay. Soil organic matter losses lead to N losses. The great losses of POM also lead to N losses, as these two properties were significantly correlated.

The amount of years since deforestation affected significantly C and the ratio, but not N. Nitrogen was mostly dependent on management practices (N fertilization). The most recent deforestation (1980) holded the greater values for all the properties in all the fractions (except for clay, where for C and N the 1st deforestation (1933) had similar values, and for the ratio there are no significant differences in values between the silt and clay fractions. Clay´s capacity to preserve carbon seems to diminish as years go by). For all the deforestation categories, clay had significantly higher values referring to C and N, whereas for the ratio the significantly higher values were found in the silt fraction. The stabilization of organic matter in the clay particles results in a steady content throughout the years, so we observed similar values for the C/N ratio in this fraction.

Management affected the properties in all the fractions, not always significantly, though overall it was a significant factor. The biggest values of SOC and N (significant for almost all the comparisons) were always found in clay, but for the C/N ratio in silt. The SOC contents in the clay fraction of the fields had no significant differences regardless management type, due to the stable behaviour of this fraction. Clay in the wheat crops showed always significantly higher values for N, but not in the other fractions. Enhanced nitrogen fertilization of the wheat fields resulted in accumulation of N on the clay particles of those fields (also resulting in lower C/N values). Referring to the fine sand fraction SOM withheld in FS was quickly lost, hence management did not affect the values significantly.

The interaction between time and management was not a significant factor.

Deforestation and further establishment of agricultural use leaded to decreases in SOC and levels of microbiological properties, suggesting a deterioration of soil quality. Despite the fact that a relatively large amount of time had elapsed since deforestation and subsequent crop establishment in the three compared deforestation stages (1933, 1971 and 1980), the amounts of SOC, MBC and SR were significantly higher in soils deforested in the most recent stage (1980). The type of crop affected the studied soil properties. Cotton plots (considered as conventional management) had significantly lower SOC and MBC values and microbiological efficiency levels than the wheat plots (considered as reduced management).

Land use changes had significant effects in SOC and N contained in the soil fractions. The amount of POM, and C and N in the fine sand fraction presented the most distinctive differences (losses, due to loss of fresh organic material) among forest (significantly higher values) and agricultural plots. The amounts of SOC and N in the clay fraction should not be considered as a sensitive indicator in order to monitor changes in land use regimes, as the contents remained rather stable depending on the nature of land use.

The amount of time since deforestation played a higher role in the order: sand>silt>clay and was adversely associated with the values of SOC and N in the particles, except for clay where after 25 years of cultivation the capacity of this fraction to preserve SOM seems to have reached an equilibrium. Soil organic matter bound in the clay particles are well protected, hence values in clay were always higher in this particle-size fraction referring to agricultural fields.

Irrespective of the management that follows, the most significant factor was the land use change for the majority of the studied properties in the soil particle fractions.

RESUMEN

La deforestación es una práctica común en muchos países en todo el mundo que se viene aplicando desde hace muchos años, con el fin de obtener tierras para fines agrícolas, siendo una de las principales razones de la liberación neta global de CO2 del suelo a la atmósfera. La conversión de la vegetación natural a tierras de cultivo a menudo conlleva una reducción del stock de carbono orgánico edáfico (SOC) debido a la reducción de la entrada de biomasa vegetal y a una mayor descomposición después de la perturbación física del suelo. El cambio de uso de masa forestal natural a cultivo lleva a menudo a una disminución en la población microbiana del suelo.

La zona de estudio de la presente Tesis doctoral se encuentra en el norte de Grecia, Filyria, que pertenece a la prefectura de Kilkis, donde han tenido lugar tres grandes actuaciones sobre las masas forestales naturales de Quercus pubescens. Se corresponde con deforestaciones llevadas a cabo en 1933, 1971 y 1980, con el fin de dotar de tierras para la agricultura a los refugiados y residentes que carecía de propiedad. Cada familia recibió entre 2 y 9 hectáreas de tierra deforestada. Los campos agrícolas han sido cultivados con trigo, algodón y tabaco, además se realizaron plantaciones de cerezos en algunas parcelas. Para el presente estudio se han seleccionado parcelas en las que se cultivan algodón y el trigo. El cultivo de trigo no se riega, mientras que las parcelas de algodón son regadas por aspersión y se alternan con el trigo cada 2 años (dos años consecutivos de cosechas de algodón, de un año de trigo).El manejo del terreno en los cultivos de algodón es un manejo convencional, mientras que en los cultivos de trigo se puede considerar como un laboreo reducido.

El estado microbiológico y bioquímico del suelo en los ecosistemas naturales y agrícolas ha sido utilizado como bioindicador del efecto que el cambio de uso provoca en el suelo. Además de las propiedades químicas y físicas, las propiedades microbianas del suelo pueden indicar si las prácticas de manejo que se llevan a cabo sobre el suelo son adecuadas y conllevan a un manejo sostenible de éste. Los microorganismos responden rápidamente a las cambiantes condiciones ambientales por lo que son considerados como indicadores sensibles de la salud del suelo y por lo tanto pueden ser utilizados para la supervisión del estado del suelo. Por otro lado, el carbono orgánico y el nitrógeno en los suelos, tienen un comportamiento diferente en las diferentes fracciones según tamaño de partícula del suelo. Así la arcilla, principalmente, y el limo en menor medida preservan los contenidos de estos elementos en los suelos.

El objetivo principal de esta tesis fue determinar el efecto en términos de propiedades microbiológicas del suelo y de estabilidad de la materia orgánica edáfica que resultaron tras el cambio de uso de masa forestal natural de Quercus pubescens. a tierras de cultivo de trigo y algodón en la región de Filiria, Kilkis, Grecia.

Cómo objetivos secundarios se plantearon:

A) determinar si el tiempo transcurrido desde la deforestación (25, 35 y 73 años) y el subsequente establecimiento del uso agrícola afecta a las características microbiológicas del suelo y a la distribución de su materia orgánica en fracciones según tamaño de partículas.

B) determinar si el tipo de cultivo/manejo (trigo vs algodón) tiene efecto en las propiedades previamente indicadas del suelo y si este efecto va a verse condicionado o no por el número de años transcurridos desde la implantación del cultivo, más que por las características de manejo del propio cultivo.

Como ya ha sido indicado el área de estudio se localizó en Filyria, prefectura de Kilkis, al norte de Grecia (40º 54’ 12’’N 40º 53’ 42’’ N, y 22º 28’48’’E- 22º 29’ 37’’E). La altitud varía desde 145 hasta 195 m sobre el nivel del mar y la pendiente media de la zona es del 2%. El clima de la zona se puede clasificar clasifica como mediterráneo templado con una temperatura media anual de 15.0ºC y una precipitación media anual de 506 mm. Los suelos se desarrollan sobre roca caliza, y se clasifican como Xeralfs según la Soil Taxonomy. La vegetación natural de la zona son bosques de Quercus (especialmente Quercus pubescens) de los cuales sólo permanecen 80 hectáreas de bosques naturales intactos. Para alcanzar los objetivos planteados se dispone de un escenario excepcional en la zona de trabajo. Concretamente se cuenta, por un lado, con tres etapas de deforestación 1933, 1971, 1980 de masas maduras naturales de *Quercus pubescens* y la masa natural remanente no deforestada y por otro lado, se cuenta con el hecho de que se adoptaran, en esos tres momentos, los mismos usos y manejos del suelo y que se hayan mantenido hasta la actualidad.

Se consideraron parcelas de 2 ha de superficie distribuidas por la zona de estudio, categorizándose en función del uso, de la fecha de deforestación en el caso de los cultivos y en el tipo de cultivo existente. Se tomaron y analizaron un total de 84 muestras compuestas de suelo procedentes de las correspondientes parcelas. Las muestras compuestas se formaron a partir de 15 sub-muestras, que se mezclaron en campo, tomadas al azar dentro de cada parcela y correspondientes a la capa superior del suelo (0-15cm). Se diferenciaron según el uso del suelo en: forestal, correspondiente a parcelas de bosque natural de Quercus pubescens que se corresponde a la masa de bosque original (12 muestras) y agrícola (72 muestras). El uso agrícola a su vez se diferenció según el año de deforestación: deforestadas en 1933 (24 muestras), deforestadas en 1971 (24 muestras) y deforestadas en 1980 (24 muestras). Cada una de las categorías según año de deforestación se diferenció según tipo de cultivo: algodón (12 dentro de cada año de deforestación, en total 36) y trigo (12 dentro de cada año de deforestación, en total 36). Las muestras de suelo se recogieron al final de noviembre de 2005, después de la cosecha.

Para el estudio de las características microbiológicas del suelo se llevó a cabo la medición y evaluación de: a) el carbono de la biomasa microbiana edáfica (MBC) y su relación con el SOC, (MBC/SOC). El MBC se determinó por el método de fumigación-incubación y el posterior análisis del C en los extractos mediante oxidación en húmedo con dicromato. El cociente microbiana (MBC/SOC) representa la fracción de SOC correspondiente a la biomasa microbiana edáfica. b) la actividad microbiana del suelo determinada mediante la respiración edáfica en condiciones controladas se humedad y temperatura (SR), c) el cociente metabólico (qCO_2) que representa el potencial de la respiración del suelo por unidad de biomasa microbiana, y se calculó como el cociente entre el SR y el MBC.

Por otro lado se llevó a cabo un estudio de la dinámica del SOC y del N según uso, manejo y fecha de deforestación. Para ello se determinó la concentración de estos elementos en las distintas fracciones según tamaño de partículas de los suelos, diferenciándose la materia orgánica particulada, POM, y las fracciones de arena fina, limo y arcilla. El fraccionamiento por tamaño de partículas se llevó a cabo utilizando ultrasonificación para provocar la ruptura de los agregados edáficos, tamizado para separar los tamaños más grandes y centrifugación para separar el limo de la arcilla. Se calculó el porcentaje en peso de POM en la fracción de arena gruesa, y en las fracciones de arena fina, limo y arcilla se determinaron las concentraciones de C orgánico y de N total. El SOC en el suelo global y en las distintas fracciones según tamaño de partícula se calculó como la diferencia entre el C total medido con autoanalizador LECO CNH2000 menos el C de los carbonatos medido por valoración con HCl. El N total en el suelo global como en las distintas fracciones según tamaño de partícula se determinó con el autoanalizador LECO CNH2000.

Para el análisis estadístico de los datos obtenidos para el suelo global se utilizó un modelo mixto lineal con tres factores inter-sujetos: el uso, el tiempo (o fecha de deforestación) y el manejo (o tipo de vegetación). En el factor uso se diferenciaron dos niveles: uso forestal y agrícola. En el factor tiempo anidado en el uso [tiempo(uso)], se diferenciaron tres niveles dentro del uso agrícola según la fecha de deforestación (1933, 1971 y 1980) y el factor manejo anidado en el uso [manejo(uso)] se diferenciaron dos niveles según el tipo de cultivo (trigo y algodón), además se consideró la interacción [tiempo*manejo(uso)]. Para el análisis estadístico de los datos correspondientes los análisis de las fracciones según tamaño de partículas se utilizó un modelo lineal mixto con los mismos tres factores inter-sujetos indicados en el modelo aplicado en el análisis de suelo global señalado anteriormente (el uso, el tiempo y el manejo) y un factor intra-sujetos (tamaño de partícula).El factor tamaño de partícula diferenció tres niveles: arena fina, limo y arcilla. Las varianzas en los modelos fueron estimadas por el método de Máxima Verosimilitud Restringida (REML). Cuando se detectaron diferencias significativas en alguno de los factores considerados o sus interacciones ($p < 0,05$), se realizó la prueba de Tukey. Todos los análisis estadísticos se realizaron utilizando PROC MIXED de SAS 9.1 (SAS Institute Inc., 2010).

La interacción entre el tiempo transcurrido desde la deforestación y el manejo no fue significativa para ninguno de los parámetros microbiológicos considerados, por lo cual se pueden considerar cada uno de los factores de forma independiente. El factor uso (forestal versus agrícola) fue significativo para los parámetros SOC, MBC, MBC/SOC, SR, qCO_2. Se detectaron valores más altos para los bosques de SOC, MBC y SR (mostrando estas tres propiedades correlaciones significativas positivas entre sí). Este resultado era esperable ya que la labranza, promueve la descomposición de la materia orgánica del suelo (SOM), la disminución de las fuentes de energía y de los sustratos disponibles para los microorganismos, lo que conlleva a la disminución del MBC y SR. Además, la cantidad de años transcurridos desde la deforestación, factor tiempo (deforestación en 1933, 1971 y 1980) mostró un efecto significativo en los contenidos de SOC, MBC y SR. Para SOC y MBC, el factor manejo (tipo de vegetación) también fue significativo. Los suelos forestales presentaron para las dos cocientes microbiológicos considerados (MBC/SOC y qCO2) valores significativamente más bajos que los suelos agrícolas. Estos valores más bajos indican que los suelos forestales presentaron condiciones de menor estrés para la comunidad microbiana, debido a que conservan niveles más elevados de SOC y una mayor acumulación de biomasa microbiana. Para la relación MBC/SOC la cantidad de años desde la deforestación también fue significativa y no lo fue el factor manejo, mientras que para qCO2 el tipo de cultivo (factor de manejo) fue significativo y no el factor tiempo transcurrido desde la deforestación. Los suelos sometidos a una deforestación más reciente (1980) presentaron los valores más altos para todos los parámetros medidos (no así para sus cocientes).

Nuestros resultados indicaron que el tiempo transcurrido desde la deforestación está asociado negativamente con el contenido de SOC, la labranza continua del suelo ha inducido la pérdida de C y ha liberado grandes cantidades de CO2 a la atmósfera. A pesar de que había transcurrido un número de años relativamente alto desde la deforestación y establecimiento del cultivo, las cantidades de SOC, MBC y SR fueron significativamente mayores en los suelos deforestados recientemente que en el resto. El tipo de cultivo afectó a algunas de las propiedades de los suelos estudiados. En los cultivos de algodón (considerados como manejo convencional) tuvieron valores significativamente menores de COS y MBC y también menor eficiencia (mayor cociente metabólico) que los suelos cultivados con trigo (considerados como de manejo reducido).

El N, la relación C/N en el suelo global y la POM siguen la misma tendencia que el SOC, es decir se obtuvieron valores significativamente más altos en los suelos bajo bosque que en los agrícolas y dentro de estos en los de más reciente deforestación (1980). Los tres factores considerados (uso, tiempo transcurrido desde la deforestación y manejo) fueron significativos para el N, pero sólo los dos primeros para la relación C/N y POM. Las cantidades de N y de POM estuvieron altamente correlacionadas con el contenido de la SOC y entre sí.

La interacción triple Tiempo*Manejo*Fracción (uso) fue no significativa para los parámetros considerados (SOC, N y relación C/N). Sin embargo, las interacciones dobles en las que se consideraba el factor fracción (tamaño de partícula) fueron siempre significativas, ello conlleva a la necesidad de estudiar que sucede con cada factor en cada tamaño de partícula, o bien considerar para cada tamaño de partícula el efecto de los factores considerados. En los suelos forestales las mayores cantidades de C y N estuvieron presentes en la fracción de arena fina, resultado esperable debido a que esta fracción se encuentra constituida principalmente por material orgánico fresco de pequeño tamaño, con valores por norma general significativamente más altos que en las fracciones de limo y arcilla. Se observó la tendencia inversa de los suelos agrícolas, donde la arcilla presentó concentraciones de C y N significativamente más altas, indicando que en esta fracción la estabilización de la SOM mediante el complejo arcillo-húmico hace que ésta se acumule. La relación C/N presentó valores significativamente más altos para el suelo de bosque en la fracción arena fina, mientras que para las parcelas agrícolas fue en la fracción limo. Los suelos forestales mostraron concentraciones significativamente más altos de C y N en todas las fracciones en comparación con las parcelas agrícolas, con excepción de los contenidos de N en la arcilla, donde las diferencias no fueron significativas. Estos resultados se explican debido a que el laboreo de los suelos en los cultivos causa la ruptura de los agregados, favoreciéndose la aireación y dando lugar a pérdidas de C y N tanto en las fracciones más lábiles como en las más estables. Las grandes pérdidas de POM también conducen a pérdidas de N, ya que estas dos propiedades se correlacionaron significativamente.

El número de años transcurridos desde la deforestación y posterior establecimiento del cultivo afectó significativamente a la concentración de C y a la relación C/N, pero no al N. Este último depende principalmente de las prácticas de manejo llevadas a cabo en los suelos (fertilización nitrogenada). Los suelos de las parcelas deforestadas más recientemente (1980) presentaron en general los mayores valores de C, N y para la relación C/N en todas las fracciones en comparación con las deforestadas en fechas anteriores. Los resultados muestran que la capacidad de las arcillas para preservar el C parece disminuir a medida que aumenta el número de años que lleva cultivándose el terreno. Para todas las fechas de deforestación consideradas, la arcilla presentó valores significativamente más altos de C y N, mientras que la relación C/N presentó valores significativamente más altos en la fracción de limo. La ausencia de diferencias significativas obtenidas para la relación C/N en la fracción de

arcilla de todos los suelos agrícolas considerados, pone de manifiesto la acumulación en esta fracción de una MO más estable que en el resto de las fracciones que no se ve afectada ni por el tiempo transcurrido desde la deforestación ni por el manejo o tipo de cultivo.

Los mayores valores de SOC y N (significativo para casi todas las comparaciones) se encuentran siempre en arcilla, y para la relación C / N en el limo. El contenido de SOC en la fracción de arcilla de los suelos no mostró diferencias significativas con el tipo de manejo, debido al comportamiento estable de esta fracción. La fracción de arcilla en los suelos de cultivo de trigo mostró siempre valores significativamente más altos para N que en las otras fracciones. La fertilización nitrogenada en el cultivo de trigo tuvo como consecuencia la acumulación e inmovilización del N en las partículas de arcilla dando también como resultado valores más bajos para la relación C/N. En relación a la fracción de arena fina, la MOS ligada a este tamaño de partícula es lábil y se ve afectada por el tiempo transcurrido desde la deforestación y posterior cultivo, pero no por el tipo de cultivo existente.

En el presente estudio se puede concluir que la deforestación y establecimiento de uso agrícola en la zona de estudio condujo a la disminución de los niveles POM, y de SOC y N y de las propiedades microbiológicas, lo que sugiere un deterioro de la calidad del suelo. Después de 25 años desde la deforestación y establecimiento de los cultivos estos sistemas han alcanzado un equilibrio, no mostrando diferencias significativas en los valores de qCO2 con los suelos de las parcelas deforestadas hace 35 y 73 años. Las prácticas de labranza reducida de los cultivos de trigo han permitido una mayor protección del SOC, en comparación con las prácticas convencionales de labranza de las parcelas de algodón. El número de años transcurridos desde el cambio de uso del suelo es más transcendente en los procesos de transformación de la materia orgánica edáfica en la zona estudiada que el tipo o intensidad del manejo que se ha llevado a cabo en el cultivo.

La cantidad de POM y la concentración de SOC y N en la fracción arena fina puede ser considerada como indicadores sensibles con el fin de monitorear los cambios en los regímenes de uso del suelo, sin embargo estas concentraciones en la fracción arcilla se mantienen bastante estables, protegiendo a la materia orgánica

asociada a estas partículas de su descomposición y no mostrando diferencias significativas ni con el efecto del tipo de cultivo ni con el tiempo transcurrido desde la deforestación y cultivo. El efecto del tiempo transcurrido desde la deforestación y puesta en cultivo afecta principalmente a las partículas de tamaño arena> limo> arcilla y se asocia negativamente con los valores de C y N en estas partículas. En las partículas de tamaño arcilla, después de 25 años de cultivo la capacidad de esta fracción para preservar SOM parece haber alcanzado un valor constante.

INTRODUCTION

1. DEFORESTATION AND IMPACTS ON SOIL ORGANIC C

Deforestation is a common practice in many countries worldwide since many years, in order to gain land for agricultural purposes, and is one of the main reasons for the global net release of CO_2 from soil to atmosphere (Veldkamp, 1994; Kucuker et al., 2015; Gómez-Acata et al., 2016). Conversion of natural to agricultural land resulted in the loss of 50-100Pg of soil organic carbon (SOC) worldwide over the past 200 years (Fließbach et al., 2007). A sustainable ecosystem depends on nutrient fluxes through the trophic levels, which are mainly mediated by microorganisms, the driving force for soil organic matter (SOM) turnover. But when forests are cleared for agriculture, the system becomes open. This creates a dependence on external inputs of nutrients in order to balance the output by harvests, leaching and erosion (Nogueira et al., 2006). Another result of deforestation for agricultural use is the altering of the biogeochemical cycling with oxidation of SOC and the establishment of a new equilibrium in soil C content. The effect of forest clearing for other land uses is often a low level of soil microbial population and enzymatic activity due to changes in soil microclimate (Sahani and Behera, 2001). The latter authors also reported that deforestation caused adverse changes in soil texture, structure, hydrological regime, nutrients and microbiological quality which included soil microbial biomass (SMB) and activity. Carbon inputs in agricultural systems are generally lower than in native forests, whereas sites under native forests have higher microbiological activities, followed by reforested areas, than in agricultural sites (Nogueira et al., 2006). Fifty years of agricultural use after forest removal showed declines in some biological activities and processes mediated by microorganisms. Devegetation led to lower microbial and biochemical quality of soil, as revealed by the lower SOM and microbial activity of the disturbed with respect to the undisturbed soil (Bastida et al., 2006). The conversion from natural vegetation to cropland often leads to a depletion of the SOC stock due to reduced input of biomass and enhanced decomposition after physical disturbance (Poeplau and Don, 2013). According to Dinesh et al. (2003), deforestation and cultivation significantly reduced microbial activity due to decline in available SOM/substrate levels. They concluded that forest clearance for agriculture:

a) decreases biodiversity

b) limits natural vegetation

c) simplifies the ecosystem structure

d) has detrimental effects (especially on biological and biochemical) soil properties, since microclimatic conditions at the ground level are modified.

Hajabassi et al. (1997) reported in their study that deforestation and subsequently tillage practices resulted to a 50 % decrease of SOM and total nitrogen (TN) and a decrease in aggregate size, overall resulting to a decrease of soil quality (SQ) and productivity of natural soils. Hence, deforestation and consequent practices of agricultural crops led to a severe damage of SQ, an increase in soil erosion and extensive nutrient losses (especially NO_3-N and Ca).

The significance of the presence of organic matter (OM) in the soils has been proved by many researchers. Organic matter is an important binding agent of soil aggregates (Angers et al., 1992). Karlen et al. (1994) refer to resistance to degradation, nutrient cycling, and water retention as soil functions affected by SOC. Beneficial influences of SOM include soil aggregate formation and stability, increased water holding capacity and improved nutrient retention (Mao et al., 1992). Organic matter has a beneficial effect on SQ and crop productivity and has the potential to sequester C from atmospheric CO_2 increases (Rasmussen and Parton, 1994). Reicosky et al. (1995) stated that productivity of soils is strongly related to SOM contents. Soil organic C and the humus fraction play significant roles in soil microaggregation leading to greater soil erosion resistance (Sahani and Behera, 2001). Organic matter could also be considered as a source for N, P, K, Fe, Mn and many beneficial elements (Panayiotopoulos, 1984; Eleftheriadis, 2005); its presence improves aeration and consequently drainage in soils with high clay contents (Eleftheriadis, 2005).

Losses of SOC often occur when converting forest to cultivated land (natural to agricultural ecosystems), mainly due to lower inputs of organic matter, reduced physical protection of SOC as a result of tillage and changes in soil temperature and moisture regime which accelerate decomposition rates (Tan and Lal, 2005). Soil tillage involves the physical disturbance of the upper soil layers, breaking down soil aggregates, thus influencing C stability in the soil (Paustian et al., 2000). According to Lantz et al. (2001), the magnitude of the decrease of SOC depends on land use factors (soil type, drainage, management practices, duration of current use), management and ecological factors.

Cultivation causes significant disturbance to soil organisms and reduction of SMB (Alvarez et al., 1998), and decreases the SOM content (Angers et al., 1992), due to:

a) dilution of SOM through mixing of the organic matter rich surface horizons with horizons low in organic matter
b) accelerated mineralization due to repeated tillage operations
c) increased erosion
d) lower amounts of C inputs.

Glaser et al. (2000) and Francini et al. (2007) stated that changes in soil management practices is one of the main factors influencing the amount, quality and turnover of SOM. Cropping and tillage systems are determinants of the amount and distribution of SOM, especially in the upper layers of the soil profile (Alvarez et al., 1998). Cultivation of soils results in the disruption of aggregates and loss of SOM compared to native sod and pasture soils (Beare et al., 1994), and conventional tillage practices can cause in the long term significant losses of SOM leading to an increase in soil erosion and loss of soil structure. These negative effects can be diminished by use of reduced tillage systems (Alvarez and Alvarez, 2000). Soil OM levels are controlled, except by climatic and pedogenetic factors, and by tillage, crop rotation, fertilization and residue management (Angers et al., 1992). Soil and crop management practices such as cultivation, crop rotation, residue management and fertilization, influence considerably the level of SOM retained over time (Chander et al., 1997). Crop management induced changes in soil moisture, soil temperature, crop rooting and crop residue input can have a large impact on SMB (and mineralizable C and N) which in turn affect the ability of soil to supply nutrients to plants through SOM turnover (Franzluebbers et al., 1994). According to Dalal et al. (1991) and Lantz et al. (2001), introducing tillage to a virgin soil decreases the amount of SOM. Reasons for this:

a) temperature change
b) moisture fluxes – aeration

c) exposure of new soil surfaces through aggregate disruption - degradation of soil structure
d) reduced additions of organic materials
e) increased soil erosion
f) removal of biomass
g) exposure of SOM to mineralization and decomposition processes

All these reasons lead to different rates of microbial activity.

On the other hand, Wardle et al. (1999) found no evidence of tillage affecting soil C loss after seven years.

Fauci and Dick (1994) stated that crop rotations, residue management, fertilization and other management practices can significantly affect SQ by changing soil physical, chemical and biological parameters.

Conservation tillage techniques seem to increase SOM in the upper layer, thus increasing the micro-aggregation and aggregate stability. These practices therefore could promote an enhancement of C sink at a global scale (Lal, 1997).

Soil systems will reach an equilibrium if both the environment and the agricultural practices remain constant over long periods. If agricultural management changes, the equilibrium will be disturbed and a new one will be reached (Anderson and Domsch, 1989). The effects of crop rotation and tillage on SOM become significant only after several years (Reicosky et al., 1995). Accumulation of organic matter in the soil confers important improvements in SQ, soil fertility and C sequestration (Roldán et al., 2007).

Soil disturbance and SOM losses are believed to be associated with reduced soil biodiversity (Wander et al., 1995).

2. SOIL MICROBIAL BIOMASS

Soil Microbial Biomass can be defined as the part of SOM that constitutes living microorganisms smaller than 5-10μm^3 (Alef and Nannipieri, 1995), or the cell mass of fungi and bacteria (Anderson, 2003), or the living microbial cells in the soil which are a constituent of total organic carbon, as Nogueira et al. (2006) argued. Soil microbial biomass (the active fraction or living component or labile pool or active form) of

SOM plays an essential role in the short term of nutrient turnover in the soil. It constitutes a reservoir of nutrients, participates in nutrient cycling and is responsible for organic matter and residue decomposition (Alvarez et al., 1995, Alvarez et al., 1998, Alvarez and Alvarez, 2000). It is a pool (a labile source and a sink) for nutrient (N and others) delivery and can be considered as an ecological indicator for structure formation and stabilization of soil (Dalal et al., 1991; Alef and Nannipieri, 1995). Soil microbial biomass is considered the living part of SOM, it is responsible for the transformations of added and native SOM (Dalal et al., 1991), correlates with aggregate stabilization (Harris, 2003) and is the most active SOM pool, whereas SOC is the active and the passive pools (Franzluebbers et al., 1994).

Soil microorganisms constitute the basic consumer trophic level of the decomposer subsystem, controlling SOM breakdown and subsequent release of nutrients and their availability for other organisms. Further, microbial activity and biomass dynamics help to regulate long term soil properties such as net fluxes and amounts of SOC and nutrients (Wardle et al., 1999). Anderson (2003) argued that microorganisms play a leading role in soil development and preservation.

The size of SMB is governed by various management practices, such as crop rotation, cultivation, organic amendments, fertilization and crop residue management (Chander et al., 1997). Karlen et al. (1994) determined the soil quality functions affected by SMB as resistance to degradation, and plant growth through nutrient cycling. With the increase of SMB and biomass turnover, more SOC is sequestered (Lantz et al., 2001). Biomass is believed to play a major regulatory role in soils (Wander et al., 1995).

Soil microbial biomass is less than 5% of organic matter volume in the soil, but it performs three critical functions (Dalal, 1998):

- it is a labile source of C, N, P, S
- it is an immediate sink for C, N, P, S
- it is an agent of nutrient transformation and pesticide degradation

Furthermore, microorganisms:

- form symbiotic associations with roots

- act as biological agents against plant pathogens
- contribute towards soil aggregation
- participate in soil formation

Soil microbial biomass comprises 2-3% of the SOC and 3-5% of the soil N. Besides serving as a source and sink for mineral nutrients and organic substrates in the short term, it acts as a catalyst for conversion of plant nutrients from stable organic forms to available mineral forms over longer periods. Crop productivity and nutrient cycling are therefore controlled by the amount and activity of SMB. Soil management, tillage methods and seasonal conditions, all affect the amount of biomass (McGill et al., 1986). Soil microbial biomass responds usually to seasonal moisture and temperature and to residue and tillage management.

Soil microbiological properties have been reported as a reliable tool in order to estimate early changes in the dynamics and distribution of soil microbial processes in different land use systems (Bending et al., 2004; Liang et al., 2012).

According to Carter (1991) microbial biomass carbon (MBC) is related to SOC. He also found that organic carbon in biomass of reduced tillage was higher than in plowed soils. Fauci and Dick (1994) agreed that SMB is closely related to SOM content. Microbial biomass carbon and microbial biomass nitrogen turnover rapidly and reflect changes in management practices long before changes in SOC and TN are detectable. This leads to the potential of SMB to serve as a SQ indicator. Usually a close relationship exists between the quantity and quality of the soil organic substance and the quantity and metabolic activity of the microorganisms (Emmerling et al., 2001). Mao et al. (1992) and Marinari et al. (2006) also stated that soil microorganisms, their growth and their activity are intimately linked and dependent to the C status (or inputs) of the soils, a statement which comes to agreement with Nogueira et al. (2006). Anderson (2003) argued that relations between functional microbial diversity and pools of SOC exist.

Microbial activity confirms the negative effect of devegetation on the microbiological quality of the soil, also reflected by MBC (Bastida et al., 2006). Residue treatments have higher total carbon and microbial activity (Karlen et al., 1994). High temperatures and low moisture lead to a decrease in microbial activity (Bastida et al., 2006).

In order to evaluate the degree and the direction of changes that occur to forest land after deforestation and subsequent cultivation, indicators have been proposed by many researchers. Microbial indices proved to be sensitive to changes that occurred to soil processes (Moscatelli et al., 2005). Measurements of the soil microbial community may be used to determine biodiversity, ecological processes and structures, and also to have a utility as an indicator of the re-establishment of connections between the biota and restoration of function in degraded systems (Harris, 2003).

Microorganisms play a leading role in soil development and preservation, in fact there is a close linkage between the biotic compartment and biogeochemical cycling. Since microorganisms respond rapidly to changing environmental conditions they are considered as sensitive indicators of soil health and could be therefore used for soil status monitoring (Pompili et al., 2006). Soil biota populations are considered an important and labile fraction of SOM involved in energy and nutrient cycling. Soil microbial biomass, soil enzymatic activity and soil respiration (SR) respond more quickly to changes in crop management practices or environmental conditions than SOM (Chander et al., 1997). Soil microbial biomass is an important source and sink for the majority of nutrients available to plants. Thus, it can influence the growth and development of crops. Nutrient fluxes through SMB are much faster than in the remaining SOM, implying that SMB could be used as an important indicator of changes in soil health and SQ produced by agricultural practices, especially in temperate regions (Roldán et al., 2007).

Soil organic carbon, TN, MBC and mineralizable C and N were highly correlated to each other. But MBC and mineralizable C appear to be more sensitive to changes in SOM content than the others, due increased crop residue input with increasing crop intensity (Franzluebbers et al., 1994). Many scientists, like Karlen et al. (1994) have suggested SMB and SR as SQ indicators for assessing long term soil and crop management effects on SQ. Emmerling et al. (2001) also stated that soil microbial properties, such as SMB and microbial activity, are suitable indicators to predict soil biological status as a part of soil fertility after transition from the high-input agricultural systems to low-input systems. When soil management changes, SMB responds more quickly that does SOM, which is relatively slow to change (Anderson and Domsch, 1989) and do not always provide adequate information of changes in soil quality that may occur (Cardelli et al., 2012). It is therefore important to identify SOM fractions more sensitive to a change of land use or management which can be applied as early indicators of the dynamics of the soil C. It can be concluded that MBC is an indicator of early changes in SOM brought by management practices, like type of tillage, straw incorporation, etc (Powlson et al.,, 1987, Angers et al., 1992). It can be used as a rapid indicator of the response of SMB to changes in soil management that affect the turnover of SOM (Perucci et al., 1997). Although SOC has been used for some time as an indirect biological indicator, its response to interferences was found less sensitive than some microbial parameters. MBC, MBN and ammonification rates responded readily and reliably to changes in soil use and strategies of reforestation, making them highly promising SQ indicators (Nogueira et al., 2006). The role of the microbial fraction in mediating soil processes and their relatively high rate of turnover, logically suggests that the microbial fraction could be an indicator and early predictor of changing SOM processes (Marinari et al., 2006).

Microbial biomass C responded rapidly to change in tillage and management prior than SOC and N (Carter, 1986), and can be used as a sensitive indicator of SQ and closely related to soil fertility (Roldán et al., 2007). But, MBC was unaffected by the direct effects of inorganic N. This may be an advantage for MBC as an indicator of soil biology, particularly for agricultural systems (Fauci and Dick, 1994). This indicator had a relatively rapid response to organic amendments, suggesting that it could be useful in identifying positive soil management effects on a temporal basis. The greater sensitivity of MBC than SOC to C input in the soil, suggests that the active SOM pool undergoes more fluctuation due to crop management practices than the passive SOM pool (Franzluebbers et al., 1994). Soil microbial biomass responded to mulching much earlier than SOC, consistent with the tendency of the soil microflora to serve as an early indicator of the effects of agricultural management (Wardle et al., 1999).

Soil microbiological quality, which determines the sustainable fertility status of soil, can be adjusted through the precise estimation of SMB and activity (Sahani and Behera, 2001).

Microbial biomass C was found higher in undisturbed than disturbed plots (Bastida et al., 2006); in both plots no correlation between SOC and MBC was found, although many other authors say the contrary, maybe due to markedly reduced efficiency of carbon use or rather reduced microbial efficiency. Deforestation reduces MBC contents (Gómez-Acata et al., 2016). The available C substrates for microbial growth decline when long-term tillage takes place (Follett and Schimel, 1989). Carter (1986) found similar biomass levels in the top 10cm under no tillage (NT) systems and moldboard ploughing. Alvarez and Alvarez (2000) found an increase of SMB under NT in the surface layer, whereas they report a reverse trend for deeper layers. At surface layers of NT and plowed soil, SMB was found 54% more under NT for the 0-7.5cm and decreased with depth up to 30cm. At the plowed soil SMB was greatest in the 7.5-15cm layer. The SMB levels are closely associated with distributions of SOC, TN, water content and water soluble C as influenced by tillage management (Doran, 1987).

Ploughing decreases SOC and MBC contents of the surface 6cm from 40-50%, but if we consider the overall 24cm, no effect is observed (Angers et al., 1992). A wheat fallow system reduced MBC and N in 0-15cm by 60% compared to a continuous wheat system, due to a decrease of returned residues (Carter, 1986). No tillage practices decreased MBC and N and microbial activity 10-23% in 0-5cm compared to shallow tillage. Arable crops and subsequent cultivation encourage biomass decline (Carter, 1986).

According to Follett and Schimel (1989), C availability for microbial growth and SMB declined with increased tillage intensity. Crop residues serve as a substrate (C, N, etc) that is converted to SMB and SOM. Calderón and Jackson (2002) mentioned the microbial community was altered within days of disturbance.

3. SOIL RESPIRATION

The CO_2 efflux is a physical process defined as the flow of CO_2 from the soil towards the atmosphere, and is measured at the soil surface (Calderón and Jackson, 2002). Frank et al. (2006) gave a definition for SR as the process whereby CO_2 evolves from the soil surface from metabolic activity of soil microbes and roots. When SR is measured in laboratory conditions only the microbial activity is taken in account. In the present study when we are referring to SR, we have measured the microbial soil activity. Insam et al. (1991) stated that SR reflects the availability of slow-flowing C for microbial maintenance and is a measure of basic turnover rates in soil. The evolution of CO_2 indicates microbial activity (Constantini et al., 1996); CO_2 is produced in the soil when microorganisms decompose organic substances to obtain energy for their growth and functioning (Wang et al., 2003; Liu et al., 2006). Soil CO_2 fluxes originate from autotrophic root respiration and heterotrophic microbial respiration (by decomposition of OM) in the rhizosphere and the bulk soil (Buchmann, 2000; Borken et al., 2002; Frank et al., 2002; Lohila et al., 2003). Soil respiration represents a major component of the soil C cycle and is an indicator of soil C storage, soil biological activity and overall soil quality (Lee and Jose, 2003a) and reflects the overall activity or energy spent by the indigenous microbial pool (Alvarez et al., 1995; Constantini et al., 1996). According to Raich (1992) SR is the total CO_2 production in intact soils resulting from the respiration of soil organisms, roots and mycorrhizae. This activity is sustained by OM inputs in the soil from aboveground and from roots. Soils play an important role in the production and consumption of CO_2. Soil-vegetation systems can act as a CO_2 sink or source, depending on decomposition rate and rate of SOC formation (Veldkamp, 1994).

Three principal components of SR may be defined: root respiration (30-70%), surface-litter respiration and the respiration of SOM (including root detritus). While CO_2 is produced in the soil, microorganisms decompose organic substances to obtain energy for their growth and functioning (Wang et al., 2003). The net CO_2 balance depends on CO_2 uptake by gross photosynthesis and CO_2 release by shoot, root and soil respiration (Lohila et al., 2003). On a global scale SR in terrestrial ecosystems is estimated to total 50-75Pg C/year (Raich, 1992).

The benefits of SR (along with MB) are increased soil aggregate formation and stability, enhanced plant litter decomposition, increased nutrient cycling and transformations, slow release storage of organic nutrients and pathogen control (Carpenter-Boggs et al., 2003). Karlen et al. (1994), when the SQ concept was under investigation and development, mentioned several soil functions that are affected by SR, such as resistance to degradation, and plant growth through nutrient cycling. Nael et al. (2004) considered SR as a reliable indicator of soil quality. Tufekcioglu et al.

(2001) considered SR as an excellent indicator of total soil biological activity, and therefore of overall soil quality.

According to Al-Kaisi and Yin (2005) and Raich (1992), the rate of CO_2 emission depends on:

- The CO_2 concentration gradient between soil and atmosphere
- Soil temperature
- Soil moisture (not linearly)
- Pore size
- Wind speed
- Agricultural practices (tillage, residue management)
- Climatic conditions

Borken et al. (2002) also mentioned as factors affecting SR forest type, soil fertility and soil texture. Tree species and nutrient availability strongly affect the decomposition of litter thus influencing SR. They indicated though that soil temperature and moisture explain most of the variation, agreeing with Howard and Howard and Howard (1993) and Klopatek (2002). They also proposed other factors (not significant though) affecting SR, such as above litter production, soil pH, SOC and TN. According to Buchmann (2000) and Lohila et al. (2003) SR depends on:

- Climate
- Land use changes
- Temperature
- Precipitation
- Shifts from forest to agricultural use (litter and organic horizons removed, roots disturbed) or changing management practices
- Root N concentration
- Soil texture
- Substrate quantity and quality

Tufekcioglu et al. (2001) considered as factors controlling the overall magnitudes of SR the availability of SOM (correlates positively), soil moisture (correlates positively) and the density of plant roots, which provide the substrates for soil biological activity. Lee and Jose (2003b) mentioned along with the above, MB, texture and root density whereas Lee and Jose (2003a) mentioned pH, CEC and nutrient availability to have strong influence on forest soils. Lohila et al. (2003) mentioned soil temperature, soil moisture, SOC quantity and quality and soil texture. Soil respiration is correlated significantly with the availability of C in the light fraction (Alvarez et al., 1998) and reflects the overall activity or energy spent by the indigenous microbial pool (Alvarez et al., 1995; Constantini et al., 1996). According to Frank et al. (2006), SR is influenced by land use, management practices and environmental conditions.

Soil respiration rates increase with soil C concentration (Witter and Kanal, 1998). Soil organic C and SR are positively related (Lohila et al., 2003; Tufekcioglu et al., 2001), as Jinbo et al. (2007) concluded in their study. Wang et al. (2003) stated that SR rate is closely and positively related to MBC, agreeing with Constantini et al. (1996). Sato and Seto (1999) on the other hand found no relationship. Earlier, Santruckova and Straskraba (1991) found no relation to MB, since SR remained on average nearly constant. The microbial populations increased their respiration rates along with temperature increase (Bastida et al., 2006). Howard and Howard (1993) claimed that the release of CO2 is a function of temperature and moisture content. Klopatek (2002) agreed with this statement. Mao et al. (1992) suggested a close relationship among SR and soil fertility. The microbial activity is an index to assess soil fertility (Sigstad et al., 2002) and reflects the nutrient status reliably (Mohr, 2004).

Conversion of forests to agriculture is responsible for a substantial increase in atmospheric CO_2 concentration (Wagai et al., 1998). The global CO_2 release due to deforestation is estimated 1-3.2 Pg/year (Veldkamp, 1994). Devegetation has a negative effect on the development of microbial activity, as revealed by the significant lower values of BR (Sahani and Behera, 2001; Bastida et al., 2006). The respiration rate was found significantly higher at forest than plantation sites (qCO_2 is well) (Dinesh et al., 2003; Nael et al., 2004). Land use change that involves deforestation and conversion into farmlands is the principle CO_2 emission source in

Ethiopia (Lemenih and Itanna, 2004). Raich (1992) mentioned that in some studies the conversion of native vegetation increased SR. As Raich and Tufekcioglu (2000) and Tufekcioglu et al. (2001) stated in their studies, SR rates were found either the same or both higher and lower in forests compared, whereas Dinesh et al. (2004) mentioned much bigger SR rates at undisturbed compared to disturbed sites. Reclaimed soil systems showed a decrease with time of successional stages (Anderson, 2003). Vegetation influences SR by influencing soil microclimate and structure, the quality and quantity of detritus supplied to the soil and the overall rate of root respiration (Raich and Tufekcioglu, 2000). Lohila et al. (2003) attributed the lower SR rates of bare soil to limited substrate supply into the soil. Cultivation accelerates microbial respiration and increases loss of SOM (Ghani et al., 2003), by improving soil aeration, increasing soil and crop residue contact and enhancing plant nutrient availability (Al-Kaisi and Yin, 2005; Angers et al., 1992), but also due to the rapid increase in microbial activities in decomposing the labile SOM pool (Al-Kaisi and Yin, 2005). Management practices influence SR through their influence on SOM (Tufekcioglu et al., 2001). Follett and Schimel (1989) found higher microbial respiration in NT than plowed sites. Cultivation strongly affected (negatively) SR at the initial stage of cultivation (Jinbo et al., 2007). The magnitude of the CO_2 losses depends on the frequency and intensity of soil disturbance caused by tillage (Al-Kaisi and Yin, 2005). Different tillage implements affect CO_2 efflux and microbial activity (Calderón and Jackson, 2002). In tillage systems, management of residues affects soil CO_2 efflux by altering soil temperature and water content, both of which affect microbial populations and activity (Frank et al., 2006). Soil disturbance leads to faster breakdown of OM. As a consequence SR raises along with soil C losses (Lee and Jose, 2003a). The differences were attributed to SOC content, greater fine root biomass and higher soil moisture content.

Reduction in soil disturbance greatly reduces soil respiratory C efflux and along with C supply are the major factors determining the magnitude of SR (Frank et al., 2006). Saggar et al., (2001) stated that the greater proportion of soil C that is decomposed in pasture soils than cultivated soils leads to higher rates of soil respiration. Soil respiration is related to C availability in the biomass, and is generally higher at the soil surface under no-till because of greater biological activity (Alvarez et al., 1995; Carter, 1986; Follett and Schimel, 1989). Differences in soil CO_2 efflux among treatments could be attributed to differences in SOC and MBC, suggesting that land use plays a significant role in soil CO_2 efflux from respiration (Frank et al., 2006). Soil respiration between tillage regimes had greater differences than MBC or SOC, indicating greater sensitivity to C substrate availability (Franzluebbers and Arshad, 1996).

4. MICROBIOLOGICAL RATIOS

Microbial biomass carbon (MBC), potential soil respiration rate (SR), metabolic quotient (qCO_2, ratio of respired C to biomass C), ratio of microbial biomass C to total organic C (MBC/SOC), are variables that have been suggested as indicators for assessing soil management effects on soil quality (Anderson, 2003). Ratios between microbiological parameters have often been used for evaluating the microbial ecophysiology implying an inter linkage between cell-physiological functioning under the influence of environmental factors (Anderson, 2003), and are suggested to be more useful than microbial variables related to soil weight when evaluating microbial populations and microbially mediated processes in soils. They also are useful tools to evaluate structure and physiology of microbial populations, microbial processes and certain properties in soil (Dilly and Munch, 1998).

4.1 The microbial quotient (MBC/SOC)

The MBC to SOC ratio (MBC/SOC) proved to be a reliable soil microbial parameter for describing changes in man-made ecosystems. For evaluating reclamation effects, the ratio can be considered superior to its single components (MBC, SOC) and to other parameters (Anderson and Domsch, 1989; Insam and Domsch, 1988) as a good index of the changes in SOM quality (Roldán et al. 2007).

According to Anderson (2003) the microbial quotient (along with the metabolic quotient) is considered as an indicator of growth, reflecting the C availability for growth and it could be also used as a stability indicator for a quick recognition of an environmental change. The ratio reflects the availability of substrate to soil microflora and the fraction of recalcitrant OM in the soil (Dinesh et al., 2003; Moscatelli et al., 2005), and also the quantum of C immobilization into the microbial standing crop (Sahani and Behera, 2001). The ratio has been proved to be an indicator of the utilization of SOC by the microbes in terms of SOM turnover rate (Agele et al., 2005). Entry et al. (1996) mentioned that the ratio along with MBC are poor predictors of annual crop yield, but the ratio may be an accurate indicator of soil health and a good predictor of long term crop yield.

Franzluebbers et al. (1994) agreed that MBC/SOC may be a more sensitive property to changes in SOM quantity and quality due to differences in organic input, than SOC alone. Soil Microbial Biomass and activity and MBC/SOC have been found more sensitive indicators of soil agricultural practices with short term reactions, compared to total SOM (Emmerling et al., 2001). The MBC/SOC ratio reflects the efficiency of conversion to MBC (Sparling, 1992) and can be considered as a useful index for changes in SOM resulting from land management changes. According to Fließbach et al. (2007) it reflects the quality of SOM for SMB establishment and it could be used as an indicator of SOM quality, or as an early indicator of soil quality (Marinari et al., 2006). These indicators (along with qCO_2 –the metabolic quotient, i.e. the ratio of basal respiration to SMB) are supposed to constitute an early warning system for soil deterioration. The microbial activity was closely related to the soil nutrient status but SMB did not consistently reflect nutrient availability (Mohr, 2004).

4.2 The metabolic quotient (qCO_2)

The metabolic quotient has been defined as the ratio of SR to MBC, the specific respiration of the biomass, specific maintenance respiration, metabolic quotient, community respiration per biomass unit, CO_2-C produced per unit biomass and time. It indicates the stress or the ecophysiological status of MB (Von Lutzow et al., 2002). According to Insam (1990), Wardle and Ghani (1995), Dilly and Munch (1998), Bauhus et al. (1998), Constanini et al. (1996), Islam and Weil (2000), Moscatelli et al. (2005), Saggar et al. (2001), Von Lutzow et al. (2002), Xu et al. (2006) and Jinbo et al. (2007), it indicates the efficiency of soil microbial populations (negatively correlated) in acquiring/utilizing OC (the efficiency of substrate utilization) and the intensity of C mineralization, and to Anderson and Domsch (1993) and Islam and Weil (2000) it reflects the stress in soils (positively correlated). Stress has as a result microbial inefficiency leading qCO_2 to rise (Wardle and Ghani, 1995). Anderson and Domsch (1993) reported it as a useful parameter in the study of bioenergetic changes in developing ecosystems. Mao et al. (1992) considered it as a valuable measure for explaining ecosystem functioning. It is an indicator of ecosystem disturbance and development, declining during the adaptation of a system to different agricultural practices (Alvarez et al., 1998). The metabolic quotient may be used to compare microbial communities and to quantify the effects caused by environmental differences (Constanini et al., 1996). It is suggested to be an adequate indicator of bioenergetic changes in developing ecosystems, and for progressive maturation of the soil system (Marinari et al., 2006). Xu et al. (2006) considered it as an alternative measure of changes in MB in response to disturbance and environmental limitations. As adaptation goes along qCO_2 decreases continuously (Anderson, 2003). According to Dinesh et al. (2003) it reflects the maturity of a soil system, with larger values at young soils, and the potential turnover of MBC (Franzluebbers et al., 2001). The ratio indicates the cell-physiological entity in a constant only under unchanging environmental conditions. Any impact on the cells (change in temperature, moisture, nutrient status, storage time etc) will be reflected in a change of the qCO_2. Along with SR, they may be important properties for explaining the differences in MBC of different soils or caused by different treatments (Insam et al., 1991). Physiological performance could be (along with MBC/SOC) employed for the characterization of the ‘baseline performance’ of a microbial community (Anderson, 2003). Along with MBC/SOC it reflects OM input and availability in the soils, efficiency of conversion to microbial C, losses of C from soil, the stabilization of OC by the mineral fractions and maintenance requirements of the soil microbial community (von Lutzow et al., 2002; Blagodatskaya et al., 2006). Therefore a rise in qCO_2 indicates soil quality degradation. Deforestation is a form of degradation, hence leading to a great rise of qCO_2 (Gómez-Ataca et al., 2016). Fließbach et al. (2007) related qCO_2 with the economy of MBC utilization, which is also connected to the complexity of the microbial food-web in soils. The metabolic quotient as a combination of microbial activity and population measurements appeared to provide more sensitive indications of soil pollution than the 2 parameters alone (Moscatelli et al., 2005). The higher the metabolic quotient, the lower the C assimilation efficiency (Bauhus et al., 1998). Overall, qCO_2 is most appropriately used as an index of adversity of environmental conditions (including both stress and disturbance for the soil microflora). In particular this index has valuable applications as a relative measure of how efficiently the SMB is utilizing C resources, and the degree of substrate limitations for the SMB (Wardle and Ghani, 1995). The more efficiently the microorganisms function, the greater the fraction of substrate C is incorporated into biomass and less C per unit biomass is lost through respiration (qCO_2 decreases) (Xu et al., 2006).

Plant dry matter production, different tillage practices, and straw incorporations or manure additions affect directly qCO_2 (Alvarez et al., 1995). Moscatelli et al. (2005) mentioned the strong relationship between qCO_2 (and MBC/SOC) with the nutritional status of the soil. Joergensen and Castillo (2001) and Noguiera et al. (2006) stated that the availability of substrate for microbial activity plays an important role for qCO_2. Anderson and Domsch (1989) mentioned the high influence on qCO_2 by the cropping practice, whereas they did not find any influence of fertilizer, previous crop cover, soil type and percentage of clay, SOC or pH. Bauhus et al. (1998) found it negatively correlated with pH, and highly affected by soil type.

The metabolic quotient was reported to be negatively correlated with SOC (Jinbo et al., 2007), MBC/SOC and MBC (Santruckova and Straskraba, 1991; Alvarez et al., 1995; Wardle and Ghani, 1995; Witter and Kanal, 1998; Joergensen and Castillo, 2001; Saggar et al., 2001; Moscatelli et al., 2005; Xu et al., 2006; Llorente and Turrión, 2009). Santruckova and Straskraba (1991) though mentioned no relation to SOC, and proposed 4 possible hypotheses for the reasons of the negative correlation between MBC and qCO_2. They argued that a) the decreased qCO_2 is caused by changes in the microbial community in the soil, b) the increase of qCO_2 is due to the effect of a stress (due to e.g. agricultural management), c) the proportion of active cells decreases with increasing MB, possibly limiting the activity by nutrient shortage, d) the inhibition of high concentrations of CO_2 produced by the microorganisms and dissolved in the surrounding medium. Low MB usually occurs in stressed or disturbed conditions, causing qCO_2 to rise (Wardle and Ghani, 1995). In some cases, higher levels of C inputs to the soil increase MB but not metabolic activity which in turn lowers qCO_2 (Insam et al., 1991; Alvarez et al., 1995). It was found to be negatively correlated with clay content (Joergensen and Castillo, 2001; Moscatelli et al., 2005), positively correlated with substrate availability, but negatively correlated with microbial pool size (Wang et al., 2003). It appears to be related to the decomposition of plant residues (higher before plowing of CT soil) (Franchini et al., 2007).

The availability of OM plays the dominant role for qCO_2 values. Decreases with time or succession in an ecosystem are mentioned (Odum's theory of succession) (when the ecosystem matures) (Insam and Domsch, 1988). Odum (1997, as referred by Harris (2003)), suggested that qCO_2 should decline during succession and increase during disturbance, because a small active biomass is present during the developing (immature) stages of an ecosystem where growth is essential for development, eventually leading to a larger less active biomass in the mature system. Wardle and Ghani (1995) considered it as an index of ecosystem development due to which it supposedly declines, and disturbance due to which it supposedly increases. The metabolic quotient decreases with increasing substrate quality (Bauhus et al., 1998) and has been reported to be higher under unfavorable than favorable conditions (Anderson and Domsch, 1993; Wardle and Ghani, 1995; Bauhus et al., 1998). Comparatively low qCO_2 values are a typical feature of diverse and highly interrelated communities (Fließbach et al., 2007). Higher values have been suggested as an indication of disturbance or stress and of soil maturity and rehabilitation (Franzluebbers et al., 2001). Joergensen and Castillo (2001) reported larger values for microbial communities in young soils. More developed soils evolve less qCO_2 than young soils (Insam et al., 1991). Higher in immature systems, decreases with maturity (Mao et al., 1992; Garcia et al., 2002; Dinesh et al., 2003; Marinari et al., 2006). Increased values of a conventionally managed soil reflect a higher maintenance energy requirement of the microbial community, and it can be used as an early indicator of energy transforming efficiency (Marinari et al., 2006). Generally, high qCO_2 and low MBC/SOC reflect a less efficient use (or difficulties in the use) of organic substates by MB, and/or suggest that the soil is an early stage of development (Pinzari et al., 1999; Moscatelli et al., 2005). On the other hand, low qCO_2 and high MBC/SOC express high stability, with the soil showing a tendency to preserve OM thereby maintaining the activity of MB at low values, and also indicate higher turnover time (Pinzari et al., 1999). When referring to organic farming, low qCO_2 and high MBC/SOC indicate better conserve of SOC (Marinari et al., 2006). The more efficiently the microorganisms function, the greater the fraction of substrate C incorporated into the biomass and the less C per unit biomass lost through respiration, leading to a decrease of qCO_2 (Sahani and Behera, 2001). Increases with stand age and showed be higher in a poor soil (Bauhus et al., 1998). The significant increase of

qCO_2 at some treatments of their study, suggested that the extra C made available for microbes was used to build up more MB (Moscatelli et al., 2005). According to Wardle and Ghani (1995) it could respond unpredictably (based on previous projects studied) and does not necessarily decline during succession, making it a not always reliable bioindicator of disturbance or ecosystem development, but it does decline during succession and following recovery from disturbance, because equilibrium conditions are approached and the soil microflora becomes more efficient on conserving C.

Many studies have reported differences in qCO_2 due to different types of management. Anderson and Domsch (1989, 1990, 1993) reported higher qCO_2 in monoculture than crop rotation, and in young compared to mature sites, mentioning that the younger the plots were, the higher qCO_2 they produced, with its values decreasing with progressing maturity of an ecosystem. According to Insam et al., (1991), it is negatively correlated with yield, due to 2 reasons: 1) for maintenance or growth, microorganisms might require more C and energy if they have to compete not only for C, but also for nutrients. 2) Clayey soils protect microbes from microbivores, so the microbial community ages. Young cells are metabolically more active, which would be reflected in a higher qCO_2. Insam and Domsch (1988) found decreases in qCO_2 with increasing time of agricultural sequence, but not under the forest. In agricultural soils, a high qCO_2 means that nutrient turnover is accomplished at high C expenses (Insam et al., 1991). Constanini et al. (1996) studied adjacent plots with same conditions and found not significant differences between tillage systems. Pinzari et al. (1999) found it higher in soils with greater diversity of plant litter. Dinesh et al. (2004) found bigger values at undisturbed compared to disturbed sites. Relatively large qCO_2 in forests is due to greater levels of readily degradable C content (for microbial degradation) and indicate that a relatively large percentage of the substrate is decomposed to supply the energetic demand of the microflora (Dinesh et al., 2003). Soil respiration did not differ between farming systems at the same intensity in their study, but when related to MB (qCO_2) then it differs up to 20%, suggesting a higher maintenance requirement of MB (Fließbach et al., 2007). The metabolic quotient is associated with soil degradation brought on by poor agricultural management (Islam and Weil, 2000).

5. DEFINING THE PROBLEM

In order to be able to make safe assumptions and assertions about the magnitude of changes, the validity of indicators and to be able to monitor reliably the changes in soil status, it is essential to run studies for several years, in order to be able to directly relate SMB dynamics to the long term sustainability of agroecosystems (Wardle et al., 1999). Soil microbial biomass and SR respond more quickly to changes in crop management practices or environmental conditions than SOM (Chander et al. 1997). Dinesh et al. (2003) come to agree with these aspects, stating that these two microbial indicators are sensitive to changes in the soil environment and could illustrate the effects of anthropogenic activities and disturbance on the soil.

Concluding, soil microbial and biochemical status in both natural and agricultural ecosystems have been used as bioindicators of soil stress or benefits from reclamation efforts. In addition to chemical and physical properties, soil microbial properties may indicate suitable management and restoration practices towards sustainability (Nogueira et al., 2006). Since microorganisms respond rapidly to changing environmental conditions they are considered as sensitive indicators of soil health and could be therefore used for soil status monitoring (Pompili et al. 2006).

Euro-Mediterranean regions are currently threatened by global changes (Ruiz Benito et al., 2010). Minetos and Polyzos (2007) carried out a regional analysis of forest land use changes in Greece during the last decades. They observed that the prefecture of Kilkis, where the present study is located, showed a high rate of depletion of their forest site. There is a scarcity of studies addressing land use change dynamics and its effect on SOC and microbial properties in these semiarid regions.

6. FRACTIONATION, RELATIONS WITH SOC AND N

Conversion of native forests to cultivation is usually accompanied by a decline in SOC and nutrients (Ashargie et al., 2007; Ratnayake et al., 2011). This depletion in SOC content is primarily due to accelerated decomposition rates caused by soil tillage, which enhances aeration and physical contact to decomposer organisms (Zinn et al., 2002). Soil OM is strongly influenced by management (Conant et al., 2001) and tillage of a soil breaks soil aggregates and exposes the previously protected SOM within aggregates to microbial decomposition (Ashargie et al., 2007), thereby increasing availability of C (Six et al., 2002). Grasslands and uncultivated soils generally have higher C and N contents than cultivated arable soils, due to the higher incorporation of SOC, the absence of soil tillage and reduced exposure to erosion (Hassink, 1997), whereas aggregate stability leads to enhanced SOM storage in soils under NT systems compared to tilled soils (Jacobs et al., 2009). Litter removal alters soil microbial processes and further accelerates soil C and nutrient losses (Mao et al., 1992). Changes in management practices are reflected by the SOC and N status, in particular by the proportion of SOM being easily transformed (active SOM) (von Lutzow et al., 2002). Forest clearing and continuous cultivation led to significant depletion of total SOC (55-63%) and N (52-60%) in the surface soils (Solomon et al., 2002). Lemenih and Itanna (2004) also stated that the soils of farmlands have significantly lower C and TN than natural vegetation. The buildup of SOC and N is determined by the amount and quality of the input of organic residues and their decomposition rate (Hassink, 1994). Wright and Hons (2004) mentioned that NT systems increase C and N storage concluding that overall, soil management strategies play significant roles in SOC and N sequestration. Tillage systems that minimize soil disturbance (minimum tillage, reduced tillage (RT), NT) generally increase the storage of SOM compared to conventional tillage (CT). This is partially due to the increased stability of macroaggregates compared to CT soils (Jacobs et al., 2010). Yang et al. (2004) reported that land use changes, especially conversion of forest to cropland, can alter C and N pools and N availability for plant uptake. Carbon and N losses mainly occurred in the top 20cm. Cultivating the soils can lead to C loss from agricultural soils because of the exposure and subsequent oxidation of previously protected OM; also it can initiate increases in soil mineral N content and denitrification within hours of the disturbance (Calderón and Jackson 2002). Management is the predominant factor determining variation in SOC stocks, but no correlation was found with texture or recent land-use changes as reported by Sleutel et al. (2006).

Soil organic matter fractions differ according to particle size distribution and the prevailing soil management system (Quiroga et al., 1996). In arable soils most of the SOM can be found in the clay and silt fractions, whereas in forest and grassland soils the contribution of sand size SOM to total organic matter is greater (Caravaca et al., 1999). With cultivation, the decline of SOM is most pronounced for the labile fractions (primarily particulate SOM). According to Leifeld and Kögel-Knabner (2005), the most sensitive fraction, with respect to land-use, was SOM in the >0.02mm fraction (part of the silt and the whole sand fraction, as defined by U.S.D.A. (Soil Survey Division Staff, 1993)). Guggenberger and Zech (1999) and Six et al. (2002) also reported that agricultural use of native soils resulted in a preferential depletion of particulate organic matter (POM), which provides an earlier indication of the consequences of different soil managements and is more sensitive than SOM and is highly influenced by the cultivation history of the soil (Six et al., 2002). Particulate OM is assumed to be associated with the sand fraction (Glaser et al., 2000). Clay contents are positively correlated with SOM concentrations (Serrano et al., 2016), when other factors such as vegetation, climate and hydrology, are similar (Schmidt and Kögel-Knabner, 2002). Carbon stored in the clay fraction contributes more to the long term stability of C than in the sand and the silt fraction (Ratnayake et al., 2011; Serrano et al., 2016). The protection of SOM and the stabilization of N by silt and clay particles are well established (Six et al., 2002; Romanya and Rovira, 2011) but fine sand (<0.2mm) also contributes (to a much lower degree) to the formation of stable organomineral complexes (Ratnayake et al., 2011). Due to structural stability of silt and clay fractions, more time is required to observe any effect of land-use changes (Saha et al., 2010). Solomon et al. (2002) reported for their project that the largest depletion of SOC and N occurred from the labile SOM associated with sand, but also from silt separates, suggesting that SOM in silt was quite susceptible to land use changes. On the other hand, small particles are particularly effective in stabilizing SOM and preserving large amounts of SOM in biologically resistant forms (Caravaca et al., 1999). As a general trend, fresh and relatively non-decomposed materials accumulate in the sand-size fractions, whereas aromatic-rich materials seem to be mainly associated with silts, and highly resistant compounds with the clays (He et al., 2009). Soil organic C associated with clay and silt sized particles in uncultivated grassland soils has been proposed to have a maximum concentration that is referred to as the soil protective capacity (Zhao et al., 2006). Hassink (1997) claimed that fine-textured soils have higher SOC and N contents than coarse-textured soils when supplied with similar input of organic material. The difference is assumed to result from the greater physical protection of SOM in fine-textured soils. One of the principal factors responsible for physical protection of SOM in soils is its ability to associate with clay and silt particles. The amount of C and N associated with clay and silt particles is mainly affected by soil texture and not by the inputs of organics in the soil, while OM in larger fractions is mainly affected by the organic inputs and not by soil texture (Hassink, 1997). Losses of SOC in topsoil were found to be more significant in soils with lower clay content (Zinn et al., 2002).

The C to N ratio suggests the level of humification and the degree of decomposition of the organic matter (Solomon et al., 2000; He et al., 2009): Forests generally tend to have higher values than agricultural sites. The basic premise behind this ratio is that organic carbon is the primary source of energy for soil microbes, but these also require nitrogen to multiply and utilize this energy. The microbes utilise this carbon via respiration, with the consequent loss of carbon dioxide from the soil. As the active fraction of the OM is thus degraded, the C/N ratio drops until a steady state (the passive fraction) is finally attained. The active fraction of the OM may have a C/N ratio between 15-30, the slow fraction typically 10-25, with the passive fraction stabilizing around 7-10 (Brady and Weil, 2002). A value of 12 is considered as typical for soils.

The C/N ratios of SOM have often been used as indices of quality; however, interpretation of trends can be difficult. Agricultural use of soils reduces total soil C/N ratios, which typically range from 14 to 8, as labile SOM is lost (Duxbury et al., 1989; Kaffka and Koepf, 1989). Typically POM has a C/N ratio of 20:1, with higher ratios in forested systems that accumulate litter. The C/N ratios decrease as labile constituents of SOM are lost.

OBJECTIVES

OBJECTIVES – BUILDING THE HYPOTHESES

The aims of this thesis were to determine the changes in terms of soil microbiological properties and soil organic carbon and nitrogen distribution in particle-size fractions that resulted from:

a) land use change from forest to agriculture
b) the amount of time from deforestation (26, 35 and 73 years)
c) type of crop stablished with different management types (wheat fields vs cotton fields)

For this purpose three stages of deforestation (1933, 1971, 1980) in the same area in Northern Greece were studied. The big amount of time transcurred since the first deforestation, the availability of three different deforestation dates and the remaining undisturbed forest, offered an interesting opportunity of studying these changes, along with assessing factors such as land use (forestry vs agriculture) and land management types correlating with the amount of time since deforestation (26, 35 and 73 years). The main hypothesis depended on whether the effects on soil chemical and microbiological properties as well as in SOC distribution in particle-size fractions, are similar in terms of amount of time since deforestation, but vary according to the subsequent type of crop installed (land management).

Secondary objectives were to determine which microbiological parameters can be considered as reliable indicators of the changes occurring in the soil, which soil particle-size fractions are mostly affected by land use change, by the amount of time from deforestation and by type of management, and which of these fractions contribute most to carbon sequestration.

MATERIAL AND METHODS

The study area: History and facts. The three deforestations.

The area of Filyria, a village which belongs to the municipality of Goumenissa, prefecture of Kilkis, province of Central Macedonia, North Greece (40°54'11.69" N to 40°53'41.9" N, and 22°28'47.97"E to 22°29'37.10"E), used to be a forest occupied mainly by *Quercus* species (and especially *Quercus pubescens*) and few other broad-leaved trees and shrubs typical of Greek climate (Photo 1). Socio-economic policies resulted to deforestation of the area at 3 stages (1933, 1971, 1980) leaving only 80 hectares of natural forest undisturbed. More specifically, great social-economical problems appeared as soon as the Greek refugees from Turkey were forced to leave their lands and come to Greece (agreed population-exchange by the two governments in 1922). Consequently, the Greek government decided to give land to the refugees. At the area of Filyria, the refugees exceeded the local population. So, new lands had to be regenerated in order to be distributed to the refugees. This led to a great scale deforestation starting in 1925 and ending officially at 1933. Each family was given 2-9 hectares of deforestated land. Those early years people were cultivating mainly wheat, but also broad beans, barley, oats and sesame. No fertilizers or pests were applied. Irrigation was possible only for the low elevated areas, where an old canal existed, supplied water by the small river which runs in the lowest altitude of the area. Fertilizers were introduced at 1950-1951. That year few people started cultivating flax. Agricultural machinery was first introduced at the area at 1959. The year 1961 brought great changes in the agricultural management of the area, as fertilizer distributor was introduced along with pumps which were pumping water out of the river. This led to the introduction of other more profitable types of cultivation, such as cotton.

The second deforestation of the area followed by distribution of land to non-land holders (people with no land property), took place in 1970 and was brought to completion in 1971. That year people started to digging (with machines) wells, up to 200m of depth, and pumping water out of the water-carrying horizons. These wells were soon spread all over the place (except at the fields of higher altitudes) along with the fields that had derived from the first deforestation. Now people were cultivating, along with wheat, cotton, tobacco and cherry-trees. Irrigation and higher altitudes was first practised at 1986-1987, but still and till today some fields were not irrigated, thus allowing only wheat to be cultivated.

In 1980 the third (and last until today) deforestation took place. No more than 70-80 hectares of forest was left. These high-elevated fields were not irrigated until 1995. Since that year some of them are irrigated. So, until 1995 the only cultivation was wheat, since 1995 tobacco altered with wheat, and at very few fields cherry-trees were established.

Maps of the three deforestations can be found in the appendix (Appendix no.2).

The agricultural fields (2-9 hectares each) are cultivated with wheat, cotton, tobacco, and cherry trees are established in several plots. The plots of the study are cultivated with cotton and wheat. The altitude of the study area ranges from 145-195 m. Mean slope of the area is 2%.

Photo 1. Photograph of the area. The *Quercus* forest plots can be seen in the back.

Climate of the area

The climate of the greater area is temperate Mediterranean with mean temperature (according to the Hellenic National Meteorological Service – EMY) of

15.0°C around the year, with absolute maximum of 40.4°C and absolute minimum of -17.4°C and mean annual precipitation of 505.9 mm (HNMS, 2006). The annual precipitation diagram can be seen in Figure 1.

Climatic data were received from a meteorological station which is 24km far away from the study area (at Trikala Imathias, prefecture of Imathia, Northern Greece). The station lies at 50m altitude. According to these data, there is an ecologically dry period of 4 months (from June to September), and snowfalls occur from December to February, snow is melting relatively fast. Fog may appear mainly at autumn and winter, but not for many consecutive days. Hail and frost may appear, but they are not of great concern.

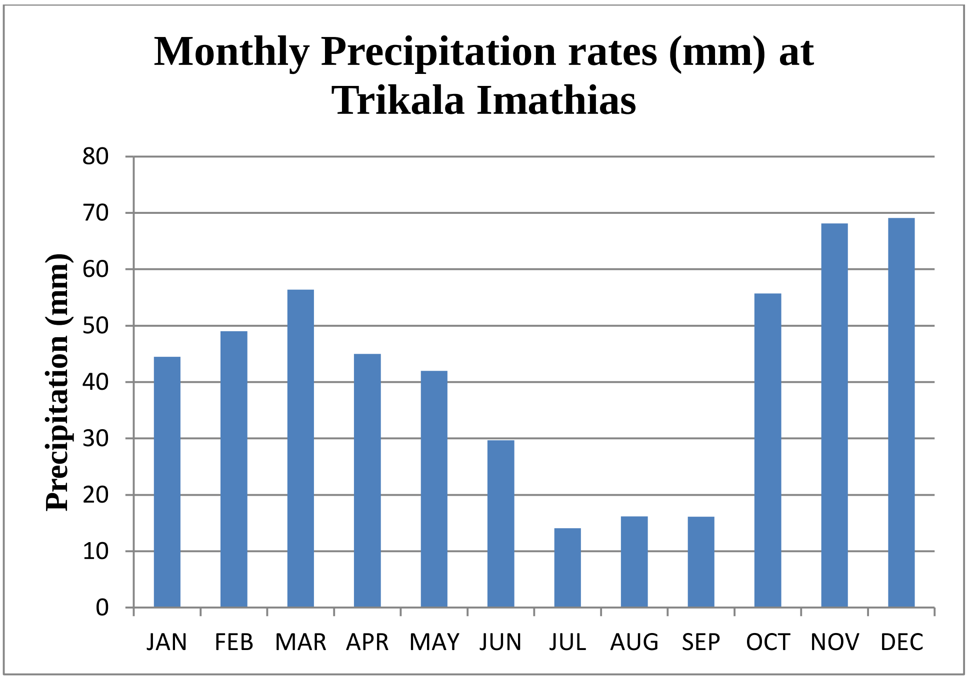


Figure 1. *Monthly precipitation rates (mm) at Trikala Imathias, 24km away of the study area (Hellenic National Meteorological Service – EMY).*

Geology and soils of the area

According to information obtained by the Institute of Geology and Mineral Exploration of Greece, the lower areas of the greater area of Filyria consist of recent to present formations, mainly alluvial deposits, eluvial mantle materials and fluvial deposits. The higher areas (i.e. the hills surrounding the river) consist of marls, mainly limestones, clayey marls, clays, sandstones, conglomerates of Neogene and locally Pleistocene age. The broad area belongs to Tertiary Neogene Upper Miocene, lacustrine-marine facies, psammitic-marly series and to Quaternary Pleistocene, fluvial-torrential deposits.

The soils of the area that have derived from the mentioned rocks are heavy clay soils, rich in Fe and Mn oxides, fertile but not productive due to bad aeration and cohesiveness. They belong to the Alfisols, Entisols and Inceptisols order of Soil Taxonomy. The soils of the study area have developed from limestone, and are classified as Xeralfs according to Soil Taxonomy (Soil Survey Staff, 1999, 2010). The A horizons have clay loam textures (% Sand: % Silt: % Clay 30:36:34 for the Forest surface horizon, and 26:38:36 for the Ap horizon at agricultural land, determinated by the International Pipette Method (USDA, 1972), mean pH (soil:water 1:2.5) is 7.82, and organic matter content of 1.9% for the cultivated plots and 2.4% for forest soils (Walkley-Black method). The $CaCO_3$ levels were determined with the method described below, in the analytical methods, and were found 1-7% at the forest sites and 1-14% at the cultivated plots.

The soil profile data for all the horizons can be seen at Table 1.

Table 1. *Soil profile data. The soil horizon depths: A (and Ap): 0-20cm; B: 20-45cm; C: more than 45cm.*

Site / horizon	%Sand	%Silt	%Clay	pH	%OM	%$CaCO_3$
Forest / A	30	36	34	7.95	2.39	0.92
Forest / B	53	30	17	8.06	1.18	1.09
Forest / C	54	34	12	8.20	0.89	47.38
Agr.site / Ap	26	38	36	7.73	1.90	1.08
Agr.site / B	27	40	33	7.63	0.83	0.42
Agr.site / C	56	29	15	7.90	0.00	51.84

Management practices

The main types of cultivation of the area are the following:

a) **Wheat** (*Triticum aestivum L.*). Management practices begin with ploughing up to 20-30cm at September. Sowing takes place until the end of November. Fertilizer (NH_4NO_3) is applied at the surface. Wheat is collected at June. No irrigation water is applied. After harvest, ploughing is again practised for mixing the remains with the soil. After 1985, 20-10-0 is used for wheat, 300-400 kg ha^{-1}, once per period, and NH_4NO_3 (on the surface) 100-150 kg ha^{-1} once per period.

b) **Cotton** (*Gossypium hirsutum L.*). Management practices begin with deep ploughing up to 30-40cm at September. At March, initially surface ploughing takes place with a ploughshare and subsequently the soil is disked. At April fertilizer (N-P-K + micronutrients) is applied and finally sowing takes place. Between the rows hoeing is practised twice for removing the weeds. Along with the first hoeing, fertilization with NH_4NO_3 is applied. 11-15-15 (or 12-12-17+micronutrients) is used for cotton, 250-300 kg ha^{-1}, once per period, and 100 kg $ha^{-1}$of NH_4NO_3 is applied twice per period at the surface. Harvest of cotton takes place at October. In terms of irrigation, 300-400 m^3 of water per hectare are applied every 10-15 days, 5-6 times at least at summer. Cotton in this area is irrigated by sprinklers, meaning that the whole soil surface of the area is watered. Cotton fields are altered with wheat every 2 years (two consecutive years of cotton crops, one year of wheat).

c) **Tobacco** (*Nicotiana tabacum L.*). The management practices are similar to those practised for cotton. Sowing begins at the end of April and the collection of the tobacco leaves at September – October. Fertilizer 11-15-15 is also used for tobacco, but in larger quantities, 500-700 kg ha^{-1}, once per period, and 200 kg ha^{-1} NH_4NO_3 at the surface twice per period. In terms of irrigation, every 15 days 500 m^3 of water per hectare should be applied. Tobacco fields are altered with wheat every 2 years (two consecutive years of tobacco crops, one year of wheat).

Wheat fields are cultivated solely with wheat. Management practices are carried out with the same principles since 1933. History data were collected from the Greek Forestry Service, the National Agricultural Service and from interviews with farmers managing the land since 1946.

Practice of irrigation is one of the main differences among the two management types, thus the cultivated are often referred as NIR plots (not irrigated, for the wheat fields) and IR plots (irrigated, for the cotton/wheat rotation fields) subcategories. This division relates with reduced tillage practices for the wheat plots (ploughing is applied once every year, no deep ploughing) and conventional tillage practices for the cotton/wheat rotation plots.

Historical, management and current state of the selected fields

The fields were selected according to the above management separation, wheat fields and cotton fields for every deforestation year category (1933, 1971, 1980). For each subcategory (management type within a deforestation category) one photograph is presented, the rest can be found in the appendix section.

All fields were first fertilized at 1960.

Photo 2. Wheat field deforestated in 1933.

Photo 3. Cotton field deforestated in 1933.

Photo 4. Wheat field deforestated in 1971.

Photo 5. Cotton field deforestated in 1971.

Photo 6. Wheat field deforestated in 1980.

Photo 7. Cotton field deforestated in 1980.

Experimental design and site sampling

Three sampling categories referring to deforestation year (1933, 1971, 1980) in cultivated field and one category to the forest were considered. For each year category two management subcategories were considered (wheat and cotton plots), and from each management subcategory, 12 plots were sampled, leading to a total amount of 72 samples for the fields. The samples consisted of 15 subsamples from the 0-15cm layer around the area, and mixed before analysis. The fields as numbered by the Municipality services are indicated in Table 2. For the control, the remaining undisturbed forest (*Quercus pubescens*), we took 12 samples. The sampling procedure was similar with the fields, from the 0-15cm layer, whereas clearing of the organic surface horizons took place before the collection of the samples.

Soil samples were collected at the end of November, after harvest, when microorganisms are fairly dormant (Wander et al., 1995).

Soil analysis - analytical methods

Samples were air-dried and sieved through a 2mm sieve. A portion of soil sample was ground for total organic C determination.

Soil organic carbon (gC kg^{-1}soil) was calculated from the total carbon measurement subtracting C from carbonates. Total Carbon was measured by dry combustion on a LECO 2000 C/N/H analyzer. In order to measure the carbonates, the HCl method was used, determination by elimination with acid previously titrated with 0.5 M NaOH (FAO, 2007).

Soil Microbial Carbon (mgC-MBC kg^{-1}soil) was determined by the fumigation-incubation method (Vance et al., 1987), and posterior C in extracts was determined by wet oxidation with dichromate (Tiessen and Moir, 1993). Briefly, samples were incubated at dark for a week at water-holding capacity, in order to activate the microorganisms. Two replicates of each sample (15g) were placed at the closed jar for chloroform ($CHCl_3$) fumigation process and left for 24 h at dark. Other two replicates were placed for the not fumigation process at the closed jar for 24 h at dark. Extraction with K_2SO_4 0.5M took place, and aliquote was digested with $K_2Cr_2O_7$ and H_2SO_4. After the digestion, the samples were titrated with Sal de Mohr. The MBC was calculated by MBC = E_C / k_{EC}, where E_C is the organic C extracted from fumigated soil minus that extracted from non-fumigated soil and k_{EC} is the extractable part of microbial biomass C after fumigation. The value k_{EC} = 0.45 was used to calculate microbial biomass C (Wu et al., 1990).

The microbial quotient (MBC/SOC) represented the fraction of MBC with respect to the SOC (Anderson & Domsch, 1993) and was expressed as gC-MBC kg^{-1} SOC.

Potential Soil Respiration (mgC-CO_2 kg^{-1}soil day^{-1}) was determined in closed jars and under laboratory-controlled conditions following the Isermeyer method (Alef and Nannipieri, 1995) modified by Llorente et al. (2008). The CO_2 respired is trapped in a NaOH solution, which is then titrated with HCl. After 1 week incubation, 50g of soil are put in the jars along with NaOH, closed firmly in the chambers under 29°C for 3 days. Subsequently, titration with HCl takes place. Soil samples were wetted to 75 % of water holding capacity.

The metabolic quotient (qCO_2) represents the potential soil respiration per unit microbial biomass, and was calculated as SR/MBC as reported by Anderson and Domsch (1993), and was expressed as [mg C-CO_2 g^{-1} C-MBC day^{-1}].

All the microbiological analyses were carried out by triplicates.

Particle-size fractionation

Particle-size fractionation was carried out in a total of 32 soil samples: 8 samples for the forest plots, 8 soil samples from plots deforested in 1933 (4 from wheat fields and 4 from cotton fields) 8 soil samples from plots deforested in 1971(4 from wheat fields and 4 from cotton fields) and 8 soil samples from plots deforested in 1980 (4 from wheat fields and 4 from cotton fields)

Soil samples were fractionated following a procedure that consisted of four steps:

1) Sonication with an energy of 60 J/ml to disrupt aggregates into single particles (sonifer Branson 450, t = 3 min 40 sec)
2) Manual wet sieving in two substeps to separate particles coarser than 0.2 mm (coarse sand) and between 0.2 mm and 0.05 mm (fine sand).
3) Sonication with an energy of 300J/ml to disrupt microaggregates (sonifer Branson 450, t = 60 min)
4) Centrifugation to separate silt from clay particles.

Briefly, 30g of air-dried soil sample was weighed and 150mL of water was added. Subsequently, the samples were ultra sonified for 3min 40sec with a Sonifier Branson 450 sonicator, inserting the sonication probe tip about 1cm in the suspension. The energy applied to the soil samples was 60 J/mL. Coarse sand (2-0.2mm) fraction was separated from the sonicated suspension by wet sieving. Subsequently, the fractions on the coarse sand were collected and further separated into mineral and particulate organic material (POM) through flotation-decantation in water. Fine sand (0.2 to 0.05mm) fractions was separated by wet seaving using a 0.05mm mesh sieve (for fine sand, we considered the very fine sand fraction and up to 0.2mm of the fine sand fraction according to the classification of U.S.D.A., as fine sand in our study). The fine sand was put in a pre-weighed glass plate and then in the oven (no more than 55°C) for drying and weighing next day. The remaining suspension, containing only silt (0.05 to 0.002mm) and clay (< 0.002mm) sized particles was ultra sonified for nearly 1 hour in order to brake the microaggregates applying an energy of 300J/mL.

To separate silt-sized particles (0.05 to 0.002mm) from clay (< 0.002mm), the remaining suspension was poured into 1L centrifuge bottles and centrifuged. The centrifuge run times and speeds to sediment particles of various diameters and densities in media of different temperature, specific gravity and viscosity were calculated using the mathematical formula of the program developed by “The coastal and Marine Geology Program of the U.S. Geological Survey based on Stoke´s Law” (Poppe et al., 1988).

$t = [8304285{,}28 * \log(18/(18-h))] / (rpm)^2$,

where t is time in minutes, h is the height of the solution in the plastic pots in cm, and rpm is the desired rounds per minute of the centrifuger, around 400-420 normally.

Silt stays in the bottom of the plastic pots, so the clay solution is emptied to a pre-weighed 2L beaker, centrifuged again, up to 3-5 times until all the clay has separated from the silt. Finally the beaker containing the clay solution is weighed. The silt from the plastic pots is placed to a pre-weighed glass plates for drying and weighing the following day. After homogenizing the clay solution (with a magnetic retriever for several minutes), some of the solution is poured into 4 pre-weighed small plastic pots, until it reaches 4 cm of height. All 4 pots with their taps are brought to the same weight, then closed, adjusted into the centrifuger (using paper, cardrobe, or plastic stuff) and centrifuged for 35min at 3500rpm. The remaining clay solution that was not used in the 2L beaker is weighed. The solution is emptied and placed at the small plastic pots containing the clay in the refrigerator until the remaining water turns into ice. Subsequently, 12 small plastic pots are placed in the lyophilizer, after more than 20-24 hours in order for the samples to have dried. The lyophilizer is closed, and the small plastic pots are weighed in order to find the clay that refers to the clay solution poured in the 4 small plastic pots and consequently find the whole amount of clay taking into account the whole clay solution that was initially weighed in the 2L beaker. Every 1-2 days the water of the lyophilizer should be taken out.

At the end, the fractions obtained were: coarse sand, fine sand, silt and clay. Particulate organic matter (POM) was separated from coarse sand.

Soil organic C and total N of the each separated fraction (fine sand, silt and clay) were determined by dry combustion using a LECO-CNH2000 autoanalizator. The ratio C/N was then calculated.

Determination of the power output of the ultrasonic equipment

For most sonicators the power output is much smaller than the displayed power output (North, 1976, Oorts et al., 2005). It is therefore necessary to measure the real power output of the ultrasonic equipment. The sonicator used in this study was calibrated by determining the real power output calorimetrically by measuring the temperature increase due to ultrasonic emission, as described in Oorts et al. (2005).

Statistical Analysis

- **Linear mixed model applied to parameters analysed in whole soil samples**

For the statistical analysis of the properties of whole soil we used a linear mixed model with three between-subjects factors: land-use, time (amount of years since deforestation) and management (type of crop). The mathematical formulation of the model was:

$$Y_{njk(i)} = \mu + \alpha_i + \beta_{j(i)} + \gamma_{k(i)} + \beta\gamma_{jk(i)} + \varepsilon_{njk(i)}$$

with i=1 (agriculture use), 2 (forest use); j(1)=1, 2 (cotton crop and wheat crop); j(2)=1 (forest use); k(1)=1, 2, 3 (1933, 1971 and 1980); k(2)=1 (forest use); n=1,…, 12 and being:

$Y_{njk(i)} =$ observed value of the dependent variable l in sample n of Land Use i with Management j and Time k.

$\mu =$ general mean effect

$\alpha_i =$ effect of Land Use i.

$\beta_{j(i)} =$ effect of Management j with use i.

$\gamma_{k(i)} =$ effect for Time k with use i.

$\beta\gamma_{jk(i)} =$ interaction effect of management j and age k with use i.

$\varepsilon_{njk(i)} =$ random error in the dependent variable in sample n of use i with management j and age k.

We considered that the random errors were independent with normal distribution and different variance for forest use and agriculture use, that is, $\varepsilon_{njk(i)} \sim N(0, \sigma_i^2)$. When significant differences were detected ($P<0.05$), Tukey's test was performed to allow separation of means.

- **<u>Linear mixed model applied to parameters analysed in particle-size fractions</u>**

For the statistical analysis of parameters determined in particle-size fractions we used a linear mixed model with three between-subjects factors (land-use, time and management) and a within-subjects factor (size of particle). The mathematical formulation of the model was:

$$Y_{njk(i);l} = \mu + \alpha_i + \beta_{j(i)} + \gamma_{k(i)} + \beta\gamma_{jk(i)} + \delta_l + \delta\alpha_{li} + \delta\beta_{lj(i)} + \delta\gamma_{lk(i)} + \delta\beta\gamma_{ljk(i)} + \varepsilon_{njk(i);l}$$

with i=1 (agriculture use), 2 (forest use); j(1)=1, 2 (cotton crop and wheat crop); j(2)=1 (forest use); k(1)=1, 2, 3 (1933, 1971 and 1980); k(2)=1 (forest use); n=1,…, 4 for agricultural use and n=1,….,8 for forest use; l=1, 2, 3 (clay, fine sand and silt) and being:

$Y_{njk(i);l} =$ observed value of the dependent variable for size l in sample n of use i with management j and age k.

$\mu =$ general mean effect

$\alpha_i =$ effect of land-use i.

$\beta_{j(i)} =$ effect of management j with use i.

$\gamma_{k(i)} =$ effect for age k with use i.

$\beta\gamma_{jk(i)} =$ interaction effect of management j and age k with use i.

$\delta_l =$ effect of size l.

$\delta\alpha_{li} =$ interaction effect between size l and use i.

$\delta\beta_{lj(i)} =$ interaction effect between size l and management j for use i.

$\delta\gamma_{lk(i)} =$ interaction effect between size l and age k for use i.

$\delta\beta\gamma_{ljk(i)} =$ three-way interaction between size l, management j and age k for use i.

$\varepsilon_{njk(i);l} =$ random error in the dependent variable for size l in sample n of use i with management j and age k.

The assumptions for the model were:

- $\varepsilon_{njk(i);l} \sim N(0, \sigma^2_{li})$
- $Cov(\varepsilon_{njk(i);l}, \varepsilon_{n'j'k'(i');l'}) = \begin{cases} \omega_i & \text{si } i = i', j = j', k = k', l \neq l' \\ 0 & \text{en cualquier otro caso} \end{cases}$

As a consequence, it has eight variance parameters which were estimated using the restricted maximum likelihood method (REML). When significant differences were detected ($P<0.05$), Tukey's test was performed to allow separation of means.

For the linear mixed models the SAS 9.1 PROC MIXED (SAS Institute Inc., 2010) was applied.

- **<u>Correlation analyses</u>**

It was also carried out a Pearson correlation analysis among all the studied parameters. For the correlation analyses the Statgraphics XVII-X64 software package was used.

RESULTS AND DISCUSSION

1. CHEMICAL AND MICROBIOLOGICAL PROPERTIES

In all the discussion that follows, except for the land use factor, we basically examined the main effects of the amount of years since deforestation and the management type (wheat, cotton) and not the interaction between them, as the double interaction (factor: amount of years since deforestation*management type) was not significant for any of the studied properties (shown in the following tables).

1.1 Soil Organic Carbon (SOC)

ANOVA results for SOC showed significant differences for the individual factors (land use, time (amount of years since deforestation) and management (type of crop)), and not significant differences for the interaction time*management (Table 2), hence we study the main effects separately.

Table 2. *ANOVA results for SOC, significance levels of the effects of land use, time (since deforestation), management (type of crop) and the interaction between time and management.*

Factor	**SOC**
Land use	***
Time (use)	***
Management (use)	*
Time*Management (use)	ns

Note: *significance levels: ***(p<0.001); **(p<0.01); *(p<0.05); and ns: not significant*

Values of SOC are shown in Table 3. Forest sites (undisturbed plots) had higher SOC concentrations, followed by the wheat plots deforested in 1980.

Table 3. *Mean values of soil organic carbon (SOC) in* [g C kg^{-1}soil] *for topsoil horizons (0-15cm) of forest, wheat and cotton crops of different deforestation year, and their standard errors (n= 12).*

Deforestation		1933		1971		1980	
	Forest	Wheat	Cotton	Wheat	Cotton	Wheat	Cotton
SOC	31.8 ± 1.4	8.5 ± 0.6	7.3 ± 0.6	8.6 ± 0.7	7.6 ± 0.7	14.0 ± 1.0	12.0 ± 0.8

In terms of land use, changing from forest to agriculture caused a significant decrease on SOC (Table 4). We assumed that the SOC level before changing the land use was the same in all soils**.**

Table 4. *Mean values of SOC for topsoil horizons (0-15cm) of forest and agriculture, and their standard errors.*

Land Use	Forest	Agriculure
SOC [g C kg^{-1}soil]	31.8a ± 1.4	9.65b ± 0.7
	n = 12	n = 72

Note: *different letters indicate significant differences (p<0.05), n=number of data*

In many studies forest soils were nearly always found to contain more SOC than deforested areas or agricultural soils (Glaser et al., 2000; Dinesh et al., 2003; Ratnayake et al., 2011). Hajabbasi et al. (1997) found higher amounts of SOM at the 0-30cm layer of forest soils than at deforested (agricultural) soils. Caravaca et al. (2004) also found higher C (and N) amounts in the forested soils of their study. According to Lemenih and Itanna (2004), changes in the amount of SOM following conversion of natural forest to agricultural land depend on several factors, such as:

- the type of forest ecosystem undergoing change
- the post-conversion land management
- climate
- soil type and texture.

Sahani and Behera (2001) argued that the low SOM levels of deforested barren sites lead to greater soil erodibility than that of natural forest, and, consequently, to loss of nutrients. Sigstad et al. (2002) stated that soil C after 15 years since deforestation with continuous cultivation is almost completely worn out.

The effect of time elapsed since deforestation was also significant for SOC ($p<0.001$, Table 2). The amount of time since deforestation was adversely associated with SOC (Table 5). Soils deforested in 1980 had significant higher values of SOC than soils deforested in 1971 and 1933, but no significant differences were observed between these latter groups (Table 5).

Table 5. *Mean values of soil organic carbon (SOC) for topsoil horizons (0-15cm) of crops of different deforestation year, and their standard errors, (n= 24).*

Year of deforestation	**1933**	**1971**	**1980**
SOC [g C kg^{-1}soil]	7.9b ± 0.6	8.1b ± 0.7	13.0a ± 0.8

Note: *different letters indicate significant differences ($p<0.05$) n=number of data*

Most losses of SOC due to introduction of a fallow system occur within 2 decades. Further loss is minimal provided soil erosion is minimal (Bremer et al., 1995). We agree with argument. Dinesh et al. (2003) argued that SOM is slow in revealing changes.

Management as a factor showed a significant effect on SOC ($p<0.05$) as can be seen in Table 2. Significantly higher SOC concentrations were found in the topsoil horizon of wheat plots than of cotton plots (Table 6). We must emphasize that we ascribe the management of the wheat plots ascribed as reduced tillage (RT) systems management, and for the cotton plots as conventional tillage (CT) systems management.

Table 6. *Mean values of soil organic carbon (SOC) for topsoil horizons (0-15cm) of wheat and cotton crops, and their standard errors.*

Crop plots	Wheat	Cotton
SOC [g C kg^{-1}soil]	10.4a ± 0.8	8.9b ± 0.7
	n = 36	n = 36

Note: *different letters indicate significant differences between wheat and cotton (p<0.005) n: number of data.*

According to Roldán et al. (2007) crop type, tillage system and soil depth had a significant effect on SOC. The authors also found SOC levels to be higher under non tillage systems (NT) than under moldboard plough practices up to 15cm. The greater amounts of SOC under NT may have been a result of reduced contact of crop residues with soil. Surface residues tend to decompose more slowly than soil-incorporated residues, because of greater fluctuations in surface temperature and moisture and reduced availability of nutrients to microbes at the surface residue. Moldboard plough incorporates residues into a larger volume of soil and therefore the rates of SOM decomposition and C mineralization increase by increasing the contact between soil microorganisms and crop residues, and by disruption of SOM protected by aggregates. Non tillage systems and generally conservation (or reduced) tillage are effective methods for increasing the levels of C sequestration, which would mitigate the atmospheric CO_2 enrichment.

Angers et al. (1992) found the highest value for SOC under pasture at the 0-15cm layer, compared to NT and CT (no differences among these two practices). These authors argue that SOC is fairly insensitive in the short term to changes in crops grown. Franzluebbers et al. (1994) found the levels of SOC in the top 5cm under NT 33-125% greater than under CT, and an increase of accumulation of SOC with increasing cropping intensity under NT than under CT. Beare et al. (1994) found 18% more SOC in NT than in CT in the plow layer. Although the input of residues was the same, the differences could be attributed to differences in assimilation and decomposition of SOM under the two tillage regimes. Repeated cultivation of soils enhances SOM decomposition, however changing the composition of the residual SOM, resulting in a more soluble, aromatic SOM with a higher content of acidic functional groups (e.g. higher fulvic/humic ratio) that substantially increases the susceptibility of soils to clay dispersion (Beare et al., 1994). In their study, Campbell et al. (1999) found significantly less SOC in the 0-7.5 cm layer under a tilled fallow-wheat system, after 4 years, no treatment effects in the 7.5-15cm layer, and no effect overall in the whole top 15 cm. After 4 more years, no more changes in SOC and N were detected, suggesting that the system may be reaching a steady state for SOC and N. Doran (1980) found significantly higher levels of SOC and N at the top 10cm of soils under NT compared to CT. He also stated that in previously tilled cropland, introduction of NT management increased the levels of SOC and N with time, but decreased them for deeper layers. The increase in OM with depth with moldboard ploughing is partially resulted from burying plant residue with plowing. According to Lal et al. (1994), SOC contents in the top 15cm were significantly affected by tillage, rotation and the interaction between them. Rasmussen and Parton (1994) found for all treatments except manure, a decline at SOC and N with time. They attributed most of the C loss with time to biological oxidation (since soil erosion is low) during the fallow year.

The absence of vegetation cover at barren sites results to a lack of organic substrate input leading to a decrease in the amounts of SOC and N (Sahani and Behera, 2001; Bastida et al., 2006). Roldán et al. (2007) stated that tillage promotes SOM decomposition through crop residue incorporation into the soil and physical breakdown of residues. When it comes to conservation tillage, SOM levels are substantially higher in the surface layer and gradually decrease to the same SOM content as CT below plow depth. This high SOM content near the surface and the surface residue are the main contributors to the improved surface soil properties that increase infiltration, decrease erosion, decrease evaporation rates and generally improve precipitation use efficiency (Reicosky et al., 1995). Conservation tillage had lower amounts of SOM than NT plots, where after 20 years SOM was less in the 7-15 cm layer but increased substantially in the top 7 cm.

Tillage effects are significant referring to C distribution with depth. Non tilled soils accumulate more SOM at the surface whereas under CT there is a uniform distribution at the top 15 cm (Alvarez et al., 1998). Angers et al. (1992) stated that repeated cultivation results in homogenization of C in the top 18 cm. Non tillage systems reduce the rate at which SOM is mineralized into its basic constituents and used by microorganisms as a source of energy. This reduction of the rate of SOM mineralization leaves more residues on soil surfaces and more SOM in the upper portions of the topsoil (Reicosky et al., 1995). Roldán et al. (2007) also stated that NT can enhance soil C sequestration and can be considered an effective management practice for carrying out sustainable agriculture under subtropical conditions, due to its improvement of soil physical and biochemical quality and soil C sequestration. They conclude that NT promoted surface accumulation of crop residues and was more effective in improving soil physical and biochemical quality than moldboard tillage.

Decreases in SOC in disturbed plots compared with the natural condition are due to lack of input by vegetal remains (Bastida et al., 2006). Non tillage improves the ability of the soil to sequester SOC due to greater physical protection of SOM, compared to CT (Alvarez et al., 1998).

Fertilizer application increases SOC content (Lantz et al., 2001). Nitrogen fertilizer effects on chemical and biological properties were relatively small compared with the effects of crop sequence and tillage (Franzluebbers et al., 1994). Management practices in the cultivated plots of this study consisted of high rates of N fertilization for the wheat plots (94-131 kg N ha^{-1} per year compared to 94-100kg N ha^{-1} per year when cotton is applied) partially reflecting the slightly higher (but not significantly) values for the management factor within each year category.

On the other hand, the effect of irrigation must be taken in account in order to explain the differences between wheat crops (not irrigated, NIR) and cotton crops (irrigated, IR) obtained in our study. Although Franzluebbers et al. (2001) found no significant differences between different precipitation levels, they stated that enhanced water levels (which is the case of irrigated plots) lead to higher plant production and C input, but also greater decomposition. In the present study soils under wheat crops showed significant higher SOC values than soils under cotton crops. Lemenih and Itanna (2004) found increases in C stocks with increase in precipitation rates, whereas excessive precipitation or increased amounts of irrigation water cause soil C leaching. According to McGill et al. (1986), the effects of tillage on biomass may be through an influence on soil moisture content or soil moisture change, or by influencing soil temperature regimes (Alvarez et al., 1998). Long periods of anaerobic conditions lead to an increase of SOC. Introducing artificial drainage adversely affects SOC levels (Lantz et al., 2001). Our results showed that the amount of water does not play that important role as the other factors of management practices, resulting (along with the higher decomposition rates) in not significantly different (slightly lower) values of the cotton (IR) subcategories compared to the wheat (NIR) subcategories, within each year category.

Concluding, differences in management did not affect the SOC contents as much as the amount of years since cultivation establishment did (Tables 2 and 3). Hence the quantity and overall amount (of years) of cultivation is more important in the SOM breakdown functions than the type and intensity of cultivation.

Soils with very low in C content tend to gain slight amounts of C after cultivation, but soils high in C lost at least 20% of C during cultivation (Mann, 1986). Soils high in C lose a greater proportion than soil with low C contents. There are strong relationships between SOM and enzyme activities and SOM and MB, but SOM is considered as a less sensitive indicator than either two (Marinari et al., 2006). Perucci et al. (1997) found significant correlations between SOC and all microbiological and biochemical parameters, indicating that SOC content plays an important role in determining the level of soil enzyme activity and, consequently, of soil fertility. In our study, SOC and MBC are positively highly correlated, with a value of $r = 0.87$ and a high significance level ($p<0.001$, Table 27).

The results of our work about SOC come to agreement with the above statements and the findings of the researchers. Forest sites (undisturbed plots) have significantly higher SOC contents. The order of the year categories are as expected, depending on the amount of years since deforestation, although the most recent category (1980) had significantly higher amounts than the other two. The slight (but not significant) difference between the two treatments (irrigated/conventionally tilled *vs* not-irrigated/reduced tillage practices, the with wheat (NIR) management type having slightly higher values) could be attributed to differences in management practices (wheat plots are considered as reduced tillage), to the rate of fertilization (fertilization mainly with N, for wheat) and to moisture effects due to enhanced water movements at the irrigated plots (irrigation is applied only at the cotton (altered with wheat) sites, once every 2 years), and, more specifically, to the combination of the effects of reduced tillage, reduced amounts of fertilizers and reduced water application by irrigation for the wheat fields than the cotton-wheat rotation plots. Practicing agriculture for many years leads to a further decrease of SOC levels, as reflected at the results of the year categories. The combination of the above comparisons leads to the results for the time*management subcategories, which followed the same tendencies with the year categories.

1.2 Microbial Biomass Carbon (MBC)

ANOVA results for MBC showed significant differences for the individual factors (land use, time of deforestation and management (type of crop)), and no significant differences for the interaction time*management (Table 7).

Table 7. *ANOVA results for microbial biomass carbon (MBC), significance levels of the effects of land use, time (amount of years since deforestation), management, and the interaction between time and management.*

Factor	MBC
Land use	***
Time (use)	***
Management (use)	***
Time*Management (use)	ns

Note: *significance levels: ***(p<0.001); **(p<0.01); *(p<0.05); and ns:not significant*

Values of MBC are shown in Table 8. Forest sites (undisturbed plots) had higher MBC concentrations, followed by wheat plots most recently deforested (1980).

Table 8. *Mean values of soil microbial biomass carbon (MBC) in* [mg C-MBC kg^{-1} soil] *for topsoil horizons (0-15cm) of forest, wheat and cotton crops of different deforestation year, and their standard errors, (n= 12).*

Deforestation		1933		1971		1980	
	Forest	Wheat	Cotton	Wheat	Cotton	Wheat	Cotton
MBC	1080 ± 28	508 ± 16	459 ± 14	471 ± 14	382 ± 10	622 ± 29	509 ± 72

Land use change from forest to crop caused a significant decrease on MBC (Table 9).

Table 9. *Mean values of microbial biomass carbon (MBC) for topsoil horizons (0-15cm) of forest and agriculture, and their standard errors.*

Land Use	Forest	Agriculture
MBC [mg C-MBC kg^{-1} soil]	1080a ± 28	492b± 27
	n = 12	n = 72

Note: *different letters indicate significant differences (p<0.05), n=number of data*

Microbial biomass carbon acts as an indicator of the microbial population size (Bastida et al., 2006). Measurements of the soil environment through MBC may minimize the number of indicators needed to predict changes in soil quality (Fauci and Dick, 1994). Sahani and Behera (2001) found 83% less MBC at deforested sites than the values under forest. Dinesh et al. (2003) reported significant declines of MBC in plantation compared to forest sites, and they attributed these decreases to differences in quantity and quality of the present and past substrate, to total dissolved organic C and N, to labile SOM among other reasons. As root growth and rooting density increases, so does MBC (McGill et al., 1986). Nogueira et al. (2006) reported higher amounts of MBC in native forests, followed by reforested sites, and finally at fallow, wheat cropped sites. These authors found MBC to be more sensitive to soil use and reforestation system than the basal respiration rate, although they were significantly correlated. They concluded that MBC is more effective indicator, and of course more sensitive than SOC to land use.

Our results confirm the affirmation of Pinzari et al. (1999) indicating that soils from sites with a greater above-ground diversity, as natural forests have higher decomposition rates and a larger amount of MBC with higher respiratory efficiency than those from natural or artificial sites with homogeneous plant coverage.

The effect of time elapsed since deforestation was also significant for MBC (Table 10). The amount of time since deforestation was adversely associated with

MBC. Among the year categories, the 1980 category had the (significantly) greater amounts of MBC compared to the other 2 categories, as expected, being the most recent deforestation. The amount of years since deforestation played a major role to the fact that the 1933 category had significantly more MBC than the 1971 category, indicating that the microbial processes after many years lead towards a system equilibrium.

Table 10. *Mean values of microbial biomass carbon (MBC) for topsoil horizons (0-15cm) of crops of different deforestation year, and their standard errors, (n= 24).*

Year of deforestation	**1933**	**1971**	**1980**
MBC [mg C-MBC kg^{-1} soil]	483b ± 15	426c ± 12	566a ± 55

Note: *different letters indicate significant differences (p<0.05) n=number of data*

The management type (type of crop) showed a significant effect on MBC ($p<0.05$) as can be seen in Table 11. Significantly higher MBC concentrations were found in the topsoil horizon of wheat plots (not irrigated plots) than cotton crop (irrigated plots).

Table 11. *Mean values of microbial biomass carbon (MBC) for topsoil horizons (0-15cm) of wheat and cotton crops, and their standard errors.*

Crop plots	**Wheat**	**Cotton**
MBC [mg C-MBC kg^{-1} soil]	534a ± 20	450b ± 32
	n = 36	n = 36

Note: *different letters indicate significant differences between wheat and cotton (p<0.005) n=nº of data.*

The increases of soil microbial biomass (SMB) in the top 5cm of soils under NT compared to previously tilled were found by Alvarez et al. (1998) to precede increases in SOC, suggesting its usefulness as a biological indicator of tillage-induced changes. Carter (1986) argued that SMB determined by the chloroform fumigation-incubation method is sensitive to tillage-induced changes in crop residue incorporation and root activity and responded to soil moisture regimes and other environmental factors, before changes could be detected by changes at SOC or N.

Soil MB is depleted from the top soil layer by cropping. As the frequency of fallowing increases, soil biochemical characteristics decrease (Campbell et al., 1999). Soil MB decreases following forest harvesting (Bauhus et al., 1998). The mean microfungal biomass also decreased in barren soils. Sahani and Behera (2001) stated that the low level of SMB indicated poor microbial growth in the deforested barren soil. Lack of a proper soil environment, altered hydrological regime and poor SOM level are the factors for such a decline in biomass size in the deforested site. According to Carter (1986), the declines in biomass encouraged by arable crops and subsequent cultivation can be handled by fertilization, crop residue management and other SOM sources. In an experiment conducted by Dilly et al. (1995) SMB values were found higher at wet grassland, followed by alder forest, dry grassland, extensive field and beech forest, and finally by intensive field (lowest value). Findings of Frank et al. (2006) also described undisturbed grasslands as larger pools of root biomass, SOC and MBC, compared to wheat systems. Chander et al. (1997) reported significant effects of crop rotation on the size of microbial C and N pools.

For the 0-5cm layer, Franzluebbers et al. (1994) found 33-125% greater MBC under NT than CT, whereas Carter (1986) for the top 5cm mentions a 10-23% increase under NT compared to shallow tillage. Roldán et al. (2007) also found greater MBC rates at NT than moldboard plough, up to 87%, stating that soils subjected to NT accumulated crop residues and SOC, which are substrates for soil microorganisms near the surface resulting to an increase in SMB and various soil processes in the surface soil under NT. They also reported that although MBC is significantly affected by soil depth and tillage system, in their study it was not significantly affected by crop type. Alvarez et al. (1998) found three times higher SMB amounts in pasture than cropped plots, but no difference between NT and CT. In the top 5cm though, all treatments had differences in SMB, and under pasture and NT this pools can be higher in the soil surface layer than in the 5-15cm layer. Balota et al. (2003) reported increased MBC by 103% when comparing NT to CT. Overall, management leads to differences in microbial communities (Wander et al. 1995). Tillage affects negatively the MBC contents, as observed and in our case, where RT wheat plots had 18.5% more than CT cotton plots. Agreeing with these results, Franzluebbers et al. (1994) reported MBC to increase with increasing cropping intensity. Burying the crop residues (instead of burning them) resulted to significantly higher MBC contents (Perucci et al., 1997). Sparling (1992) suggested that MBC much increased under fertilized pasture. Overall, management leads to differences in microbial communities (Wander et al., 1995).

Soil microbial biomass can be increased directly by soil amendments (from 80-400%) or indirectly by the return of more residues, created as a result of improved plant nutrition (Fauci and Dick, 1994). The differences in SMB response to organic amendments were, according to the authors, probably the result of substrate availability or amount of C input. Although SMB reflects past inputs over many years, it can be strongly influenced by a single organic input. McGill et al. (1986) suggested that average annual biomass quantities in each plot within the tillage depth should be more closely related to long term C inputs than to C additions during the current year. Joergensen (1995, as referred by Emmerling et al., 2001) found a mean SMB of 345μg/g soil at 27 agricultural sites. The increase on SMB can contribute to decrease nutrient losses from soil by leaching (Nogueira et al., 2006). Moscatelli et al. (2005) found that N fertilization promoted SMB enrichment, lowering energetic maintenance requirements, whereas Fauci and Dick (1994) reported no significant differences. Guo and Gifford (2002) gave an explanation of the latter report, stating that although fertilization can increase biomass production, it may also enhance decomposition. Moreover, it may reduce the relative allocation of C belowground.

Reductions of MBC at sites, in which herbicides were used, were attributed to losses in vegetation rather than to toxic effects of the herbicides to MBC (Li et al., 2004, as referred by Nogueira et al., 2006). Herbicides, and external inputs in general, may affect the bacterial structure and function in agricultural soils.

Significant differences observed in our study between type of crops can be attributed to reduced tillage practices at wheat plots compared to cotton/wheat rotation plots and to the increased amount of crop residues at wheat practices, (Franzluebbers 1994, Roldán 2007), enhanced application of N fertilizer which promotes MBC enrichment (Moscatelli et al., 2005), to the overall decrease of the amount of herbicides applied (herbicides affect bacterial structure and function

(Nogueira, 2006)), and to differences in hydrological regime (Sahani and Behera, 2001) (the soil remains wet much more days at the cotton (IR) plots due to irrigation application at the fields, suggesting microbial nutrient immobilization in the dry season, although drying appears to be correlated with the death of microorganisms). Hence, irrigation was not the main factor influencing the highest rate of MBC at wheat (NIR) plots, compared to the effects of reduced tillage practices and in general the management of the wheat fields.

In terms of year subcategories (time*management as a factor), the highest amounts are (as expected) found in the 1980 wheat plots, as the most recent plots with reduced tillage practices. Year of deforestation played a major role in MBC contents among the cultivated sites, although its influence at the order of significance when referring to subcategories seemed not as strong as the type of management.

One year of cultivation of a native soil resulted in a reduction of SMB on the surface 7.5 cm by approximately the same amount as 25 years of cultivation (Angers et al., 1992). Campbell et al. (1999) reported that MBC was not affected by tillage or fallow frequency after 4 and 8 years of their experiment, but after 12 years significant differences were detected at both 0-7.5cm and 7.5-15cm depths. Bauhus et al. (1998) mentioned declines in MBC from the upper to the lower soil horizons. Doran (1980) found much more amounts at the 0-7.5cm layer under NT than CT, and reported a rapid decrease under the 7.5cm under NT practices. Insam and Domsch (1988) reported that 30 years after reclamation, the MBC levels rose very fast to levels characteristic of undisturbed soils.

Microbial biomass carbon contents were generally higher during the early raining season in tropical China than before the onset of rainfall (Mao et al., 1992). It can be therefore assumed that nutrients derived from litter that accumulated on the soil surface during the dry season were immobilized by microorganisms as soon as water availability allowed microbial growth. But the higher amount of MBC during the dry season suggests microbial nutrient immobilization in dry conditions with subsequent release in the wet season. These observations come to agreement with the ones Singh et al. (1989, as referred by Mao et al., 1992) and Yang and Insam (1991, as referred by Mao et al., 1992) made, who found that SMB and nutrient pools declined during the period of the most rapid plant growth, which is the wet season. Singh et al. (1989, as referred by Mao et al., 1992) concluded that the principal function of SMB is to accumulate nutrients during the dry period and to release them rapidly upon onset of the wet period. The desiccation of the soil appears to be related to death of biomass (McGill et al., 1986). Its regrowth is associated with remoistening. Hence, environmental conditions and management practices affect the amount of SOM by controlling both C input and rate of biomass turnover. The effects of tillage on biomass may be through an influence on soil moisture content or soil moisture change. According to Bauhus et al. (1998), seasonal fluctuations of SMB and its activity were closely related to the substrate water content and temperature rates. Joergensen and Castillo (2001) concluded that humidity and soil water affect extremely biological properties and state that drying is able to kill microorganisms.

Microbial biomass carbon correlates with N content (Anderson and Domsch, 1989) positively through increased root biomass, especially when the levels of N fertilization are high (Spedding et al., 2004). We obtained a value of $r = 0.80$ (with a p value less than 0.001, Table 27) for these two properties, and the trends were exactly the same as will be seen in the nitrogen discussion chapter. Nitrogen is a basic nutrition element for the SMB. Soil Microbial Biomass can be protected by clay, but the correlations are not always significant compared to other types of texture (Marinari et al., 2006). We have the similar results ($r = 0.35$, not significant correlation, Table 27). According to Emmerling et al. (2001), soil texture of the top horizon significantly affects SMB, along with SOM and the ratio MBC/SOC. Soil MB increases along with increases at silt or clay contents and decreases at loamy sands or sandy loams. For their study, clay content was strongly positively correlated with SMB, as well as in ours, as can be seen at the Fractionation chapter. Microbial (and especially fungal) biomass may be responsible for aggregation following cultivation (Angers et al., 1992).

The forest values of MBC were, as expected, significantly higher than all the cultivated plots, whereas use of agriculture at deforested sites result to significantly lower amounts, by decreasing MBC amount, growth, activity, the available substrates for the microorganisms (substrate quantity and quality are adversely affected), restricting root growth, decreasing MBC amounts through the application of herbicides (affecting bacterial structure and function), altering the hydrological regime and by decreasing the above ground diversity leading to lower decomposition rates and to lower amounts of SMB.

1.3 The MBC to SOC ratio

ANOVA results for MBC/SOC ratio showed significant differences for land use and time of deforestation and no significant differences for management (type of crop) and the time*management interaction (Table 12).

Table 12. *ANOVA results for MBC/SOC, significance levels of the effects of land use, time since deforestation, management and the interaction between time and management.*

Factor	MBC/SOC
Land use	***
Time (use)	***
Management (use)	ns
Time*Management (use)	ns

Note: *significance levels: ***(p<0.001); **(p<0.01); *(p<0.05); and ns:not significant*

Values of MBC to SOC ratio are shown in Table 13.

Table 13. *Mean values of MBC/SOC ratio in* [g C-MBC kg^{-1} SOC] *for topsoil horizons (0-15cm) of forest, wheat and cotton crops of different deforestation year, and their standard errors, (n= 12).*

Deforestation		1933		1971		1980	
	Forest	Wheat	Cotton	Wheat	Cotton	Wheat	Cotton
MBC/SOC	37 ± 5	63 ± 4	67 ± 5	59 ± 6	57 ± 7	46 ± 3	44 ± 3

The values of the ratio in the studied soils varied from 3.7-6.7% (i.e. 37-67g C-MBC / kg SOC), whereas, as already has been previously presented, most of the researchers mention values from 0.5-7%. Nevertheless, some researchers, as Deng and Lü (1984, as referred by Mao et al, 1992), Yang and Insam (1991, as referred by Mao et al, 1992) and Emmerling et al. (2001) reported values greater than 10%. Sparling (1992) and Alef and Nannipieri (1995) stated that the ratio varies from 1-5%. Anderson and Domsch (1989) referred values of 0.3 to 7%, depending on: culture type, vegetation cover, soil management, amendments. Emmerling et al. (2001) reported a mean 4.1% ratio in their experiment, where they dealt with systems reduced N fertilization and rational amounts of pests. Joergensen (1995, as referred by Emmerling et al., 2001) found a mean 2.2% ratio at 27 arable soils. Mäder et al. (1993, as referred by Emmerling et al., 2001) reported mean values for the ratio 2.4-2.7% in conventionally managed soils. Marinari et al. (2006) mention values 3.2-5.0% for organic soils and 2.0-3.2% for soils under CT. The differences could be attributed to differences in cropping management than to soil texture (i.e. clay content). Considerable values of the ratio are probably due to easily available C fraction when organic material is introduced (Marinari et al., 2006; Moscatelli et al., 2005).

Reasons for the differences in the values could be attributed to:

- Environmental conditions (temperate climate)
- Season of sampling (Anderson and Domsch, 1989)
- Analyses techniques (Anderson and Domsch, 1989)
- Long term effects, as in this project (72, 34 and 25 years since the deforestations)
- High pH values (>7.5)
- Differences in soils (mineralogy, genesis factors)
- Vegetation cover (Anderson and Domsch, 1989)
- Management practices
- N fertilization rates
- Amounts of carbonates (Emmerling et al., 2001)
- Crop intensity (increases the ratio, as referred by Franzluebbers et al., 1994)
- Local conditions (Sparling, 1992)

Land use change from forest to crop caused a significant increase on the MBC to SOC ratio (Table 14). The forest values of the MBC/SOC ratio of this study were found significant lower than the values of the crop plots.

Table 14. *Mean values of MBC/SOC ratio in* [g C-MBC kg^{-1} SOC] *for topsoil horizons (0-15cm) of forest and agriculture, and their standard errors.*

Land Use	**Forest**	**Agriculture**
MBC/SOC [g C-MBC kg^{-1} SOC)	37b ± 5	56a ± 5
	n = 12	n = 72

Note: *different letters indicate significant differences (p<0.05), n=number of data*

The ratio of MBC to SOC could indicate if SOC is in equilibrium, accumulating or decreasing (Anderson and Domsch, 1989). According to Sahani and Behera (2001) it reflects the linkage and interaction between MBC and SOC, and it can serve as an index for monitoring soil perturbation. There is a very close quantitative relationship between MBC and SOC (Sparling, 1992; Anderson, 2003; Agele et al., 2005). When soil management changes, SMB responds more quickly than SOM, which is relatively slow to change. Hence, Anderson and Domsch (1989) argued that the ratio will increase for a time if organic matter input to a soil increases, and will decrease otherwise. The ratio declines strongly as the concentration of available organic matter decreases (Dinesh et al., 2003). Mohr (2004) found negative correlations between the ratio and SOC, and therefore high ratios in soils subjected to disturbance regimes following nutrient depletion and low ratios at plots with accumulation of easily degradable organic compounds. This is our case, as we observed significantly higher ratios of every subcategory of our study compared to forest values, except for the 1980 cotton plots. There was a strong negative correlation between the values of the ratio and SOC, due to the differences of the MBC contents in each soil, as presented in the previous paragraph of the MBC analysis. On the other hand, Agele et al. (2005) found positive correlations between the ratio and SOC in their experiment. Moscatelli et al. (2005) argued that the ratio decreases along with the contents of available SOM. Sparling (1992) mentioned a significant correlation between MBC and SOC, as in our case (r = 0.87, with a p value less than 0.001, Table 27).

Sahani and Behera (2001) ascribed the low ratio that was found generally at deforested sites and especially at deforested barren sites of their experiment, to less microbial immobilization of nutrients. Low ratios at deforested sites indicate loss of SOC and clearly reveal soil perturbation due to forest loss. The high ratios at forests could be due to the fact that more diversified organic substrate production and input in the soil subsystem support a more interdependent food chain leading to maintenance of a higher ratio (Sahani and Behera, 2001).

Our soils presented a low quality of SOC in terms of MBC support and establishment, leading to lower MBC/SOC dynamics in the forest plots. Although the high correlation between the two properties that form the ratio, the increase in SOC did not have a respective increase in the values of MBC, leading to lower ratios.

Sparling (1992) considered texture (clay content), mineralogy, SOC content (and especially stabilized C in the SOC fraction), vegetation and management history as the factors influencing the ratio. The amount of years since all three deforestations is big enough to smoothen these differences in the rapidity of changes between the two properties. The authors argued that changes in the ratio reflect:

- Organic matter input to these soils
- The efficiency of conversion to MBC
- Carbon losses from the soil
- The stabilization of SOC by the soil mineral fractions.

Sparling (1992) mentioned values from 1-4.3%, with higher values (and higher SOC values) at pastures than forest or arable crops, suggesting that the input of SOC was greater under pasture and that this OC was of a quality resulting in a greater MBC fraction. Regarding pasture as a baseline, maize plots had lower MBC, SOC and MBC/SOC values due to much decreased organic input. He also found at fertilized arable crops greater ratios than in the forest, probably due to fertilization. On the other hand, Yang and Insam (1991, as referred by Mao et al., 1992) found ratios from 10-12% in mature rain forests (mature soils are supposed to be in C equilibrium, with the greater values found in the A horizon). Deng and Lü (1984, as referred by Mao et al., 1992) found ratios up to 19% for mixed forests. Bauhus et al. (1998) reported an average of 1.64% in forest soils, with the ratio being higher under tropic soils (2.53%) than under temperate (1.39%) or boreal (1.42%). He mentioned that as the ratio increases, SOM becomes less recalcitrant. The author also stated that the ratio is dependent not only in SOM quality but also at the extent to which microbes are physically protected in substrates. Powlson et al. (1987) reported a value of 1% at sandy soils and 2-4% for soils with higher clay contents. Dinesh et al. (2003) found no significant differences between MBC/SOC in plantations and forest sites.

The effect of time elapsed since deforestation was significant for the MBC to SOC ratio (Table 15). Significant higher MBC/SOC ratios were observed in crop plots reforested in 1933 and 1971 than in 1980.

Table 15. *Mean values of MBC/SOC ratio for topsoil horizons (0-15cm) of crops of different deforestation year, and their standard errors, (n= 24).*

Year of deforestation	**1933**	**1971**	**1980**
MBC/SOC [g C-MBC kg^{-1} SOC]	65a ± 5	58a ± 7	45b ± 3

Note*: different letters indicate significant differences (p<0.05) n=number of data*

The SOC of our study is of a quality resulting in a greater ratio in the fields. Nitrogen fertilization and generally cultivation and management practices can have significant effects in the ratio of the deforested soils compared to forest soils, probably resulting in accumulation of C in the fields whereas in the forest an equilibrium has been reached; evenmore, MBC responds quickly when soil management practices change, compared to the undisturbed throughout the year forest plots. According to Anderson and Domsch (1990) and Anderson (2003), increased below-ground species diversity results to less community respiration consequently leading to more C available for biomass production, which is reflected in a high MBC/SOC ratio. At continuous crop rotations during years lower community respiration is leading to increased values of the MBC/SOC ratio with time.

Management as a factor showed no significant effect on the MBC/SOC ratio as can be seen in Tables 12 and 16. No significant differences for the MBC/SOC ratio were found between the topsoil horizon of wheat plots (NIR) and cotton plots (IR) (Table 16).

Table 16. *Mean values of MBC/SOC ratio for topsoil horizons (0-15cm) of wheat and cotton crops, and their standard errors.*

Crop plots	**Wheat**	**Cotton**
MBC/SOC [g C-MBC kg^{-1} SOC]	56a ± 4	56a ± 5
	n = 36	n = 36

Note: *different letters indicate significant differences between wheat and cotton (p<0.005) n=number of data.*

Under NT, Roldán et al. (2007) found 4.6% at the top 5cm, whereas under moldboard plough 3.1%. Balota et al. (2003) reported increased MBC and the ratio MBC/SOC, by 103% and 44% respectively, when comparing NT to CT.

The higher ratio under mixed cultivation rather than continuous cropping was caused by the quality of the OM input under mixed cropping being more suitable for microbial growth and survival (Anderson and Domsch, 1989; Sparling, 1992). If the conditions for microbial activity are optimal, the ratio will probably increase (Franzluebbers et al., 1994). Low values of the ratio indicate low availability of SOM to microorganisms (Joergensen and Castillo, 2001). This may be caused indirectly by an increased C input rate due to improved growth conditions for plants. Another reason could be a direct effect on the soil microorganisms i.e. the reduced demand for maintenance energy so that the same C input rate is able to maintain a higher level of MBC. Bastida et al. (2006) found the ratio higher in undisturbed plots (*Pinus halepensis* and natural Spanish herbs) than disturbed ones suggesting a higher C accumulation in the undisturbed plots.

The ratio can be significantly affected by the farming systems, regardless their fertilization intensity (Fließbach et al., 2007). Franzluebbers et al. (1994) stated that the ratio increases when cropping intensity increases, though not always. They also argued that increase in the ratio indicates an enlarging pool of SOM and accumulation of the most active fraction of SOM. Emmerling et al. (2001) reported a 10-15% increase on MBC/SOC, MBC and SOM at a site where intensive agriculture gave way to a low-input system (over 10 years). Insam and Domsch (1988) found forest soils to have a much smaller contribution of MBC to SOC the soil of the agricultural sequence of their research. The contribution of MBC decreased with time, more rapidly on the forest sites than on the agricultural ones. Values of MBC/SOC and MBC contents could also decrease in the long term due to reduced yield efficiency (Joergensen and Castillo, 2001). The ratio increases with ploughing and heap tillage compared to NT (Agele et al., 2005). The microbial communities of long term crop rotation systems are energetically more efficient (lower qCO_2) with a higher MBC/SOC value (suggesting increased biomass) as compared to monoculture soil systems (Anderson, 2003). If little biomass is produced, the ratio is low (Anderson, 2003). Anderson and Domsch (1989) reported a value of 2.3% for permanent monocultures and 2.9% for continuous crop rotations. With organic fertilizer the ratio rose up to 4 and 3.7% respectively. They also stated that the rise is a transient rather than an absolute phenomenon and is probably due to the easily available C fraction of the introduced organic material. The differences between the two management systems are probably caused by differences in management and not in texture. After green manure application the ratio increased to 4.04%. In organic soil management, the increase of the MBC/SOC ratio (with consequent decrease of qCO_2) indicates better conserves of SOC (Marinari et al., 2006). Carter (1991) reported increase in the ratio due to reduced tillage compared to grassland sites. Johnson and Williamson (1994) found at opencast mine sites very small values of the ratio up to 3 years after restoration. With straw incorporation practices, the ratio increases (Anderson and Domsch, 1989). Mixing of crop residues has a positive effect on the ratio (Franzluebbers et al., 1994). The relative high ratio found in the experiment by Emmerling et al. (2001) might be caused by methodological causes, since some soils of their study showed a significant content of carbonates, as in this case. According to this, an unknown amount of abiotic CO_2 release from these samples probably occurred. If there are no differences in the ratio, then a relative stabilization in the relations between SMB and SOC pools seems to be present (Mataix-Solera et al., 2006).

In mineral fertilized treatments a significantly negative correlation was found between the ratio and SOC, but not when organic fertilization was applied (Steiner et al., 2007). Considering the mineral fertilizations of our soils, this statement comes to agreement with our case, when taking the land use factor into account. Moscatelli et al. (2005) stated that fertilization increases the ratio, which along with qCO_2 seems to be strongly affected by the nutritional status of the soil; the microbial pool is strongly dependent on N and probably suffered from a competition with plants for this element. This competition is greater in forests where flora quantity and diversity is higher. This nutritional 'stress' could explain according to the researchers the decrease of the ratio in not fertilized plots to values lower than 2% that also Anderson (2003) reports, which is a critical threshold for soils with neutral pH. The ratio parallels the trend of inorganic N, as Moscatelli et al. (2005) observed in their study.

Franzluebbers et al. (2001) reported that mean annual precipitation affects the ratio adversely. At another of their studies (1994) they stated that when the water-filled porosity at the 12.5-20cm layer exceeded 80%, this fact could be considered as supraoptimal conditions for microbial activity and may hid reduced the MBC/SOC ratio. In our case the amount of water applied in the soil did not seem to have significant effects, as in general the management factor does not play a significant role (Tables 12, 13, 16).

Cultivation and management practices seem to affect significantly the cultivated plots of all the year categories only when taking into account the land use factor, resulting in significantly higher values for the agricultural sites than the forest ones. The differences between the two management types (wheat-cotton), although not significant, could be attributed to N fertilization rates (which also affect the differences between both management types compared to forest), reduced tillage practices (leading to higher MBC levels) of the wheat plots, and to the amount of crop residues accumulating different amounts of C.

As we will see in the soil respiration discussion chapter that follows, our results showed that the Quercus forest plots had higher respiration rates, leading to less C (proportionally) available for biomass production and to a final decrease in the MBC/SOC ratio. This theory was introduced by Anderson (2003). The correlation we obtained for the two properties (the microbial quotient and SR) are highly negatively correlated, with a p value less than 0.001 (Table 27).

1.4 Soil Respiration (SR)

ANOVA results for SR showed significant differences for land use and time of deforestation and no significant differences for management (type of crop) and the time*management interaction (Table 17).

Table 17. *ANOVA results for soil respiration (SR), significance levels of the effects of land use, time since deforestation, management and the interaction between time and management.*

Factor	SR
Land use	***
Time (use)	***
Management (use)	ns
Time*Management (use)	ns

Note: *significance levels: ***(p<0.001); **(p<0.01); *(p<0.05); and ns:not significant*

Values of SR are shown in Table 18. Soils under forest (undisturbed plots) show higher SR concentrations, followed by the wheat plots deforested in 1980.

Table 18. *Mean values of soil respiration (SR) in* [mg C-CO_2 kg^{-1} soil day^{-1}] *for topsoil horizons (0-15cm) of forest, wheat and cotton crops of different deforestation year, and their standard errors, (n= 12).*

Deforestation		1933		1971		1980	
	Forest	Wheat	Cotton	Wheat	Cotton	Wheat	Cotton
SR	39.1 ± 3.3	23.5 ± 1.2	23.7 ±1.2	22 ± 1.2	20.8 ± 1.2	30.3 ± 1.2	26.7 ± 1.2

Land use change from forest to crop caused a significant decrease on SR (Table 19). Soil respiration values in forest soils were, as expected, significantly higher than in the cultivated plots.

Table 19. *Mean values of SR for topsoil horizons (0-15cm) of forest and agriculture, and their standard errors.*

Land Use	Forest	Agriculture
SR [mg C-CO_2 kg^{-1} soil day^{-1}]	39.1a ± 3.3	24.5b ± 0.5
	n = 12	n = 72

Note: *different letters indicate significant differences ($p<0.05$), n=number of data*

This significantly higher value of SR in forest than in crop soils can be explained by the dominant role for SR played by the availability of SOM (Joergensen and Castillo, 2001). Rampazzo and Mentler (2001) stated that SR is strongly correlated to the amount of SOM in the soils. In our case there is a very strong correlation between SR and SOC (r = 0.77, Table 27), with a p value less than 0.001. According to Frank et al. (2006), higher SOC and MBC lead to higher SR, and lowest values of SR corresponded to sites with lowest MBC (Dinesh et al., 2004), in our study forest and agricultural soils, respectively. In most cases, soil moisture and substrate properties were more important in controlling the overall rate of SR than vegetation type (Raich and Tufekcioglu, 2000). As Lee and Jose (2003a) stated, SR was found to be positively correlated with SOM and MBC. Our results showed very strong positive correlations (for both SOC and MBC the p value was less than 0.01, Table 27). Bastida et al. (2006) argued that metabolic activity was much greater in the undisturbed plot of their experiment even 15 years after devegetation. Soil respiration correlates positively with litterfall rates in forests (Raich and Tufekcioglu, 2000). Tufekcioglu et al. (2001) found higher soil respiration rates in riparian buffers covered with different grasses than in adjacent crop fields. The differences were attributed to SOC content, greater biomass and higher soil moisture content. Our forest plots satisfy these terms. Fields under permanent grass had 50% greater respiration and MBC than CT or NT (Carpenter-Boggs et al., 2003).

The effect of time elapsed since deforestation was significant for SR (Table 20). Significant higher SR ratio was observed in crop plots reforested in 1980 than in 1933 and 1971.

Table 20. *Mean values of SR for topsoil horizons (0-15cm) of crops of different deforestation year, and their standard errors (n= 24).*

Year of deforestation	**1933**	**1971**	**1980**
SR [mg C-CO_2 kg^{-1} soil day^{-1}]	23.6b ± 0.9	21.4b ± 0.9	28.5a ± 0.9

Note: *different letters indicate significant differences (p<0.05)*

Campbell et al. (1999) found that SR activity was significantly affected from tillage practices only after 12 years. The authors also stated that cropping resulted in higher SR activity, likely associated with higher crop residue production. This seems to reflect the tendency of the 1980 plots to have significantly higher values than the 1971 and 1933 plots, probably due to more recent historical of tillage. The SR rates followed the MBC and SOC tendencies in terms of time since deforestation. The 1980 plots have significantly more SR due to more recent historical of tillage. The 1933 plots have higher (although not significantly) rates than the 1971 plots, probably due to the status near or towards equilibrium.

Type of crop showed no significant effect on SR (Tables 17 and 21). No significant differences for SR were found between the topsoil horizon of wheat plots (not irrigated plots, NIR) and cotton crop (irrigated plots, IR).

Table 21. *Mean values of SR for topsoil horizons (0-15cm) of wheat and cotton crops, and their standard errors.*

Crop plots	**Wheat**	**Cotton**
SR [mg C-CO_2 kg^{-1} soil day^{-1}]	25.3a ± 0.7	23.7a ± 0.7
	n = 36	n = 36

Note: *different letters indicate significant differences between wheat and cotton (p<0.005) n=number of data.*

Other authors have obtained a significant effect of management; Constantini et al., (1996) indicated that tillage impacts on SR were due to variations caused in MBC. Microbial stress and changes in microbial community structure after tillage may lead to decreases in SR (biological production of CO_2 by organisms in the soil) (Calderón and Jackson, 2002). Greater root biomass along with lack of soil disturbance by tillage raise SR levels (Frank et al., 2006), by providing an energy rich C supply for microbial activity, thereby increasing respiration rates.

Insam et al. (1991) found no relationship between SR and crop yield. As in our results, Fließbach et al. (2007) found SR not to differ between farming systems. In their study, SR and MBC at the top 15cm remained statistically indistinguishable between tilled treatments and the control (Calderón and Jackson, 2002).

A sudden increase of CO_2 output from soil is generally observed after the addition of easily available organic substrates or of inorganic N fertilizers to the soil. This phenomenon (the 'priming effect') is due to an increase of microbial activity resulting in an acceleration of SOM mineralization as substrate and energy source (Moscatelli et al., 2005). The authors also mentioned a significant response of microbial respiration to N fertilization. The fertilization impacts on SR generally appear to be small, but sometimes it appears to depress SR rates (Raich, 1992).

Soil respiration rates could be either higher or lower in CT compared to NT, according to the decomposition rate of residues near the surface (if slower then CT<NT) and to the level of soil temperature (if lower then CT<NT) (Frank et al., 2006). Generally, SR in grasslands is higher than in annual crops. Soil respiration along with MB are not expected to increase immediately after tillage (Calderón and Jackson 2002). No tillage regimes lead to higher rates of SOM, influencing positively SR (Lee and Jose 2003a). Alvarez et al. (1995) recorded 2-3 times more SR in NT than plough tillage, whereas Hendrix et al. (1988) recorded higher production of CO_2 under NT than CT. Alvarez and Alvarez (2000) observed 80% higher CO_2-C production under NT compared to CT. Significantly greater amounts of CO_2-C were released from NT and RT plots than CT; the release was positively correlated with MBC (Constantini et al., 1996). Actively cropped fields were found to have up to 20% higher SR than did adjacent fields without plants (mostly fallow fields), but the differences were not significant in their study (Raich and Tufekcioglu, 2000). Less intensive tillage treatments lead to lower CO_2 emission than moldboard plough (where more C is lost as CO_2 resulting in a decrease of SOC), especially immediately after tillage operations (Al-Kaisi and Yin 2005). So reducing the tillage intensity leads in enrichment of 0-15cm surface TC. Calderón and Jackson (2002) mentioned that

tillage of soils increased CO_2 efflux within 24h of the disturbance, but this fact may be due to physical degassing of dissolved CO_2 from the soil solution, since microbial respiration did not increase in tilled soils. Francini et al. (2007) reported ploughing to increase CO_2 by 57% on average. Moldboard plough treatment buried nearly all the residue and left the soil in a rough, loose, open condition and resulted in maximum CO_2 loss (Reicosky, 1997). There are contradictory results about the effect of increasing plowing and total respiration. Grabert (1968, referred by Reicosky, 1997) and Reicosky (1997) observed an increase in total respiration, whereas Richter (1974, referred by Reicosky, 1997) and Hendrix et al. (1988) observed greater CO_2 output from NT than CT. Wagai et al. (1998) like us, did not find any significant differences in soil CO_2 under tilled or no tilled corn.

Varying soil water content affects microbial respiration by limiting diffusion of substrates in water films, desiccating stress at low water contents and by limiting the diffusion of oxygen in pore spaces at high water contents (Davidson et al. 1998, Davidson et al. 2000). Xu et al. (2006) also stated that humidity affects microbial activity. High water content can impede diffusion of oxygen, which impedes decomposition and CO_2 production (Davidson et al. 1998); on the other hand, low water contents can inhibit CO_2 production in soils (Davidson et al. 2000). The latter authors also stated that SR and soil moisture correlate positively as long as aeration is sufficient, and CO_2 emissions correlated with the cube of volumetric water content. In our case the respiration assay was performed under controlled laboratory conditions, hence the observed significant differences must be caused mainly by the quality and quantity of SOM.

Irrigation was followed by an increase in SR partly due and to increased biological activity (Calderón and Jackson 2002). The authors also found that CO_2 efflux was greater after irrigation when tillage had not previously occurred. Soil respiration rates were similar under most moisture conditions for soils varying widely in texture (Bouma and Bryla, 2000), suggesting that differences in soil texture do not affect significantly SR rates. Wang et al. (2003) stated that clay had no significant effects on CO_2 production rate. Our results agree with the above statements.

Overall, the amount of time since deforestation played a bigger role than management.

1.5 Metabolic quotient (qCO_2)

ANOVA results for qCO_2 showed significant differences for land use and the management factor (type of crop), and no significant differences for time of deforestation and the time*management interaction (Table 22).

Table 22. *ANOVA results for metabolic quotient (qCO_2) significance levels of the effects of land use, time since deforestation, management and the interaction between time and management.*

Factor	qCO_2
Land use	***
Time (use)	ns
Management (use)	*
Time*Management (use)	ns

Note: *significance levels: ***(p<0.001); **(p<0.01); *(p<0.05); and ns:not significant*

Values of qCO_2 are shown in Table 23. Soils under forest (undisturbed plots) had lower qCO_2 than other studied soils.

Table 23. *Mean values of qCO_2 in [mg C-CO_2 g^{-1} C-MBC day^{-1}] for topsoil horizons (0-15cm) of forest, wheat and cotton crops of different deforestation year, and their standard errors (n= 12).*

Deforestation		1933		1971		1980	
	Forest	Wheat	Cotton	Wheat	Cotton	Wheat	Cotton
qCO_2	36.4±3.2	46.5 ± 2.9	52.1 ± 2.9	47.1± 2.9	54.7 ± 2.9	50.0 ± 2.9	53.1 ± 2.9

Land use change from forest to crop caused a significant decrease on qCO_2 (Table 24).

Table 24. *The metabolic quotient and the Land Use factor: Mean values of qCO_2 for topsoil horizons (0-15cm) of forest and agriculture, and their standard errors.*

Land Use	Forest	Agriculture
qCO_2 [mg C-CO_2 g^{-1} C-MBC day^{-1}]	36.4b±3.2	50.6a ± 1.2
	n = 12	n = 72

Note: *different letters indicate significant differences (p<0.05), n=number of data*

Higher maintenance energy requirement and lower growth yield of SMB lead to an increase of qCO_2 (Alvarez et al., 1998). Highest values occur when the microbial C is low (at high temperatures self consumption of MBC takes place) (Alvarez et al., 1995). That is what happened in our case, as the microbial biomass reduced significantly in crops compared with forest as seen in previous chapter (Table 9). The correlation between MBC and qCO2 was significant, with a p value less than 0.001 (r = -0.53, Table 27).

According to Dilly and Munch (1998), increase in qCO_2 could mean: a) stress or unfavorable conditions for the microbiota (which is a fact in the deforested plots), b) increase in the bacteria/fungi ratio, c) prevalence of zymogenous in contrast to autocthonous microbiota, supposed to arise under agriculture. Decrease in qCO_2 means that per unit C allocated in the soil, less C is lost through respiration, and more is retained as SOM or biomass, with all its beneficial effects (Mao et al., 1992). The metabolic quotient reduces as degradation of plant cover is more pronounced, perhaps due to reduction in specific respiration due to reduction of the water soluble substrates directly available to the microorganisms (Garcia et al., 2002). The metabolic quotient is strongly influenced by the content in the soil of compounds which can be used as an energy resource by microorganisms. Low values suggested that during mineralization of SOM, microbes divert less C to respiration than to new microbial biomass causing less C loss from microbes in cultivated soils (Saggar et al., 2001; Jinbo et al., 2007), probably indicating the presence of more efficient microbial populations in conserving C. Low values also suggested preserve of SOC in the soil and higher accumulation of resistant SOC pool (Rudrappa et al., 2006), that is what happens in the undisturbed plots. High values indicate short turnover times of microbial biomass

(Joergensen and Scheu, 1999). Saggar et al. (2001) observed that the increase of qCO_2 agreed with previous findings (especially referring to Anderson and Domsch, 1990) in less diverse agroecosystems with fewer C inputs from roots and crop residues. Decreased with increasing concentrations of MBC (Bauhus et al., 1998), the microorganisms were more C efficient on substrate supporting high concentrations of microflora. Insam and Domsch (1988) reported higher values in non equilibrated environments. Low values are related with more efficient utilization of C by the microbial community (Witter and Kanal, 1998). The metabolic quotient could raise when dry soil is rewetted, herbicides have been applied and substrate has been added (Wardle and Ghani, 1995). Garcia et al. (2002) found values 1.68-7.49. Anderson (2003) and Moscatelli et al. (2005) mentioned that qCO_2 values greater than 2 indicate an energetically less efficient microbial community.

Our results showed that forest soils have significant lower qCO_2 values than the cultivated (Tables 22 and 24), no matter how the cultivated plots are categorized. The metabolic quotient was found higher in cultivated soils than in pasture (Saggar et al., 2001), reflecting an increase in the ratio of active:dormant components of the biomass. Islam and Weil (2000) reported higher values in cultivated soils than in forests. Enhanced microbial activities in soils under natural forest, reforestation and grass are related to greater levels of available SOC. Thus, reforestation of degraded land not only increased total MBC and active MBC contents, but also the labile fraction of SOC. As a result, microbial communities under natural forest, reforestation and grass were more biologically active and less stressed than in the cultivated soils. Furthermore, the intense competition for the available C may favor those microorganisms which use more C energy for cell integrity and maintenance than for growth under perturbed or disturbed ecosystems. As a result, cultivated soils favor bacteria-based food webs which have low C assimilation efficiencies and faster turnover rates than the more efficient fungal-based food webs dominant in untilled or natural ecosystems. This explains the significant differences between forested and agricultural plots of our study. Li et al. (2007) reported no significances among land uses and depths, although it was found higher in annual and perennial pastures. The effect of time elapsed since deforestation was not significant for qCO_2 (Tables 22, 25).

Table 25. *Mean values of qCO_2 for topsoil horizons (0-15cm) of crops of different deforestation year, and their standard errors, (n= 24).*

Year of deforestation	1933	1971	1980
qCO_2 [mg C-CO_2 g^{-1} C-MBC day^{-1}]	49.3 ± 2.1	50.9 ± 2.1	51.5 ± 2.1

These results could be attributed to approaching equilibrium conditions thus stress has not a significant effect.

Management as a factor (type of crop) showed a significant effect on qCO_2 (Table 26) with a $p<0.05$. Significantly lower qCO_2 values were found in wheat crop (not irrigated plots, NIR) than in cotton crop (irrigated plots, IR).

Table 26. *Mean values of qCO_2 for topsoil horizons (0-15cm) of wheat and cotton crops, and their standard errors.*

Crop plots	Wheat	Cotton
qCO_2 [mg C-CO_2 g^{-1} C-MBC day^{-1}]	47.9b±1.7	53.3a ± 1.7
	n = 36	n = 36

Note: *different letters indicate significant differences between wheat and cotton ($p<0.005$) n=number of data.*

These results show that management type, e.g. reduced or conventional tillage, attributes more to the qCO_2 values than the year of conversion (amount of time since deforestation), with more intensive agricultural practices leading to more stressed conditions. Reaching or heading to an equilibrium after several years, the management practices affect more the behavior of the microbial biomass.

Tilled soils, having lower SOC contents, can sometimes generate greater amounts of CO_2 and sustain a MB with a higher qCO_2. The different accessibility of the C substrate to microorganisms or metabolic changes in the flora could be responsible for these results (Alvarez et al., 1995). Santruckova and Straskraba (1991) found the highest values in field soils, with lowest values in forests. The metabolic quotient was found lower in zero tilled than conventionally tilled plots, indicating greater accumulation of potentially more active and decomposable in field-disturbed cultivated soil, which therefore may actually have a slower in situ decomposition rate than soil under zero tillage (Franzluebbers and Arshad, 1996). The study took place in Boralfs. Bastida et al. (2006) found the disturbed plot of their study showing lower qCO_2 values than the undisturbed (*Pinus halepensis* with indigenous Spanish shrubs) plot, claiming that inputs of vegetal rest could act as substrate for respiration, increasing qCO_2 in the undisturbed plots. Low values are related with more efficient utilization of C by the microbial community (Witter and Kanal, 1998). Furthermore, the intense competition for the available C may favor those microorganisms which use more C energy for cell integrity and maintenance than for growth under perturbed or disturbed ecosystems. As a result, cultivated soils favor bacteria-based food webs which have low C assimilation efficiencies and faster turnover rates than the more efficient fungal-based food webs dominant in untilled or natural ecosystems. In this study, forest soils were found to have significant lower qCO_2 values than the cultivated, no matter how the cultivated plots are categorized.

The metabolic quotient was found lower in no tilled than conventionally tilled plots, indicating greater accumulation of potentially more active and decomposable in field-disturbed cultivated soil, which therefore may actually have a slower *in situ* decomposition rate than soil under NT (Franzluebbers and Arshad 1996), and 55% lower in NT than CT because of the higher efficiency of the microbial community (Franchini et al., 2007). The authors stated that the reduction of qCO_2 along with the raise of MBC in NT systems, resulted in greater SOM with time. Nitrogen fertilization decreased microbial mortality lowering energetic maintenance requirements (qCO_2) (Moscatelli et al., 2005). The metabolic quotient was found higher on reduced or not fertilized than adequately fertilized plots (Insam et al., 1991). Bauhus et al. (1998) found that it was negatively correlated with total N. Moscatelli et al. (2005) reported qCO_2 to be strongly affected the nutritional status of the soil.

Referring to irrigated fields, the not irrigated ones referential of reduced tillage (wheat crops), had slighter lower values than the irrigated ones, on which conventional tillage is practiced more (cotton plots), as expected referring to the above discussion.

Table 27. The correlation coefficients (r) and their significance (p-value) between all the studied properties (n = 84).

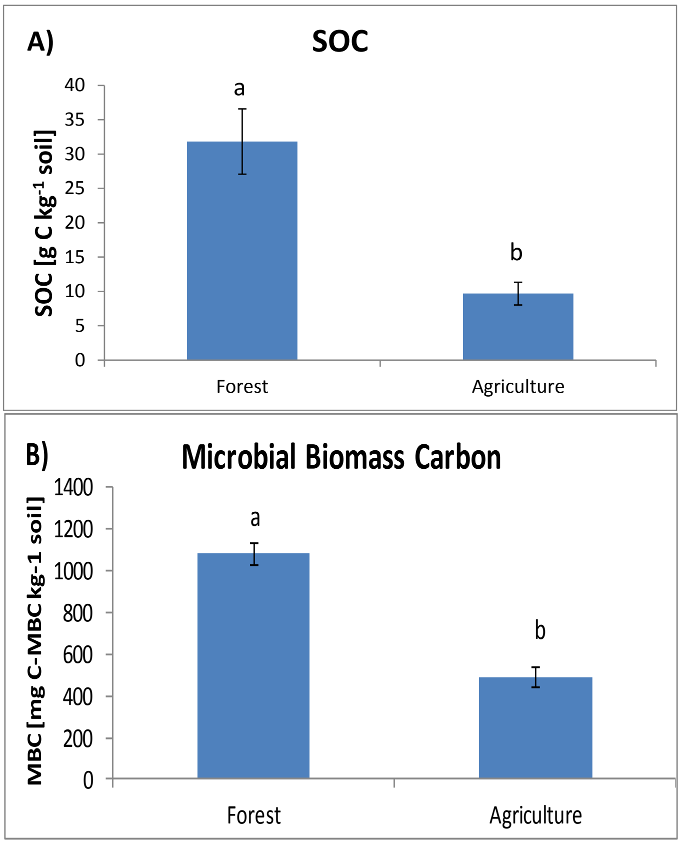
PROPERTY	POM	% Clay	% Silt	% Fine Sand	% Gross Sand	C/N	N	qCO_2	SR	MBC/SOC	MBC
SOC	0.9277	0.4027	-0.060	-0.0504	-0.2745	0.1272	0.936	-0.328	0.7701	-0.597	0.8697
	***	*	ns	ns	ns	ns	***	***	***	***	***
MBC	0.8158	0.3459	0.0278	-0.0365	-0.3024	0.2152	0.8016	-0.531	0.6926	-0.3817	
	***	ns	ns	ns	ns	*	***	***	***	***	
MBC/SOC	-0.5656	-0.1757	-0.0284	0.0914	0.114	0.0382	-0.5977	0.1712	-0.361		
	***	ns	ns	ns	ns	ns	***	ns	***		
SR	0.7684	0.3566	-0.044	0.0181	-0.2941	0.1622	0.7376	0.1869			
	***	*	ns	ns	ns	ns	***	ns			
qCO_2	-0.255	-0.023	-0.159	0.0398	0.1166	-0.0218	-0.285				
	ns	ns	ns	ns	ns	ns	**				
N	0.9042	0.426	-0.053	-0.0193	-0.3221	-0.0091					
	***	*	ns	ns	ns	ns					
C/N	0.0963	0.0352	-0.0066	-0.0665	0.0203						
	ns	ns	ns	ns	ns						
% Gross sand	-0.203	-0.455	-0.581	-0.207							
	ns	**	***	ns							
% Fine sand	-0.023	-0.460	-0.104								
	ns	**	ns								
% Silt	-0.139	-0.141									
	ns	ns									
% Clay	0.3701										
	*										

Note: *significance levels: ***(p<0.001); **(p<0.01); *(p<0.05); and ns: not significant*

2. EFFECTS OF LAND USE, TIME FROM DEFORESTATION AND MANAGEMENT

2.1 Land use change effect: forest *versus* agriculture

In terms of land use, forest sites had significantly higher SOC concentrations than the cultivated sites in the present study (Figure 2), more than three times higher. In many studies, forest soils were nearly always found to contain more SOC than deforested areas or agricultural soils (Hajabbasi et al., 1997; Glaser et al,. 2000; Dinesh et al., 2003, Llorente and Turrión, 2009). Reasons for this are presented in the first part of the discussion.



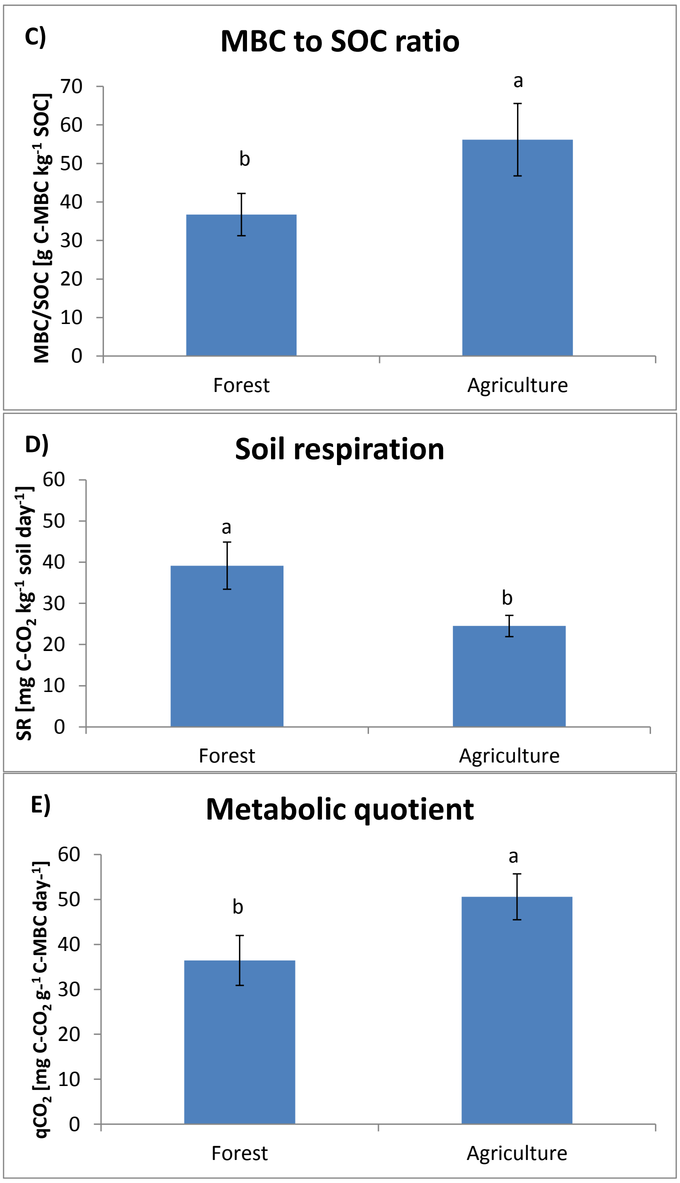


Figure 2. *Effect of deforestation and crop establishment on A) soil organic carbon (SOC), B) microbial biomass carbon, (MBC), C) the MBC to SOC ratio (MBC/SOC), D) potential soil respiration (SR), and E) the metabolic quotient (qCO_2), for topsoil horizons (0-15 cm). Different letters indicate significant differences between forest and crop ($p<0.05$).*

Soil microbial biomass constitutes a reservoir of nutrients, participates in nutrient cycling and is responsible for organic matter and residue decomposition (Alvarez and Alvarez, 2000; Turrión et al., 2012). The concentrations of MBC in the cultivated soils obtained in this study (382–622 mg kg^{-1}) fall well within the range observed in cultivated calcareous soils under Mediterranean conditions (Melero et al., 2009;

López-Garrido et al., 2012) who obtained for traditional tillage mean MBC values between 470 and 624mg kg^{-1} for traditional tillage and between 694 and 805mg kg^{-1} for conservation tillage. Vittori Antisari et al. (2010) found values of MBC between 853 to 1491mg kg^{-1} for soil profile epipedons of typic Calciustepts under *Quercus petraea L* in Italy. The MBC values obtained in our study for forest topsoil horizons are into this range (Table 9). The size of MBC is governed by various agricultural management practices, such as cultivation and fertilization (Chander et al. 1997). Our results showed that deforestation following by crop establishment caused a significant decrease by half of the MBC pool (Figure 2). In our study MBC trends followed changes in SOC contents, with which they were positively correlated (with a p value of less than 0.001, Table 27) (also as Shahbazi et al. (2013) stated). Sahani and Behera (2001) found 83% less MBC at deforested sites, compared to values under forest. The authors stated that the low level of MBC indicated poor microbial growth in the deforested soil. Lack of a proper soil environment and poor SOM level are the factors for such a decline in biomass size in the deforested site. Angers et al. (1992) reported a 50% decrease in MBC due to ploughing.

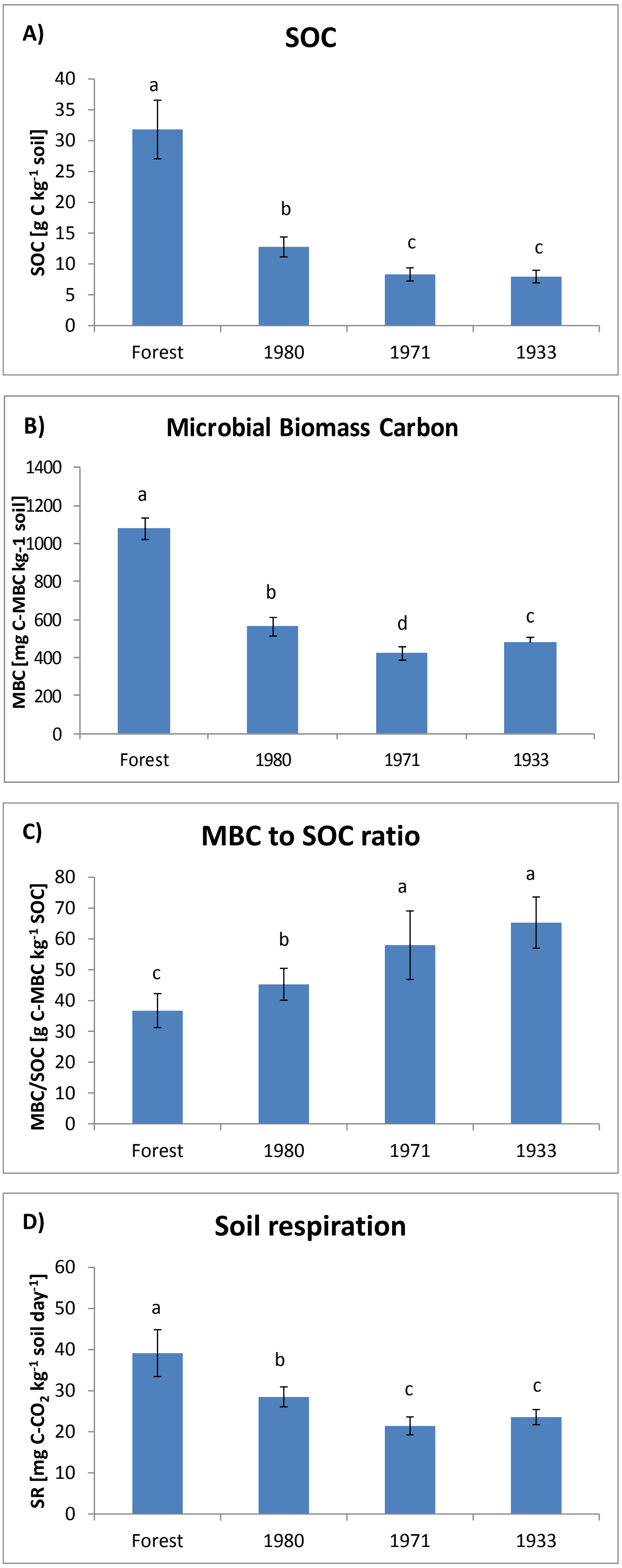
There is a very close quantitative relationship between MBC and SOC (Anderson, 2003). The microbial quotient (MBC/SOC), defined as the ratio of MBC to SOC, proved to be a reliable soil microbial parameter for describing changes in man-made ecosystems (Anderson and Domsch, 1993). The values of the MBC/SOC ratio in the studied soils varied from 3.7 to 6.7% (Table 13); most of the researchers mention values from 0.5-7%. For evaluating reclamation effects, the MBC/SOC ratio can be considered superior to its single components MBC and SOC, especially when compared to SOC, and is a good index of the changes in SOM quality (Insam and Domsch, 1988). However there are contradictory results reported by bibliography about the effect of land use change from forest to crop in the MBC/SOC ratio. Anderson and Domsch (1989) argued that the ratio will increase for a time if organic matter input to a soil increases, and will decrease otherwise. Dinesh et al. (2003) indicated that the ratio declines strongly as the concentration of available SOM decreases. Sahani and Behera (2001) ascribed the low MBC/SOC ratio that was found generally at deforested sites and especially at deforested barren sites of their experiment, to less microbial immobilization of nutrients. They indicated that low MBC/SOC ratios at deforested sites show loss of SOC and clearly reveal soil perturbation due to forest loss. The high MBC/SOC ratios at forests found by these authors could be due to the fact that more diversified organic substrate production and input in the soil subsystem support a more interdependent food chain leading to maintenance of a higher MBC/SOC ratio (Sahani and Behera, 2001). Nogueira et al. (2006) did not detect significant effects on the MBC/SOC ratio by different sites (fallow, wheat, eucalyptus forest). On the other hand, Moscatelli et al. (2005) and Fließbach et al. (2007) stated that fertilization increases the MBC/SOC ratio, which seems to be strongly affected by the soil nutritional status. These authors indicated that the microbial pool is strongly dependent on N (as in our case, Table 27) and probably suffers from a competition with plants for this element. According to this affirmation the nutritional improvement caused by fertilization could explain the significant higher values of the MBC/SOC ratio observed in our study for cultivated plots in comparison with the ratio values of forest plots (Figure 2), although the amount of SOC in the last ones were significantly higher than in the crops.

The SR values obtained in this study are similar to those reported by Fliessbach et al. (1994) for crop soils, but are lower than those showed by Pascual et al. (2001) for degraded soils of aridic areas and for crop soils, respectively. Rampazzo and Mentler (2001) stated that SR is strongly correlated to the amount of SOM in the soils. According to Frank et al. (2006), higher SOC and MBC lead to higher SR and lowest values of SR corresponded to sites with lowest MBC (Dinesh et al. 2004). In our soils we had very strong correlations among SR and SOC and MBC, with p values of less than 0.001 for both SOC and MBC, respectively (Table 27). Devegetation has a negative effect on the development of microbial activity, as revealed by the significant lower values of SR (Sahani and Behera, 2001; Bastida et al., 2006). Hence, as expected, changes in land use strongly affected soil CO_2 efflux, as our results showed that forest rates in terms of SR were significantly higher than the corresponding agricultural rates (Figure 2).

The metabolic quotient (qCO_2), defined as the ratio of SR to MBC, indicates the intensity of microbial metabolism and reflects the maintenance energy requirements (Anderson, 2003) or generally stress by different factors (Bastida et al., 2006). Our results showed that deforestation following by crop establishment caused an increase of 40% in the qCO_2 values (Table 24). Moscatelli et al. (2005) indicated that the efficiency of soil microbial populations in acquiring or utilizing SOC and the intensity of C mineralization are measured by qCO_2. The metabolic quotient is strongly influenced by the soil content of compounds which can be used as an energy resource by microorganisms. Low values suggest preserve of SOC and lead to a high accumulation of resistant SOC pool (Rudrappa et al., 2006). Insam and Domsch (1988) reported higher values in non equilibrated environments. Low values are related with more efficient utilization of C by the microbial community (Witter and Kanal, 1998). Islam and Weil (2000) reported higher values in disturbed soils than in forests. In the present study, forest soils showed significant lower qCO_2 values than the cultivated soils, hence a more efficient microbial community than crop soils.

2.2. Effect of time transcurred since deforestation and subsequent cultivation

Sigstad et al. (2002) stated that SOC after 15 years since deforestation with continuous cultivation is almost completely worn out. Our results showed that practicing agriculture for many years after deforestation leads to a further decrease of SOC levels, more specifically after 25, 34 and 72 years of cultivation the SOC decrease was 60%, 74% and 77% respectively (Table 5, Figure 3). As can be seen in Figure 3 the most recent year category (1980) showed significantly higher amounts of SOC than the other two categories (1971 and 1933, no significant differences were observed between these categories).


A)
SOC
SOC [g C kg-1 soil]
40
35
30
25
20
15
10
5
0
a
b
c
c
Forest
1980
1971
1933
B)
Microbial Biomass Carbon
MBC [mg C-MBC kg-1 soil]
1400
1200
1000
800
600
400
200
0
a
b
d
c
Forest
1980
1971
1933
C)
MBC to SOC ratio
MBC/SOC [g C-MBC kg-1 SOC]
80
70
60
50
40
30
20
10
0
c
b
a
a
Forest
1980
1971
1933
D)
Soil respiration
SR [mg C-CO2 kg-1 soil day-1]
60
50
40
30
20
10
0
a
b
c
c
Forest
1980
1971
1933

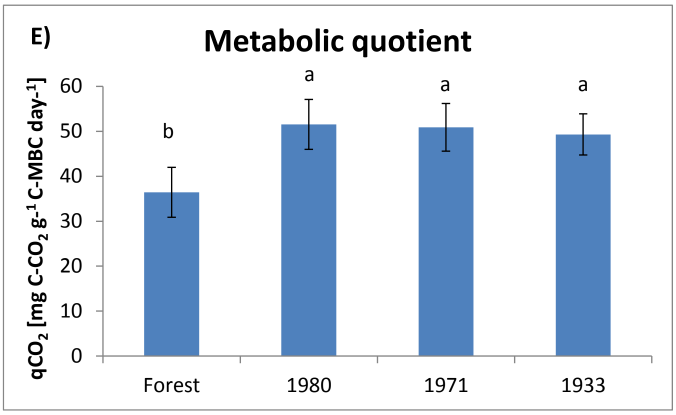


Figure 3. *Effect of time transcurred from deforestation and subsequent cultivation on A) soil organic carbon (SOC), B) microbial biomass carbon (MBC), C) the MBC to SOC ratio (MBC/SOC) D) potential soil respiration (SR), and E) the metabolic quotient (qCO_2). Different letters indicate significant differences between deforested plots on 1933, 1971, 1980, and forest plots ($p<0.05$).*

During the first 30 years after deforestation and subsequent cultivation, SOC decreased with a rate of 0.71 g kg^{-1} soil y^{-1}, the equation obtained for SOC versus number of years since deforestation (t) was: SOC = -0,71*t+ 31,6; with an r^2 of 0.996. Much of this loss in SOC can be attributed to soil tillage, which induces soil C loss by acceleration of organic C oxidation, responsible for the release of large amounts of CO_2 to the atmosphere (La Scala et al., 2006). Another tillage-related factor that contributes to soil C losses is soil aggregate disruption, which exposes once-protected organic matter to decomposition (Paustian et al., 2000; Grandy and Robertson, 2007).

The significantly highest amounts of MBC and SR were found in the most recent crop plots, as expected (Figure 3, Tables 10 and 20) whereas the lowest ones in plots deforested in 1971. There were significant differences among the three year categories and with the soil under forest (Figure 3).

For the MBC/SOC ratio the effect of the time transcurred from deforestation followed by subsequent cultivation was also significant. Higher MBC to SOC ratios were observed in the older cultivated plots (1933 category). There was a significantly good correlation between the number of years since deforestation and the microbial quotient, (MBC/SOC = 0.36*t + 40.6; r^2 = 0.774). The y-intercepts (40.6 g C-MBC kg SOC) are very close to the microbial quotient for forest soil (37 g C-MBC kg^{-1} SOC, Tables 13 and 15), which could be considered with 0 years since deforestation.

The 1933 category holded the highest values among treatments, followed by the 1971 and 1980 categories (Figure 3). The amount of time since deforestation affected the results more than the management type also at the subcategory level.

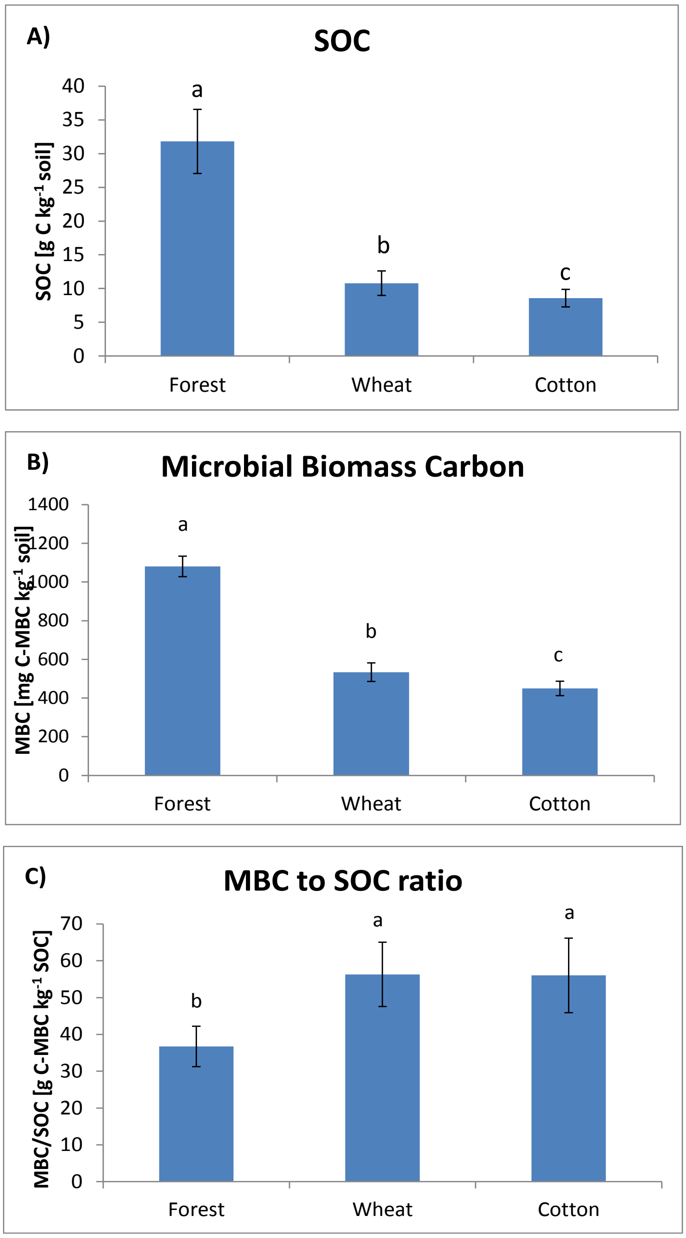
When studying different farming systems, Fließbach et al. (2007) found SR not to differ. Campbell et al. (1999) found that SR activity was significantly affected from tillage practices only after 12 years. Soils will tend towards a state of equilibrium if both environment and agricultural practices remain constant over long periods (Anderson and Domsch, 1989). The MBC, SR and MBC to SOC ratio indicate that at least in the 1933 and 1971 plots this new equilibrium has been achieved. The most recent deforestation plots had the highest (significantly different) values among the year categories. The amount of time since deforestation played a more significant role comparing to management/crop type for this property.

On the other hand the amount of years since deforestation and subsequent cultivation did not show significant differences in qCO_2 values (Tables 22 and 25). The 1980 category values were the greatest, followed by 1971, but the differences were not significant.

2.3. Land management effect: wheat *versus* cotton crops

The main differences between the two management types compared in this work are: a) the irrigation practice carried out in the cotton (IR) plots *versus* no irrigation of the wheat (NIR) plots, b) the ploughing practices, with those in the wheat plots to be considered as reduced tillage (where only once every year a not deep ploughing is applied), and those in the cotton plots to be considered as conventional tillage practices, and c) the N fertilizer applications, which are higher in the cotton plots than in the wheat plots.

Our results showed that management (the type of crop) played a role (though not very important) on the SOC amount, obtaining significantly lower values of this parameter in the cotton plots compared to the wheat subcategories ($p<0.05$, Figure 4). Madejón et al. (2009) found significant higher SOC values under reduced tillage plots than conventional tillage plots at the surface layer (0-5cm), however these differences were not significant at intermediate layers (5-10cm). Roldán et al. (2007) stated that tillage promotes SOM decomposition through crop residue incorporation into the soil and physical breakdown of residues. When it comes to conservation tillage, SOM levels are substantially higher in the surface layer and gradually decrease to the same SOM content as conservation tillage below plow depth (Madejón et al., 2009). In our case, the management effect probably was diluted by the depth considered, 0-15cm (Figure 4).



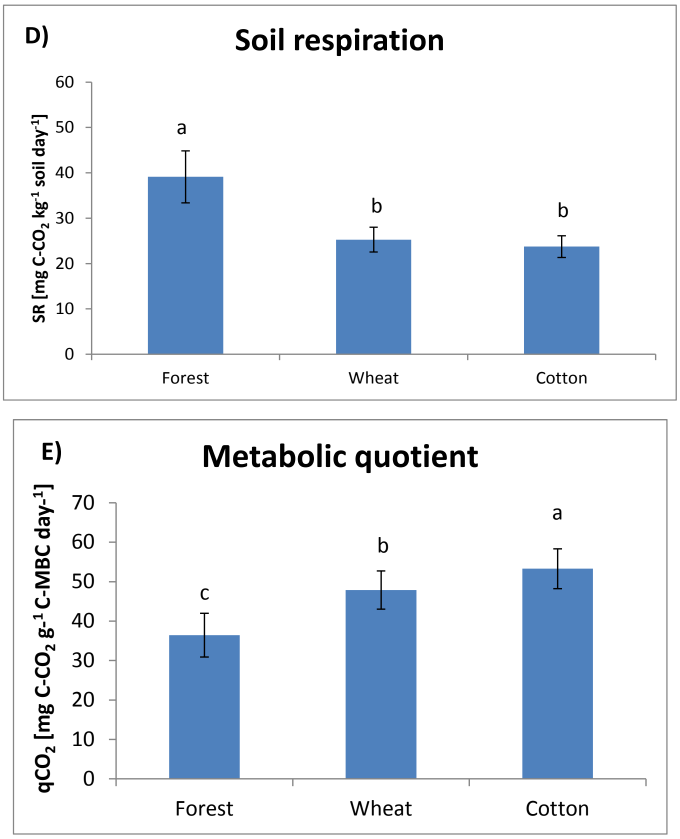


Figure 4. *Type of vegetation effects on A) soil organic carbon (SOC), B) microbial biomass carbon (MBC), C) the MBC to SOC ratio (MBC/SOC) D) potential soil respiration (SR), and E) the metabolic quotient (qCO_2). Different letters indicate significant differences between wheat, cotton, and forest plots ($p<0.05$).*

On the other hand, the effect of irrigation must be taken in account in order to explain the differences between cotton and wheat crops obtained in our study. Franzluebbers et al. (2001) indicated that if only irrigation effect occurred, higher values on SOC in the cotton crops should be observed, however in the present study soils under wheat crops showed significant higher SOC values than soils under cotton crops (Figure 4, Table 6). Franzluebbers et al. (2001) observed increasing levels of SOC with increased water content rates, leading to higher plant production and SOC input, but also greater decomposition. Lemenih and Itanna (2004) found increases in C stocks with increase in precipitation rates, whereas excessive precipitation or increased amounts of irrigation water cause soil C leaching (Lemenih and Itanna, 2004).

Wheat and cotton crops of this study showed significant differences (*$p<0.001$*) in terms of MBC (Figure 4, Table 11) that can be attributed to tillage and irrigation practices, such as reduced tillage practices at wheat plots compared to cotton/wheat rotation plots, enhanced application of N fertilizer which promotes MBC enrichment (Moscatelli et al., 2005), increased amount of crop residues (that has as consequence a significant increase in SOC) at wheat/NIR plots, and differences in hydrological soil regimes caused by irrigation. It is not easy to determine which of these causes has the most important repercussion on MBC values in our study, but considereing the above, irrigation could not be the main factor influencing the highest rate of MBC at the wheat plots, compared to reduced tillage practices and in general the management of the wheat fields. Roldán et al. (2007) and Madejón et al. (2009) reported that reduced tillage accumulated crop residues and SOC, which are substrates for soil microorganisms near the surface resulting to an increase in MBC and various soil processes in the surface soil. The ANOVA results for MBC showed significant differences for time and management (type of crop) factors but not for their interaction (Table 7). This microbial property was more sensitive than SOC regarding the type of crop. The same behavior/pattern was observed for both types of crop regardless of the deforestation date.

Many authors indicated that when soil management changes, MBC responds more quickly than SOM, which is relatively slow to change, so the MBC/SOC ratio is a good indicator of these changes (Fauci and Dick, 1994, Moscatelli et al., 2005, Chen et al., 2009), However, our results showed than both parameters separately were sensitive to management changes, but not the MBC to SOC ratio. No significant effects of management as a factor and of the interaction time*management were observed for the MBC/SOC ratio (Table 16). Franzluebbers et al. (1994) stated that the MBC to SOC ratio increases when cropping intensity increases. Franzluebbers et al. (2001) reported that an increase in the mean annual precipitation decreased the MBC to SOC ratio.

Land use, management practices and environmental conditions influence SR processes (Frank et al., 2006), however in our study potential SR was not significantly affected by management/crop type (Table 21 and Figure 4) or for the interaction time*type of crop (Table 17). Contradictory results are reported in many studies about the effect of increasing plowing in SR. Reicosky et al. (1995) observed an increase in soil respiration with tillage, whereas Hendrix et al. (1988) observed greater CO_2 output from no-tillage than conventional tillage. The water factor (irrigation) does not seem to enhance substantially SR (Gömöryová et al., 2013), although it increases (Raich, 1992) due to increased biological activity. There is a significant positive correlation between SR and mean annual precipitation (Raich, 1992). Varying soil water content affects microbial respiration by limiting diffusion of substrates in water films, desiccating stress at low water contents and by limiting the diffusion of oxygen in pore spaces at high water contents (Davidson et al., 1998; Davidson et al., 2000). Xu et al. (2006) also stated that humidity affects microbial activity. High water content can impede diffusion of oxygen, which impedes decomposition and CO_2 production (Davidson et al., 1998); on the other hand, low water contents can inhibit CO_2 production in soils (Davidson et al., 2000). The latter authors also stated that SR and soil moisture correlate positively as long as aeration is sufficient, and CO_2 emissions correlated with the cube of volumetric water content. Respiration rates generally decrease with decreasing water content (Davidson et al., 2000). According to Raich (1992) and Bouma and Bryla (2000) irrigation appears to stimulate SR, although the latter claimed that it recovers in 1 day. In their study, irrigation was followed by an increase in SR (it rose by 40-60%) that appears to be associated with both physical processes and increased biological activity (Calderón and Jackson 2002). All of these affirmations are referred to soil respiration measured in the field, in laboratory assay the humidity conditions are identical for all the samples.

Insam et al. (1991) reported significantly higher metabolic quotient on reduced or not fertilized than on adequately fertilized plots. Franzluebbers et al. (1994) found it greater in wheat crops (in the top 20cm) without N fertilization than rotated wheat or not cultivated land, but not under conventional cultivated crops. Moscatelli et al. (2005) reported qCO_2 to be strongly affected by the nutritional status of the soil. The metabolic quotient was found lower in zero tilled than conventionally tilled plots, indicating greater accumulation of potentially more active and decomposable in field-disturbed cultivated soil, which therefore may actually have a slower *in situ* decomposition rate than soil under zero tillage (Franchini et al., 2007). The wheat plots, considered in our study as reduced tillage, had significant lower values than the cotton plots (the performed ANOVAs for qCO_2 were significant), on which conventional tillage is practiced. The latter fact agrees with the above discussion.

The mainly differences between the two crop types compared in this work are: a) the irrigation practice carried out in the cotton plots *versus* no irrigation of the wheat plots, b) the ploughing practices, with those in the wheat plots to be considered as reduced tillage (where only once every year a not deep ploughing is applied), and those in the cotton plots to be considered as conventional tillage practices, and c) the N fertilizer applications, which are higher in the cotton plots than in the wheat plots. As both tillage and irrigation effects occur simultaneously in the cultivation plots, our results did not allow to distinguish clearly between the effect of tillage and irrigation separately.

3. SOIL PROPERTIES IN SOIL PARTICLE-SIZE FRACTIONS

In Table 28 the concentrations of SOC, total N and C to N ratio obtained for the different studied soils are shown. The percentages of the particle size fractions considered: Coarse Sand (CS), Fine Sand (FS), Silt and Clay as well as the results of Particulate Organic Matter (POM) are also presented in Table 28.

Table 28. *SOC, N, C/N ratio, % of Coarse Sand (CS), % of Fine Sand (FS), % of Silt, % of Clay and POM (g/kg soil) results.*

		SOC	N	C/N	CS	FS	Silt	Clay	POM
		(g/kg soil)			-------------- (%)			------------	(g/kg soil)
Land use	Agriculture	9.65	0.96	10.01	20.30	16.67	30.65	32.38	2. 77
	Forest	31.8	2.75	11.56	17.24	16.46	28.97	37.33	14.82
Time	1933	7.9	0.86	9.21	23.44	20.31	28.63	27.61	1.83
	1971	8.1	0.83	9.77	18.54	14.65	31.44	35.36	2.68
	1980	12.95	1.21	10.75	18.92	15.04	31.87	34.17	3.80
Management	Wheat	10.4	1.05	9.87	16.42	17.60	34.40	31.58	2.81
	Cotton	8.9	0.87	10.18	24.19	15.73	26.89	33.19	2.73
	1933 wheat	8.5	0.94	9.08	19.58	24.40	32.39	23.63	1.45
	1933 cotton	7.3	0.78	9.36	27.31	16.22	24.88	31.60	2.22
	1971 wheat	8.6	0.92	9.36	16.42	15.30	35.25	33.03	3.28
	1971 cotton	7.6	0.74	10.28	20.67	14.00	27.63	37.70	2.07
	1980 wheat	14.0	1.31	10.72	13.25	13.10	35.56	38.09	3.71
	1980 cotton	11.9	1.10	10.78	24.59	16.98	28.17	30.26	3.89

In the following Table 29 the ANOVA results corresponding to SOC, N, C to N ratio, and particulate organic matter (POM) are presented.

Table 29. *ANOVA results for SOC, N, C/N ratio, and POM (g/kg soil) parameters.*

	SOC	N	C/N	POM
ANOVA				
Land use	***	***	**	***
Time (use)	***	***	*	*
Management (use)	*	*	ns	ns
Time*Management (use)	ns	ns	ns	ns

Note: *Significance levels: *** ($p<0.001$),** ($p<0.01$), * ($p<0.05$), and ns: not significant; n = 12 for each group.*

Mean values of SOC, N and the ratio C to N contained in Fine Sand, Silt and Clay for all the Categories and Subcategories are presented in Table 30.

Table 30. *Presentation of all the laboratory results referring to SOC, N, C/N ratio, in Fine Sand, Silt, and Clay, referring to all the Categories and Subcategories.*

		FINE SAND			SILT			CLAY		
		SOC	**N**	**C/N**	**SOC**	**N**	**C/N**	**SOC**	**N**	**C/N**
		- (g/kg FS) -			**- (g/kg Silt) -**			**- (g/kg Clay) -**		
Land use	Agriculture	8.6C	1.0c	8.21*B*	14.1B	1.2b	11.53*A*	15.5A	2.2a	7.16*C*
	Forest	46.0A	2.4a	19.16*A*	28.7B	1.75b	16.44*B*	21.2C	2.4a	9.01*C*
Time	1933	5.8C	0.9c	6.63*B*	12.0B	1.14b	10.48*A*	15.5A	2.1a	7.47*B*
	1971	7.7B	0.9c	8.12*B*	13.2A	1.1b	11.75*A*	13.7A	2.0a	6.93*C*
	1980	12.1B	1.2c	9.88*B*	17.0A	1.4b	12.35*A*	17.2A	2.5a	7.09*C*
Management	Wheat	8.6C	1.1b	7.77*B*	12.4B	1.17b	10.45*A*	16.4A	2.4a	6.99*B*
	Cotton	8.5B	1.0c	8.65*B*	15.8A	1.24b	12.61*A*	14.5A	2.0a	7.34*C*
Time and Management	1933 wheat	5.9C	1.0b	6.15*B*	9.6B	1.1b	8.82*A*	17.2A	2.3a	7.41*AB*
	1933 cotton	5.7B	0.8c	7.12*B*	14.5A	1.2b	12.14*A*	13.8A	1.8a	7.54*B*
	1971 wheat	7.7B	1.0b	7.56*B*	12.6A	1.1b	11.30*A*	14.7A	2.4a	6.33*B*
	1971 cotton	7.7B	0.9c	8.67*B*	13.8A	1.13b	12.20*A*	12.6A	1.7a	7.52*B*
	1980 wheat	12.3B	1.3b	9.60*B*	15.0A	1.34b	11.21*A*	17.4A	2.4a	7.21*B*
	1980 cotton	12.0B	1.2c	10.17*A*	19.1A	1.4b	13.48*A*	17.0A	2.5a	6.98*B*

Note: *Different letters indicate significant differences among fractions for each type of sample, upper case for SOC, lower case for N, and italic upper case for C/N.*

In the following Table 31 the ANOVA results for the fractionated samples are presented.

Table 31. *Results of the statistical linear mixed model applied to the parameters: SOC, N and the ratio C to N (C/N).*

Factor / Property	SOC	N	C/N
Land Use	***	***	***
Time (use)	**	*	**
Management (use)	ns	ns	**
Time*management (use)	ns	ns	ns
Fraction	*	***	***
Land use*Fraction	***	***	***
Time*Fraction (use)	***	ns	***
Management*Fraction (use)	***	**	*
Time* Management*Fraction (use)	ns	ns	ns

Note: *Significance levels: *** ($p<0.001$),** ($p<0.01$), * ($p<0.05$), and ns: not significant; n = 12 for each group.*

3.1 SOC IN THE FRACTIONS

3.1.1 General remarks and comments

The agricultural soils of our study showed a tendency to increase in SOC contents in the order sand<silt<clay (Table 30) (agreeing with the tendency referred by Christensen (1992) and Solomon et al. (2000)), though not in every comparison and not always significantly (significances were observed when comparing all the agricultural soils as a whole factor, land use, Figure 7), compared to the reverse trend for the forest soils, where particulate organic matter is withheld in the sand fraction (Table 30). The amounts of C and N in coarser size fractions are related to the amount of debris that is incorporated into the soil and not to soil characteristics (Caravaca et al., 1999). Soil organic matter associated with sand is composed of undecomposed and macromorphologically identifiable particulate plant residues (coarse sand) and partially decomposed organic debris (fine sand). This macro-organic matter, when compared with silt and clay bound SOM, is much more susceptible to mineralization. The SOM associated with clay and silt size fractions is characterized by an intimate association with the mineral phase (Christensen, 1992). In the forest soils that were studied in the present work, SOC in sand represented almost the 48.97% of total organic carbon in the soil, however in crop soils the SOC in sand fractions represented only 3.21% of total SOC (Figure 5). On the other hand, SOM associated with clay interact forms organo-mineral complexes and microaggregates, which make the organic matter less accessible to decomposers. In the forest soils of our study, the contribution of the silt and clay fractions to SOM was lower than the contribution of sand fraction, suggesting that C input provided by plant debris in forest soils was accumulated in the sand fraction. Caravaca et al. (1999) indicated that the redistribution of SOM between particle-size fractions is most probably affected by land use. Our results corroborate this affirmation, with land use being the most important and influencing factor for changes in SOM distribution into the soil fractions of our soils (Table 31). Generally, as referred by Solomon et al. (2000), the distribution of SOM in particle size fractions of the different land use systems showed that clearing and cultivation of the native tropical woodland resulted in a decline of SOM contents in all size fractions. The authors reported that during the first 3 years the decline was rapid in the coarse and fine sand fractions whereas the reduction of SOM which was intimately associated with the silt and clay fraction (i.e. the stable SOM) was relatively small. However, a more pronounced

decline in the stable SOM, especially from clay, was observed after 15 years of cultivation. Therefore, with continued cultivation, the SOM in the more stable fractions is also affected.

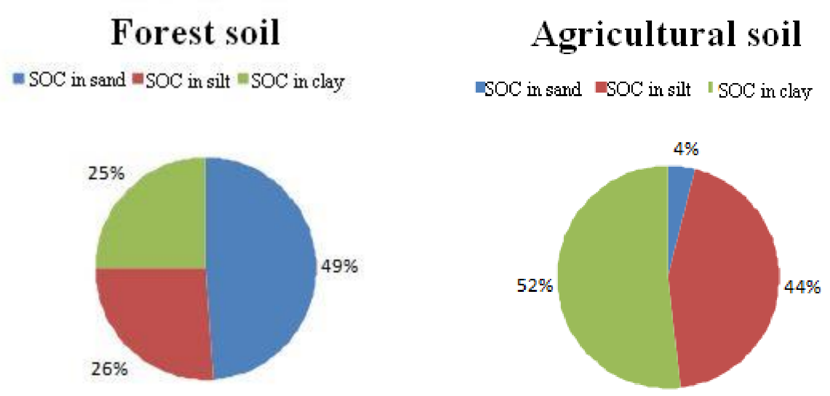


Figure 5. *Percentages of soil organic carbon in the fractions for each land use.*

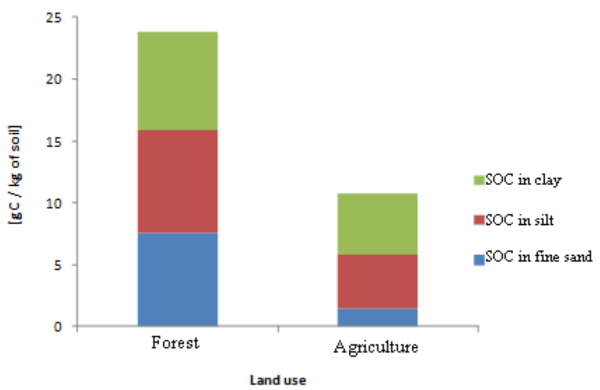


Figure 6. *Contents (g/kg of soil) of soil organic carbon in the fine sand, silt and clay fractions of forest and agricultural soils.*

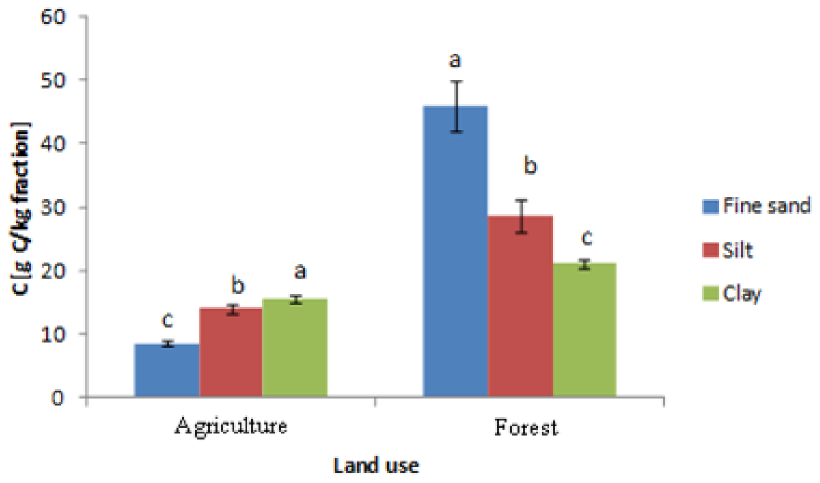


Figure 7. *Soil organic carbon concentrations in fine sand, silt and clay fractions of forest and agricultural soils.*

3.1.2 Particulate Organic Matter (POM) and SOC in the Fine Sand fraction

It is reported in several studies that practicing agriculture after forest use results in a depletion of the POM pool, whereas the silt- and clay-bound SOM is less affected (Guggenberger and Zech, 1999, Zinn et al., 2002). The unprotected organic matter of POM responds more rapidly to agricultural management than the total SOM pool (Zhong et al., 2015). Our results showed that the amount of POM reduces intensely when changing from forest to agricultural use (Table 28, Figure 8). Conversion of native forests to cultivation leads to a decline in total SOM which has been largely attributed to losses of POM (Ashargie et al., 2007). Studies carried out by Beare et al. (1994) and Zhong et al. (2015), indicated that POM concentrations in sand-free aggregates were significantly affected by tillage, responding more rapidly than the total SOM pool.

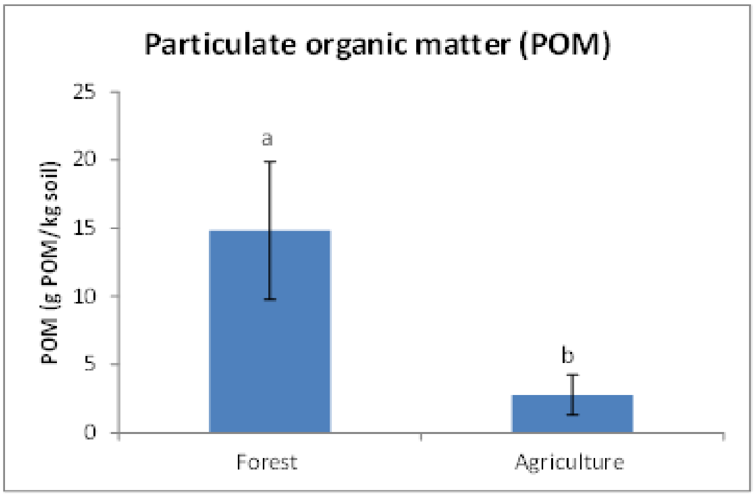


Figure 8. *Effect of deforestation and crop establishment on particulate organic matter (POM) (g/kg of soil) for topsoil horizons (0-15 cm), and the standard deviations. Different letters indicate significant differences between forest and agriculture (p<0.05).*

The amount of years since deforestation affects POM, as it decreased substantially after 60 years of cultivation (Tiessen and Stewart 1983, referred by Six et al., 2002). This seems to be our case, as POM decreased as the amount of years since deforestation increased (Table 28, Figure 9). The amount of years affected the presence of POM, by eliminating the contents the more years the soil is cultivated (Table 28). Big differences in the amount of years since deforestation and subsequent cultivation result in significant differences in POM contents (1933 year category compared to 1980 has significant differences, whereas in all the other inter-comparisons -except of course when referring to forest plots- the differences are not significant). It could be argued that sites deforested more recently seem to have a capacity to preserve more POM (and contain actually more amounts of POM) and SOC overall. The size of labile pools of SOM (e.g. POM) is usually sensitive to changes in land use and management that reduce input or increase output of OM from soil (Li et al., 2006). Especially coarse POM (>250µm in size) is relatively easily decomposable and is greatly depleted upon cultivation (Six et al., 2002). Large stocks of POM in a soil are associated with pronounced mineralization or organically bound nutrients as N, and are therefore intimately linked.

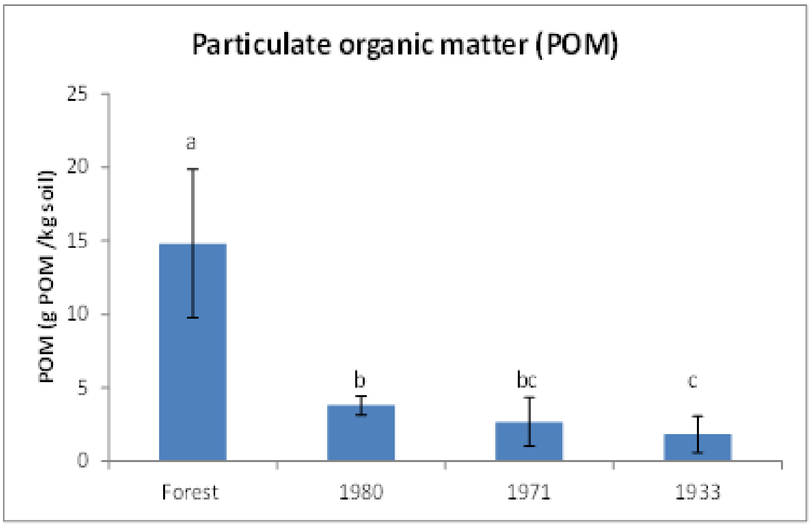


Figure 9. *Effect of time since deforestations and subsequent cultivation on particulate organic matter (POM) (g/kg of soil), and the standard deviations. Different letters indicate significant differences between deforested plots on 1933, 1971, 1980, and forest plots (p<0.05).*

Christensen (1992) and Guggenberger and Zech (1999) argued that POM is important in the short-term turnover, clay-bound SOM is dominating the medium-term turnover and silt-bound SOM participates in longer-term turnover. Chan (2001) found POM to account significantly for the changes in SOM caused by differences in land use and management. The author argued that measuring changes in POM rather than in total organic matter, is more appropriate in terms of land use monitoring. This implies for short term observations, in our case the effects seem to smoothen after practicing agriculture for many years (Table 28).

Particulate organic matter often responds more rapidly to management-induced changes in the SOC pool than more stabilized, mineral-associated fractions with longer turnover times (Leifeld and Kögel-Knabner, 2005). It was found less under conventional tillage than NT practices, as reported by Beare et al. (1994) and Jacobs et al. (2009). The amount of recently incorporated residues (POM) was high in NT and low in conventional tillage systems, therefore highly variable among soils (Buschiazzo et al., 2001). As can be seen in Table 28 and in Figure 10, in our case 5% higher (but not significantly) values were observed at the reduced tillage practices (wheat plots) compared to the conventionally tilled (cotton plots); management did not affect the POM concentrations significantly (Figures 10 and 11). This means management (type of cultivation practices or type of crop) did not affect POM contents in the soil, as in our case type of land use (Forest – Agriculture) was the important factor determining POM contents in the soil. Other researchers mentioned similar findings, for conventional tillage sites Chivenge et al. (2003) reported that the largest decline in SOC was in the coarse sand fractions and least at all depths in sand fractions for the conventional tillage (CT) plots compared to the other management practices of their study (mulch ripping and weedy fallow). Jacobs et al. (2009) did not find any significant differences caused by tillage regarding POM concentrations, but they reported minimum tillage practices to affect significantly C and N concentrations at the mineral fractions.

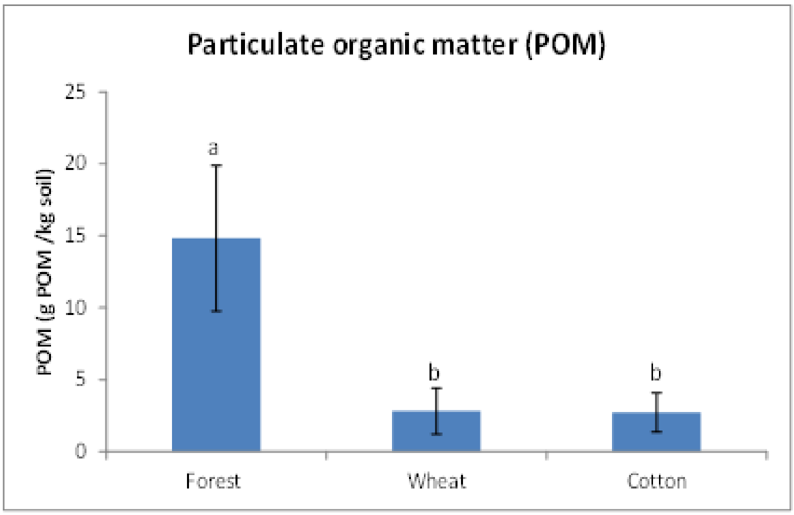


Figure 10. *Type of management effects on particulate organic matter (POM) (g/kg of soil), and the standard deviations. Different letters indicate significant differences between wheat, cotton, and forest plots ($p<0.05$).*

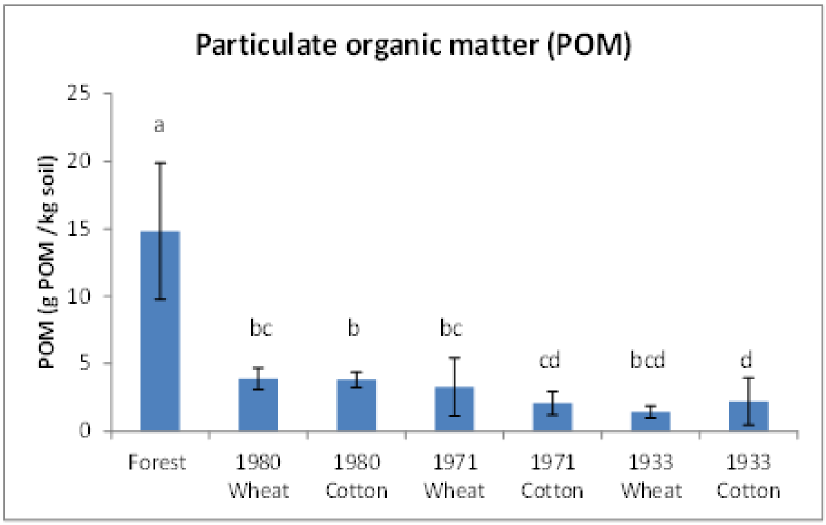


Figure 11. *Particulate organic matter (POM) (g/kg of soil) concentrations obtained in the studied plots, the comparison among the treatments, and the standard deviations (different letters indicate significant differences between categories, $p<0.05$).*

Sand fractions contain the recent C depositions in the soil; therefore these soil pools are sensitive to changes in SOM dynamics with time (Saha et al., 2010). Land use changes primarily affect SOC in the sand fraction, which contains labile SOM, whereas stable SOM pools are also affected by deforestation, but to a lesser extent. Hence the contents of SOC in the sand fraction of a soil could be considered as a reliable and sensitive indicator of changes in SOC after deforestation of the studied site. Leifeld and Kögel-Knabner (2005) stated that the fine sand fraction (0.2-0.02 mm) is the most sensitive fraction after POM in terms of C contents, with more pronounced differences when referring to land use.

The results of our work showed clearly great and significant differences between POM in the forest sites and POM in the crop fields, as well as between fine sand in forest and fine sand in agricultural plots (Tables 28, 29, 30 and 31, and Figure 12). Forest plots have 4-8 times greater POM amounts (depending on the amount of years since deforestation), with the values following exactly the same tendencies with SOC in the whole soil. This is expected according to the above analysis and discussion.

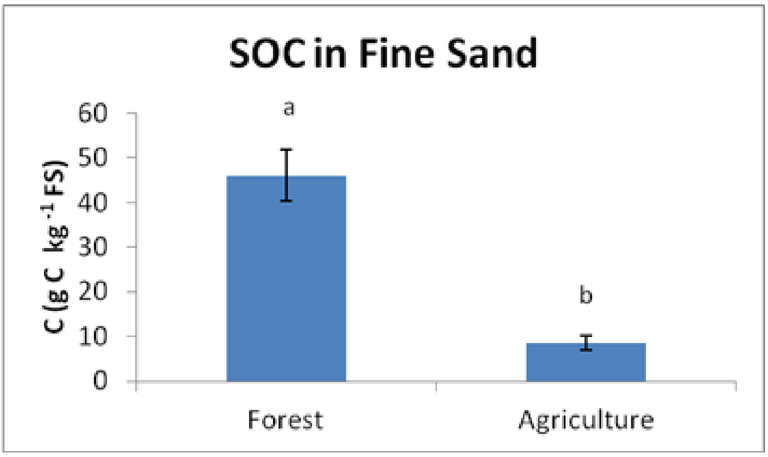


Figure 12. *Effect of deforestation and crop establishment on soil organic carbon (SOC) of the Fine Sand fraction for topsoil horizons (0-15 cm), and the standard deviations. Different letters indicate significant differences between forest and agriculture ($p<0.05$).*

In the case of fine sand, all the year categories had significant differences between them (Figure 13). The amount of years since deforestation leads to greater losses of SOC in all the fractions (except of the clay fraction, as will be discussed below), and of course for the fine sand fraction. Along with POM the fine sand fraction appeared to be the most sensitive from the studied fractions in terms of SOC loss (as mentioned above) when converting forest land to cropland, thus it could be considered as a more sensitive indicator when studying changes in land use regimes.

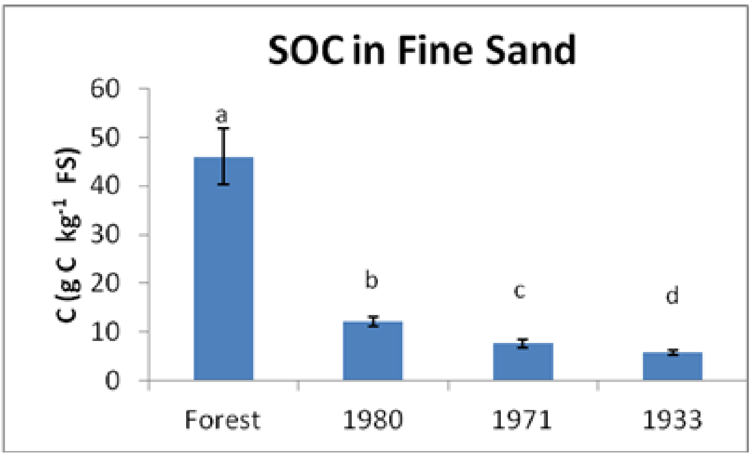


Figure 13. *Effect of time since deforestations and subsequent cultivation on soil organic carbon (SOC) of the Fine Sand fraction, and the standard deviations. Different letters indicate significant differences between deforested plots on 1933, 1971, 1980, and forest plots ($p<0.05$).*

Management did not play a significant role for fine sand SOC concentrations (Figures 14 and 15). Soil OM associated to the sand fraction was quickly lost when the land use regime was changed, hence management did not affect it significantly. Differences in the amount of water applied by irrigation in the different management practices affected the groundwater table, for which Hassink (1994) mentioned higher SOC contents in sandy soils with a low groundwater table than sandy soils with a high groundwater table. The increased amounts of irrigation water every two years that is applied in the cotton plots is not that sufficient to affect SOC in the fine sand fraction.

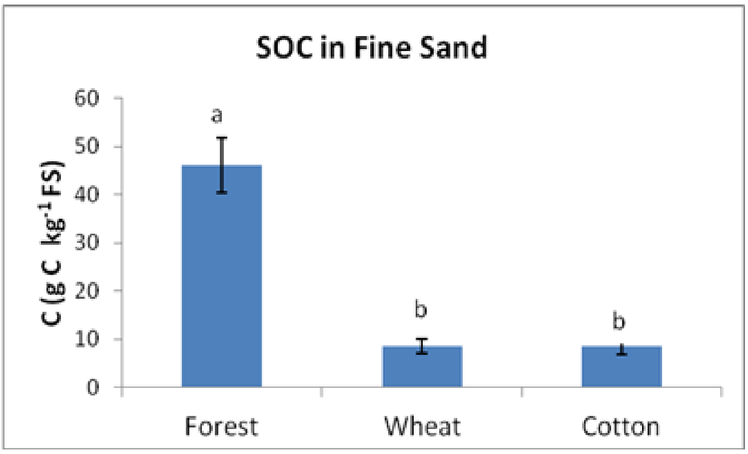


Figure 14. *Type of management effects on soil organic carbon, SOC [g C-SOC kg^{-1} soil] of the Fine Sand fraction, and the standard deviations. Different letters indicate significant differences between wheat, cotton, and forest plots (p<0.05).*

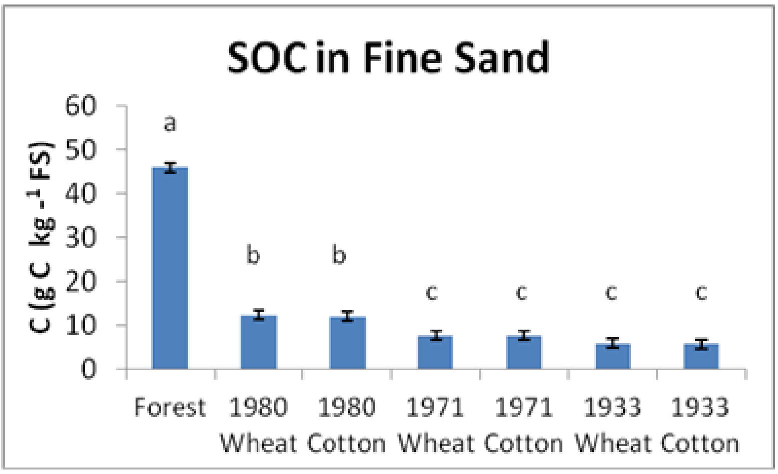


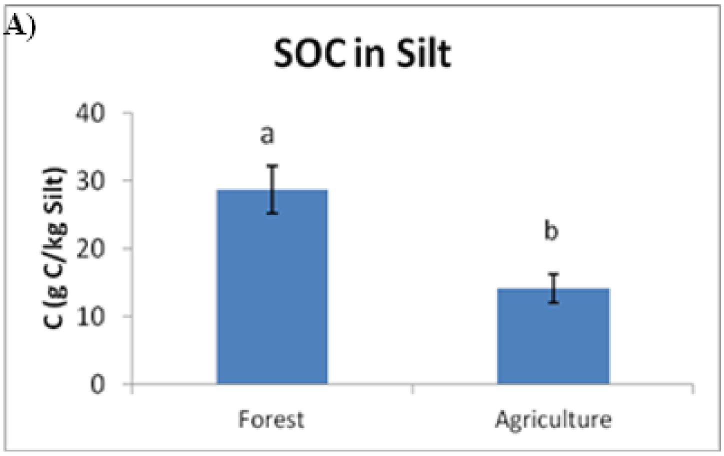
Figure 15. *Soil organic Carbon concentrations of the Fine Sand fraction, obtained in the studied plots, the comparison among the treatments and the standard deviations (different letters indicate significant differences between categories, p<0.05).*

3.1.3 SOC in the Silt and Clay fractions

Studying the conversion of forest land to pasture, Glaser et al. (2000) reported carbon losses to affect all particle-size classes, but the silt and clay fractions contributed to the highest amounts of SOC and N to the soil samples. The capacity of a soil to preserve SOC by its association with clay and fine silt particles depends on the clay and silt content of the soil (Hassink, 1997; Sleutel et al., 2006). Zhao et al. (2006) also argued that total SOC is significantly related to silt and clay contents. The percentages of whole soil C and N in the silt and clay fractions in the study of Caravaca et al. (1999) and Lehmann et al. (2001) were higher than in the sand fraction confirming that the SOM associated with silt and clay is physically better protected against microbial decomposition, when dealing with arable soils or soils under agroforestry. Zinn et al. (2002) mentioned that in the afforestated tropical soils of their study, the silt fraction showed the highest C content, most likely due to accumulation of C. Silt- and clay-associated OM fraction is stabilized and is therefore relatively insensitive to management (Sleutel et al., 2006).

Cultivation leads to a decrease of soil C associated with the silt and clay fractions, but not to such a great extent than in the sand fraction in our soils (Table 30). Caravaca et al. (2004) also mentioned similar observations. Hassink (1997) reported that for the silt and clay fractions the proportions of C and N left after conversion to arable land were generally more than 60%. Although in this study grassland soils contained higher amounts for total C and N, and C and N in the sand fraction, C and N amounts in the silt and clay fractions were similar for the cultivated soils. This also suggests that the amounts C and N that can become associated with these fractions had reached a maximum. Generally in arable soils most of SOM is found in the silt and clay fractions, whereas in forest and grassland soils the contribution of sand size OM to total SOM is greater (Christensen, 1992; Hassink, 1997). Six et al. (2002) attributed the silt and clay protected C losses upon cultivation to the breaking up of the aggregates. Saha et al. (2010) also mentioned higher levels of SOC in the silt and clay fractions of forest rather than disturbed sites. Caravaca et al. (1999) reported higher percentages (but not amounts) of clay-C at cultivated sites than under natural vegetation but lower for silt-C. They found that clay-associated SOC on forest soils was lower than cultivated sites (18% compared to 30%) whereas silt-associated SOC was higher. The average SOC content was higher in the silt than in the clay fraction for the calcareous and forest soils of their study, but lower in the cultivated sites where it is negatively correlated with particle size. Overall, the amounts of SOM in the silt and clay fractions were less affected by cultivation in most soils of their study, whereas Jiménez et al. (2008) mentioned that in all the forest stands of their study, the fine silt and clay fractions contained the highest values of SOC. Chan (2001) found significant differences between pasture and cropped soils in the C content in the <53μm fraction, that is fine sand, silt, and clay. Arable soils generally contained less than 50% of the amount of C and N in the sand fraction of uncultivated and grassland soils (Caravaca et al., 2004); in our case the cultivated plots had less than 20% (18.7% exactly) in SOC contents of the value of forest soils. This indication of better protection of SOM in the silt and clay fractions is present in the cultivated plots of our study (in silt 49% of SOC is preserved comparing cultivation plots to forest land (14.1g compared to 28.7g), and in clay 73% (15.5g compared to 21.2g)), whereas the great and significant losses of SOC in the sand fraction are attributed to the fresh organic material of the surface horizons of the forest soils (which is more characteristically present in the sand fraction) that is lost. Dalal and Mayer (1986) found 48% of SOC in the clay fraction, the rest equally helded in the sand and the silt fractions, whereas most of the SOC in surface soils was found in the silt and clay fractions of their study (Nelson et al., 1994). Carbon is transferred from the sand-associated fraction to the silt- and clay-associated fractions during decomposition (Six et al., 2002). Compared to the whole soil contents, the C and N contents in the clay and silt fractions were increased whereas the sand fractions were all depleted (Christensen, 1985). Christensen (1985) found less than 10% C in sand, 22-38% in silt and 49-69% in clay. Changes in C of the <53μm fraction were much smaller than those of POM, accounting only 5.7-30.9% of the total changes in TOC (Chan 2001). Clay was found to contain up to 65% of the TC content (Anderson and Domsch, 1989).

The concentration of C for the silt and clay fractions was found to be more related to the soil management than to the soil characteristics (Caravaca et al., 2004). The clay fraction was equally affected during long term cultivation, although it is more stable against rapid decomposition than other fractions (Ratnyake et al., 2011). The latter authors reported the SOC content of the clay fraction to decrease significantly with cultivation, although it is mentioned to be more stable against land use changes compared to other fractions; our results corroborate this affirmation (Figure 16).



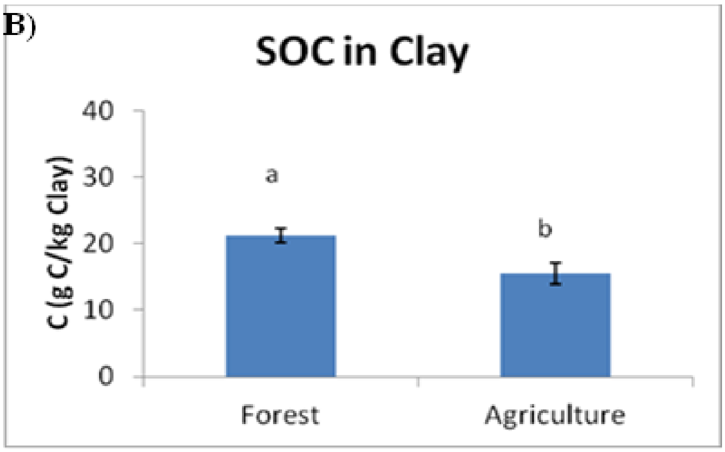


Figure 16. *Effect of deforestation and crop establishment for topsoil horizons (0-15 cm) on soil organic carbon (SOC) of the Silt and Clay fractions A) and B) respectively, and the standard deviations. Different letters indicate significant differences between forest and agriculture (crop) ($p<0.05$).*

Clay and carbon contents of our soils are highly positively correlated with a p value of less than 0.05 (Table 27). Clay particles create small pores (<1µm) where OM can be stored and can remain unreachable to decomposing organisms (Saha et al., 2010). Maximum physical protection capacity for SOM is determined by the maximum microaggregation, which in turn is determined by the clay content (Six et al., 2002). Clay is the determining factor for SOM contents, whereas the amount of sand is not correlated (Emmerling et al., 2001). Humified SOM content is positively related to clay contents and not highly variable (Buschiazzo et al., 2001). The protective effect of clay on SOM decomposition became significant as the substrate supply and microbial demand reached an equilibrium state (Wang et al., 2003). Carbon in clay is bonded strongly to colloid surfaces, being of low availability to decomposers, and resulting in little changes in C contents (Zinn et al., 2002). Hassink (1997) stated that the amount of organics that can be bound to clay particles is limited. The amount of C that can become associated with the clay fraction reaches a maximum and then any extra input of C from crop residues or vegetative cover would accumulate in the silt fraction (Caravaca et al., 2004). Hassink (1997) found arable soils to contain less C and N than grassland soils, but the amounts of C and N associated with clay and silt particles was the same, indicating that the amounts C and N that can become associated with these fractions had reached a maximum. Apparently SOC inputs into the soil defined in his study were enough to saturate the clay particles under arable farming. The extra input of C under grassland management could not be bound to clay particles and accumulated in fractions with a greater particle size (Hassink 1997).

Clay and carbon contents of our soils are highly positively correlated with a p value of less than 0.05 (Table 26). Clay particles create small pores (<1μm) where OM can be stored and can remain unreachable to decomposing organisms (Saha et al., 2010). Maximum physical protection capacity for SOM is determined by the maximum microaggregation, which in turn is determined by the clay content (Six et al., 2002). Clay is the determining factor for SOM contents, whereas the amount of sand is not correlated (Emmerling et al., 2001). Humified SOM content is positively related to clay contents and not highly variable (Buschiazzo et al., 2001). The protective effect of clay on SOM decomposition became significant as the substrate supply and microbial demand reached an equilibrium state (Wang et al., 2003). Carbon in clay is bonded strongly to colloid surfaces, being of low availability to decomposers, and resulting in little changes in C contents (Zinn et al., 2002). Hassink (1997) stated that the amount of organics that can be bound to clay particles is limited. The amount of C that can become associated with the clay fraction reaches a maximum and then any extra input of C from crop residues or vegetative cover would accumulate in the silt fraction (Caravaca et al., 2004). Hassink (1997) found arable soils to contain less C and N than grassland soils, but the amounts of C and N associated with clay and silt particles was the same, indicating that the amounts C and N that can become associated with these fractions had reached a maximum. Apparently SOC inputs into the soil defined in his study were enough to saturate the clay particles under arable farming. The extra input of C under grassland management could not be bound to clay particles and accumulated in fractions with a greater particle size (Hassink 1997).

The amount of years since deforestation (time) was a significant factor in most cases, for both silt and clay fractions (Figure 17 A) and B)). Especially the soils of the most recent deforestation (1980) had the highest SOC values, suggesting a reduction of the amounts of SOC withheld in the silt and clay fraction with time; this shows a tendency of reduced capacity of these fractions to hold SOC equally through time. The amount of SOC in the clay fraction should not be considered as a sensitive indicator in order to monitor changes in land use regimes, as the contents remain rather stable depending on the nature of land use. This report applies for our case, although the SOC forest values in clay compared to the agricultural values were significantly higher (hence this significant difference cannot be used as a reliable indicator of evaluating changes in different land use regimes) (Figure 17 B). The year categories did not have a certain pattern of the changes in SOC contents in the clay fraction (Table 30) as our results did not show a clear tendency of organic matter to be less in the clay fraction as the amount of years transcurred from deforestation increases. The latter year category (1980) did have the highest values as could be expected, but the amount of time did not play a role as the next higher value is that of the 1st deforestation year category (1933). The 1933 and the 1971 plots had as mentioned above the least values (the latter having the lowest), but still there is a greater capacity of the clay particles to withhold SOC compared to the silt particles. Hence clay appears to be more stable in terms of SOC amount accumulation, but as years go by this capacity starts to diminish. The significance of the values between the 2nd deforestation year category (1971) and the 3rd deforestation category (1980), could be attributed to local and individual management practices (especially for cotton, as the 1971 cotton subcategory had the lowest values) referring to irrigation amounts, fertilization amounts and dates, and ploughing amounts, dates and techniques, slight microclimate differences, possible laboratory analyses deviations and to plant growth and development, pathogens, dates of harvest, but at no case could SOC withheld in clay could serve as an indicator at deforested sites.

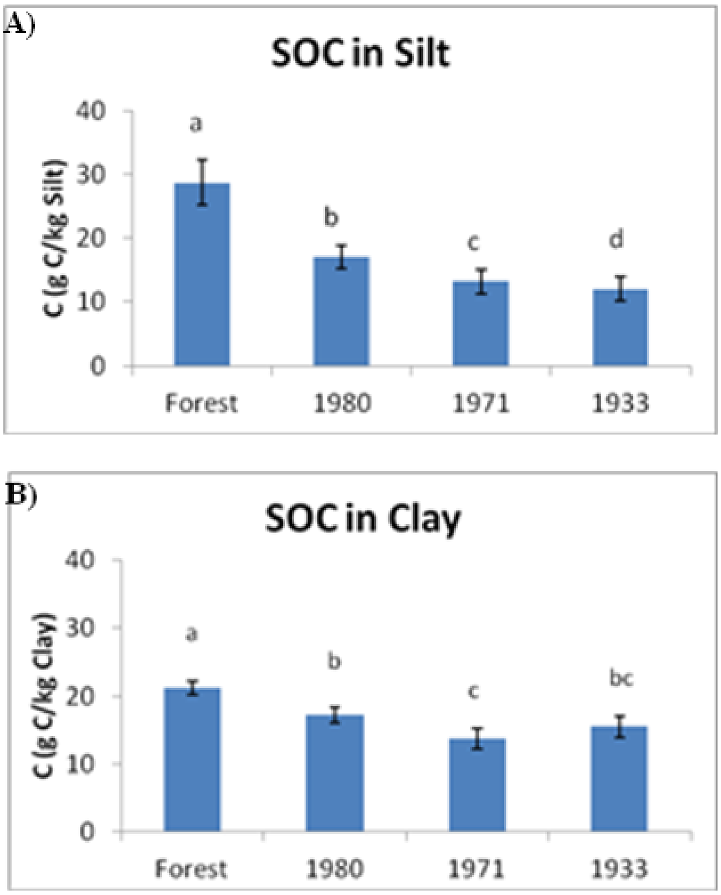


Figure 17. *Effect of time since deforestations and subsequent cultivation for topsoil horizons (0-15 cm) on soil organic carbon (SOC) of the Silt and Clay fractions, A) and B) respectively, and the standard deviations. Different letters indicate significant differences between deforested plots on 1933, 1971, 1980, and forest plots ($p<0.05$).*

Silt is the only fraction for which we have found more SOC (in many cases significantly) withheld for the cotton subcategories (Table 30). The differences in management have as result in the long-term more SOC proportionally to be present in the silt fraction than in the other soil fractions. The excess of water due to the practice of irrigation, and the more intensive management techniques resulted in mechanisms that enhance decomposition and possible SOM translocation from the clay particles. Soil OM withheld in silt is more cumbrous than the other two fractions, as Christensen (1992) and Guggenberger and Zech (1999) mentioned about time and SOM presence in different fractions (as discussed above). Guggenberger and Zech (1999) also mentioned that relatively high C concentrations in sand- and silt-sized separates could be due to incomplete soil dispersion. For both the cultivated and forested soils of their project, Caravaca et al., (2004) found the highest percentage of SOC to be associated with the silt fraction, concluding that in some cases SOM associated with silt can be considered less decomposed than that with clay and better protected against microbial and enzymatic degradation. Dalal and Mayer (1986) found that upon cultivation SOC declined in all fractions, but the contents in the silt fraction were least affected. The year categories showed in the silt fraction the same tendencies as the fine sand results (Table 30), although 1933 and 1971 categories did not differ significantly (Figure 17 A).

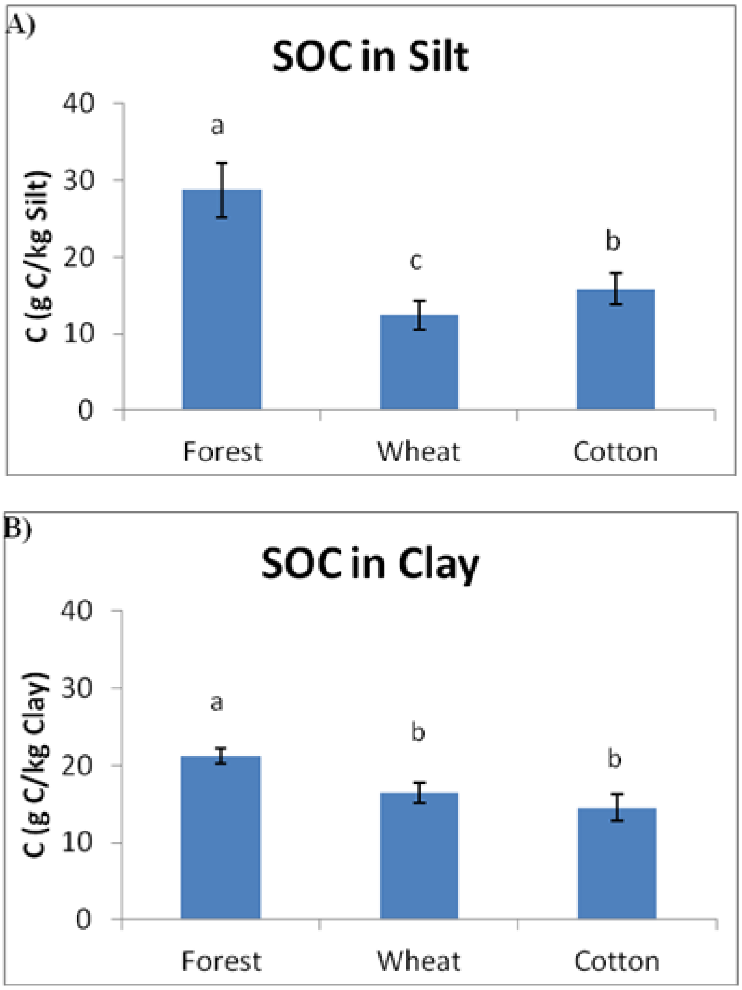


Figure 18. *Type of management effects for topsoil horizons (0-15 cm) on soil organic carbon (SOC) of the Silt and Clay fractions A) and B) respectively, and the standard deviations. Different letters indicate significant differences between wheat, cotton, and forest plots ($p<0.05$).*

According to our findings, silt-bound SOM was more sensitive to management and time factors than fine sand and POM (Figure 19). Soil OM in sand was swiftly affected by changes in land use, whereas SOM withheld in the silt fraction was much less affected in the short-term changes. The more the years, the less SOM is withheld in silt, but the changes are slow.

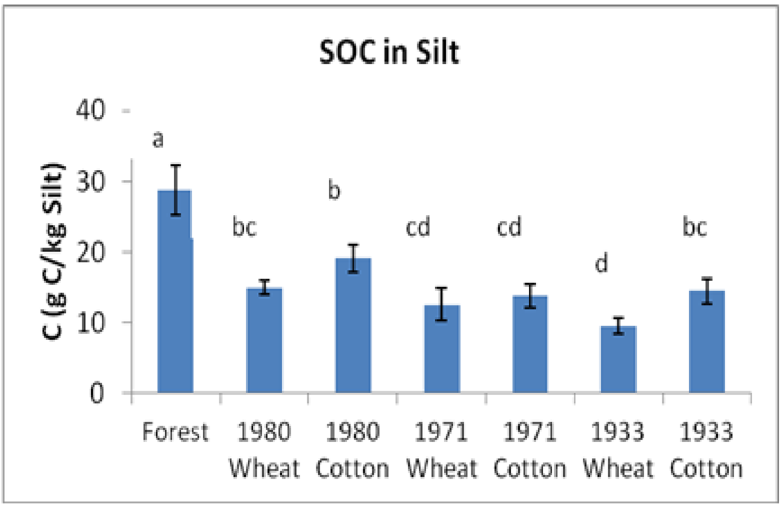


Figure 19. *Soil organic Carbon concentrations of the Silt fraction obtained in the studied plots, the comparison among the treatments and the standard deviations (different letters indicate significant differences between categories, p<0.05).*

Significant differences were found when comparing SOC in the silt fraction between the forest sites and the crop plots (26.14% and 44.77% respectively) as well as SOC in the clay fraction (24.87% and 52.02% respectively) (Table 30). Solomon et al. (2002) reported (for their subhumid highland soils of Ethiopia) 4-13% of total SOC to be found in the sand-size fraction, 29-52% in silt and 40-63% in clay (the first number refers to cultivated sites, the second to the forest plots of their study), stating that besides the reactivity and specific charge characteristics of clay minerals, the higher active surface area controls the enrichment of SOM in the finer-size particles. They also found that the largest SOC (78-79%) losses due to continuous cultivation occurred from particulate SOM associated with the sand fraction; our agricultural soils showed an 81.3% reduction in SOC contents of the fine sand fraction compared with the forest soils (Table 30). The silt fraction as Solomon et al. (2002) mentioned for their study lost

63-78% of SOC, indicating that SOM associated with silt is also quite labile, whereas we found a reduction of only 49.8% (Table 30), hence silt is quite protective of the SOC contents. Clay-bound SOM lost 42-44% of the SOC after 25-30 years of cultivation at the sites Solomon et al. (2002) studied; in our case agricultural soils generally showed a 27% reduction suggesting that clay is highly protective of SOC contents and more stable compared to the other two fractions. This fact may be attributed to physical and chemical stabilization of the SOM against biodegradation by the clay. Hence, clay presence protects SOC contents in the soil. In agreement to the above findings, Clapp and Hayes (1999) and Puget et al. (2000) found higher SOC concentration in the clay than in the silt fraction of an agricultural soil.

Reduced tillage practices in the wheat plots had as effect greater amounts of SOC withheld in clay compared to conventional tillage techniques, with values up to 15% more, not significantly higher though.

Irrigation might affect the SOC status in fractions, probably by leading to higher decomposition rates, as long as anaerobic conditions are not present (in the latter case decomposition rates are slower). In this thesis our results showed that generally SOC in fractions is higher at the wheat than at the cotton plots, but significant differences can only be found at the silt fraction, where the reverse trend is present (cotton plots have higher values than wheat plots). Overall, management was a significant factor, along with land use ($p<0.001$) and the amount of years since deforestation (Tables 30 and 31).

In the subcategory (time*management as a factor) level, few significances among all the comparisons were found (Figure 20). The time*management as well as the time*management*fraction factors do not affect significantly the SOC amounts and the tendencies.

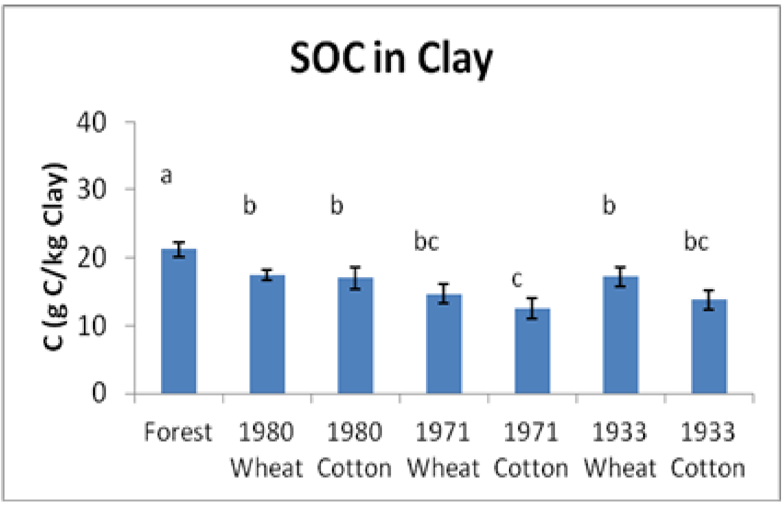


Figure 20. *Soil organic Carbon concentrations of the Clay fraction, obtained in the studied plots, the comparison among the treatments and the standard deviations (different letters indicate significant differences between categories, p<0.05).*

3.1.4 Comparison of SOC contents between the fractions

The SOC contents between the fractions depended significantly on land use and age (amount of time since deforestation) as factors; on the other hand, management, time*management and time*management*fraction as factors did not affect significantly the differences in SOC contents between the fractions (Table 32). For the forest values, and for those of agriculture as a land use factor, the differences were significant for each fraction, i.e. the amounts of SOC between those present in clay and in silt, the amounts of SOC between those present in clay and in fine sand, as well as the amounts of SOC between those present in silt and in fine sand differed significantly. The tendency though between forest sites and agricultural plots was opposite; for the forest plots, SOC contents in fine sand were significantly greater than SOC in silt which in turn were significantly greater than SOC in clay. This is expected, as the majority of the SOC amounts are present in sand, as fresh material. In the agricultural plots the SOC amounts in fine sand were much less than those in the forest, and significantly lower than the amounts withheld in silt. The clay fraction in this case acts as a protector of SOC, having the significantly greater values. Overall, land use changes play very important roles in SOC contents.

In the age level (time as a factor), all the inter-comparisons were significant, except the comparison between the silt and clay values of the 1971 and 1980 deforestation plots. As years pass by (from the most recent to the earlier deforestation), the differences in the amounts between the silt and clay values increased. The SOC losses were less pronounced in clay, as this fraction obtains increased capacity of SOC holding.

Management as a factor did not play a significant role overall, but the wheat plots followed the same tendency as agriculture as a whole. The insignificance of this factor is due to the increased SOC contents in the silt fraction or the cotton plots, fact which has been analyzed above. Generally, all the SOC contents in fine sand of the agricultural plots were significantly lower than the silt and/or clay values. Cultivating the land for many years has resulted in the depletion of SOC in the fine sand fraction. Concerning time*management as a factor, the only significant differences between the amounts of SOC in silt and clay can be observed in the 1933 wheat plots. This subcategory had the lowest value of SOC in the silt fraction, affecting the significances for the subcategory level, management level, and age level. The amount of SOC is mostly present and protected in the clay fraction. In every other subcategory, silt and clay had nearly similar and not significantly different values, suggesting that the capacity of silt to protect and withhold organic carbon is not significantly lower than the respective capacity of clay.

Table 32. *ANOVA significances for SOC contents among fractions (FS: Fine Sand, Silt and Clay) for each factor*.

Factor	FS *versus* Silt	FS *versus* Clay	Silt *versus* Clay
Land Use			
Forest	**	***	**
Agriculture	***	***	*
Time since deforestations			
1933	***	***	***
1971	***	***	ns
1980	***	***	ns
Management			
Wheat	***	***	***
Cotton	***	***	ns
Time*Management			
1933wheat	**	***	***
1933cotton	***	***	ns
1971wheat	***	***	ns
1971cotton	***	***	ns
1980wheat	*	***	ns
1980cotton	***	***	ns

Note*: Significance levels: *** (p<0.001),** (p<0.01), * (p<0.05), and ns: not significant.*

3.2. TOTAL SOIL NITROGEN

3.2.1 Nitrogen in whole soil

According to Franzluebbers et al. (1994), total N is highly correlated with SOC; this is also what we observed, with a significantly high correlation between the two properties (r = 0.936 and the p value is less than 0.001, Table 27). Huang et al. (2008) found significant correlations among labile SOM C and N pools. Hence, we expected N to follow the same trends as SOC in whole soil, with significantly higher values in the forest sites (N contents in the forest are always significantly greater than any category or subcategory, Figures 21, 22, 24), greater values for the latest deforestations (with the 1980 category having significantly higher values than the other two categories which between them had almost similar and not significantly different values, Figure 22), and the wheat plots to have higher values compared to the cotton plots (with significant differences, Figure 23). Indeed, our results (Tables 28 and 29) showed three factors to play a significant role for N contents in soil: land use, time (the amount of years since deforestation), and management of the cultivated plots (type of crop).

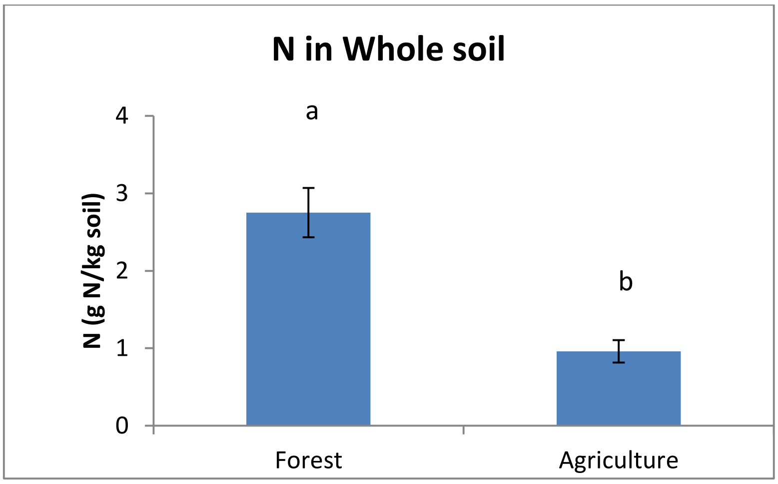


Figure 21. *Effect of deforestation and crop establishment on whole soil Nitrogen concentrations for topsoil horizons (0-15 cm), and the standard deviations. Different letters indicate significant differences between forest and agriculture (crop) (p<0.05).*

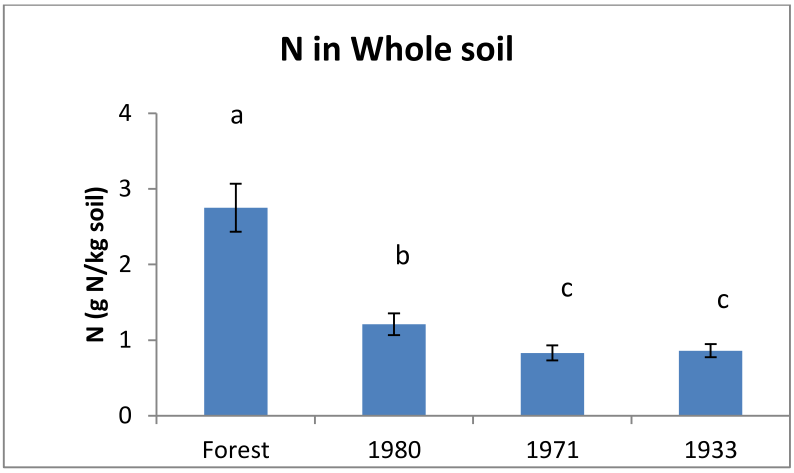


Figure 22. *Effect of time since deforestations and subsequent cultivation on whole soil Nitrogen concentrations. Different letters indicate significant differences between deforested plots on 1933, 1971, 1980, and forest plots ($p<0.05$).*

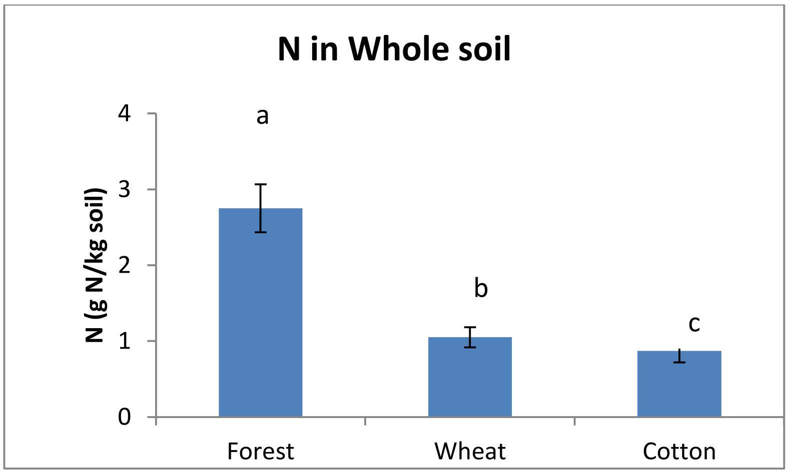


Figure 23. *Type of vegetation effects on whole soil Nitrogen [g kg^{-1} soil]. Different letters indicate significant differences between wheat, cotton, and forest plots ($p<0.05$).*

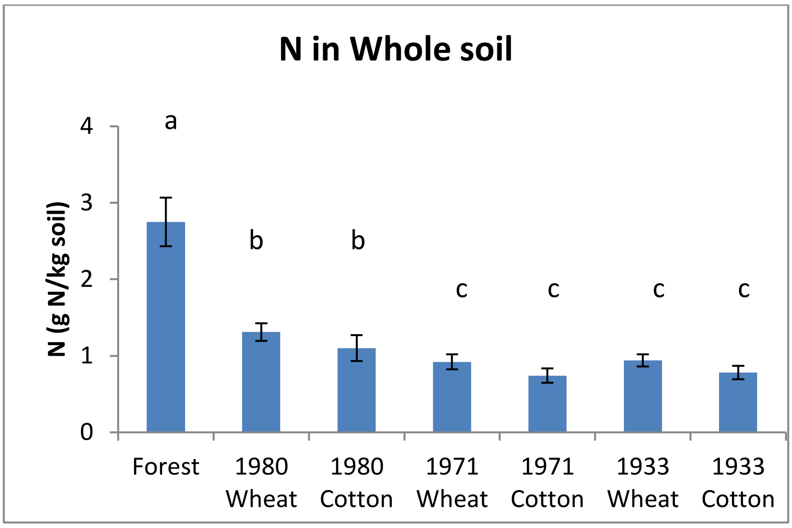


Figure 24. *Whole soil Nitrogen concentrations obtained in the studied plots (w for wheat, c for cotton), the comparison among the treatments and the confidence intervals (different letters indicate significant differences between categories, p<0.05).*

Many studies have indicated that converting forest to agriculture reduces organic matter input from litter and enhances C output via breaking the protection of SOM resulting in a decrease of TN and SOC (Rasmussen and Parton, 1994; Constantini et al., 1996; Islam and Weil, 2000; Buschiazzo et al., 2001; Sahani and Behera, 2001; Guo and Gifford, 2002; Caravaca et al., 2004; Dinesh et al., 2004; Rudrappa et al., 2006; Ashargie et al., 2007). Our results previously discussed in the SOC chapter corroborate this affirmation for SOC; Franzluebbers et al. (1994) and Ashargie et al. (2007) also mentioned that N losses at deforestated sites are important and significant. In the former authors´ work, the 0-30cm agricultural horizon was found to contain half of the amount of the upper forest horizon N. Clearing and subsequent cultivation of tropical woodland resulted to a decrease of the N contents of the soil by 51% (Solomon et al., 2000). In our case the decrease was 65.1% (Table 28). Land use changes have significant effects, since the organic matter inputs in the soil decrease substantially. Another explanation of the decrease in N contents following deforestation in our study is the rapid and important loss of POM. Particulate OM levels are significantly correlated with the macroaggregate stability and mineralizable N and are therefore intimately linked (Chan, 2001). Ashargie et al. (2007) argued that losses of POM and N due to cultivation were highest in comparison to whole soil SOC and N, suggesting that POM constitutes a more sensitive SOM fraction to the effects of cultivation.

The amount of years since deforestation played a significant role on total N in the whole soil in our study, since the most recent deforestation had significantly higher values than the oldest one. As time marches on, these differences seem to moderate. Campbell et al. (1999) mentioned that the systems may reach a steady state for SOC and N, as they observed no further changes 4 years after the first 4 years of managing their sites. In our case the equilibrium could probably be reached after many years, since the categories derived from the first two deforestations (1933 and 1971) had similar values whereas the latter deforestation (1980) had significantly higher values. The relatively higher total amounts of N added from fertilization of the soils throughout the years did not seem to affect the values of the long-term tilled soils compared to the N losses due to reduction of organic matter. Hassink (1994) reported the rate of N fertilization to have no significant effects on the SOC and N amounts of their study.

Tillage promotes SOM decomposition through crop residue incorporation into the soil and physical breakdown of residues (Roldán et al., 2007) rendering soil management the main factor affecting N (as well as SOC) levels (Franchini et al., 2007). This is the case of our study, as the differences between wheat and cotton plots were significant, with the values of the reduced tillage (RT) plots being higher, as wheat plots are not so frequently tilled. Practice of NT showed a greater impact on N than on SOC storage, suggesting that N cycling is an important factor related to soil C sequestration potential (Wright and Hons, 2004). Franzluebbers and Ashrad (1996) mentioned greater values at NT compared to CT, whereas N increased significantly under minimum tillage practices compared to CT (Jacobs et al., 2009). Furthermore, tillage differences may affect soil porosity and air diffusion with possible impacts for loss of N by denitrification (Carter, 1986). In our case, one main reason for the differences observed in the total N values of the wheat and cotton plots could be the different amount of N fertilizers applied in each management practice. As was indicated in the material and methods section, wheat plots received a mean of 111.875 kg N ha^{-1} through annual fertilization, whereas cotton plots received 98.750 kg of N ha^{-1}. Wheat plants absorb 69.88-119.26 kg ha^{-1} of N (according to Kumbhar et al., 2007), whereas cotton plants 67.40-113.43 kg ha^{-1} (Kumbhar et al., 2008). We could consider most of the amounts of fertilizer used by the plants, and the slightly higher amounts not absorbed could participate in the slightly higher amounts of N that were observed in the wheat plots.

Nitrogen was reported to be significantly correlated with MBC (Insam et al., 1991). Our results imply a good correlation between these parameters (r = 0.802). We observed the same tendencies in amounts, but not in all the types of significance of the differences. There was a difference in management, although the values had the same tendencies (higher in the wheat plots). It seems in our case N was slightly more significantly correlated to SOC amounts and trends.

3.2.2 Nitrogen in Fractions

3.2.2.1 General remarks and comments

As N contents in soils are intimately related to SOC contents (Haney et al., 2012), the general trend of N in fractions of cultivated soils is similar to that of SOC increasing in the order: sand<silt<clay (Solomon et al., 2000; Solomon et al., 2002). Cultivation leads to a decrease of soil C and N associated with the silt and clay fractions (Caravaca et al., 2004), but not to such a great extent than in the sand fraction. Hassink (1997) reported cultivated soils to contain less than 50% of the amount of C and N in the sand fraction than uncultivated and grassland soils. The latter author also stated that the slight differences between the amounts of N associated with clay and silt particles in the uncultivated and cultivated plots of their study (the proportion of C and N left after conversion to arable land was generally more than 60%), indicated that the amounts C and N that can become associated with these fractions had reached a maximum (Hassink, 1997). After 25-30 years of cultivation of tropical soils the largest losses in the amounts of N (75-81%) due to continuous cultivation occurred from particulate SOM associated with the sand fraction, the silt fraction lost 60-73% of N, indicating that SOM associated with silt is also quite labile, and clay-bound SOM lost 42-53% of the N (Solomon et al., 2002).

Our results come to agreement with the above remarks, as N followed the SOC trend with the exception in the silt and clay values of the forest plots, where N amounts in clay were higher than in silt, whereas the SOC contents in silt were higher than in clay. These facts are discussed in the following paragraphs of the discussion, analyzed for each fraction separately.

The fact that cultivation decreased the amount of C and N in the sand fraction to a greater extent than in the silt and clay fractions, indicates that C and N associated with the latter fractions is better protected against decomposition. This indication of better protection of SOM in the two former fractions was present in the cultivated plots of our study, whereas in the forest plots fresh organic material was more characteristically present in the sand fraction.

In the agricultural sites of our study, 13.4% of N was associated with sand, 29.7% with silt and 56.9% with clay (Figure 25), whereas at the forest soils 41.6% of the N contents was associated with sand, 21.1% with silt and 37.3% with clay (Figure 26). Other researchers mentioned similar values; Solomon et al. (2000) mentioned 2-12% of total N to have been found in the sand fraction, 25-40% in silt and 47-71% in clay, and Christensen (1985) mentioned 20-33% of N to be found in the silt fraction and 47-78% in clay. It is obvious that most of the N was withheld at the clay particles in the agricultural soils, whereas in forest soils sand had the biggest influence on N contents.

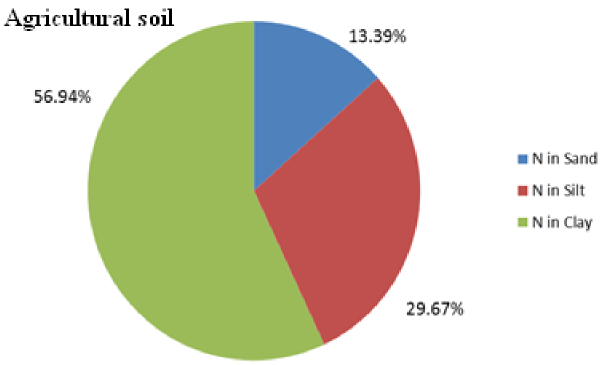


Figure 25. *Percentages of Nitrogen in the fractions of the agricultural land use.*

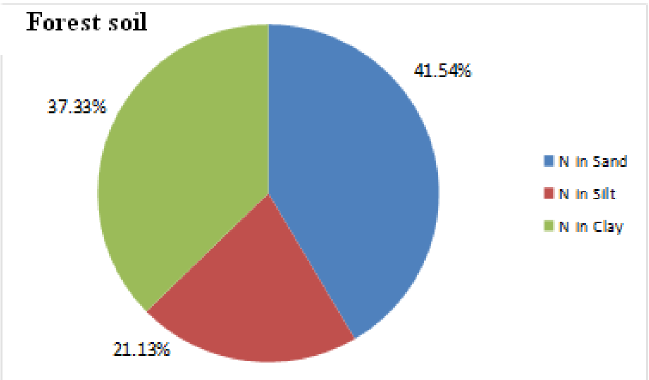


Figure 26. *Percentages of Nitrogen in the fractions of the forest land use.*

3.2.2.2 Nitrogen in the Fine Sand fraction

Forest soils were expected to and actually did have significantly higher amounts than any year category, subcategory and management type (Table 30, Figures 27 to 30). The total N concentration in the fine sand fraction was significantly higher in forest soils than in crop soils (Figure 27) probably due to the higher amount of OM residues incorporated to soil in forests than in crops. The 1980 year category sites had significantly greater values than the other two year categories which do not differ between them (Figure 28). The wheat plots (referring to management type) had higher values than the cotton plots, but not significantly (although p=0.0528) (Figure 29).

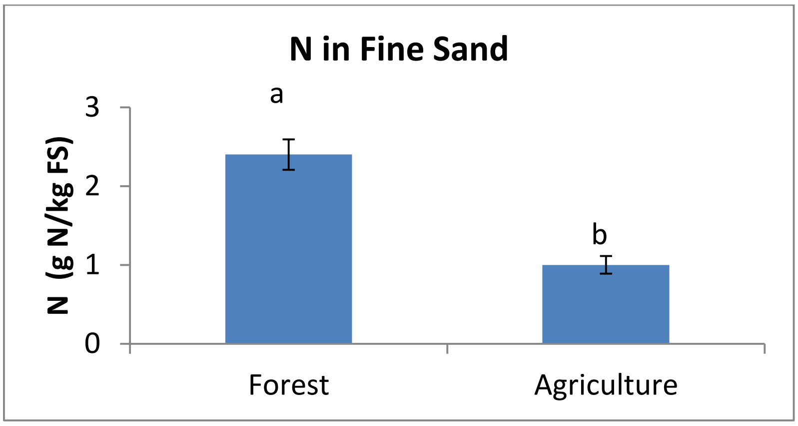


Figure 27. *Effect of deforestation and crop establishment on Nitrogen concentrations of the Fine Sand fraction for topsoil horizons (0-15 cm), and the standard deviations. Different letters indicate significant differences between forest and agriculture (p<0.05).*

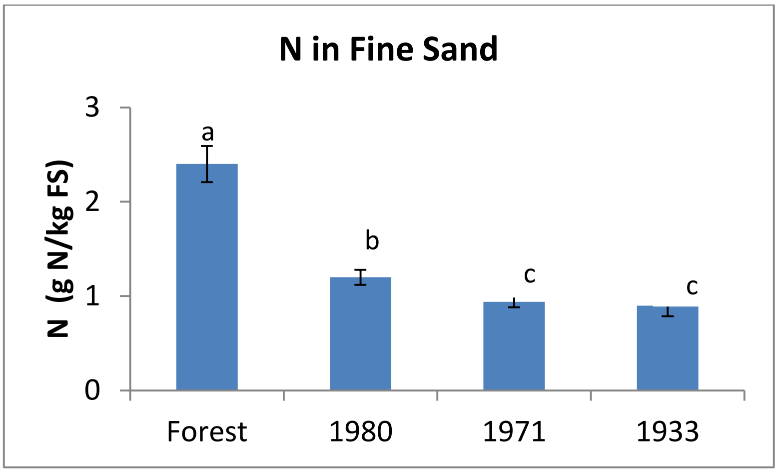


Figure 28. *Effect of time since deforestations and subsequent cultivation on Nitrogen concentrations of the Fine Sand fraction, and the standard deviations. Different letters indicate significant differences between deforested plots on 1933, 1971, 1980, and forest plots ($p<0.05$).*

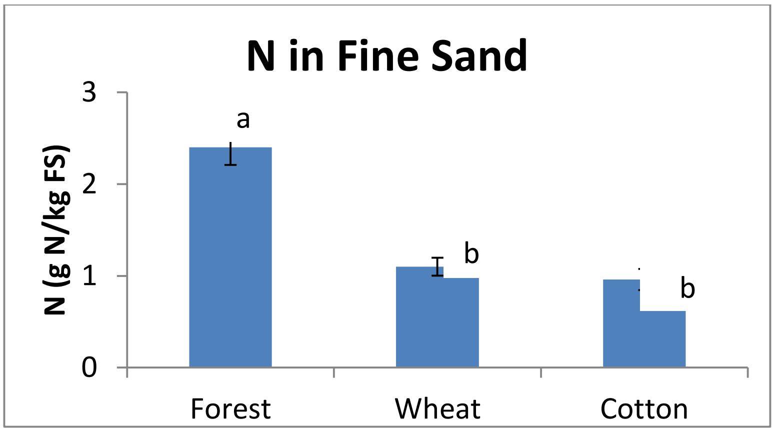


Figure 29. *Type of vegetation effects on Nitrogen [g kg^{-1} soil] of the Fine Sand fraction, and the standard deviations. Different letters indicate significant differences between wheat, cotton, and forest plots ($p<0.05$).*

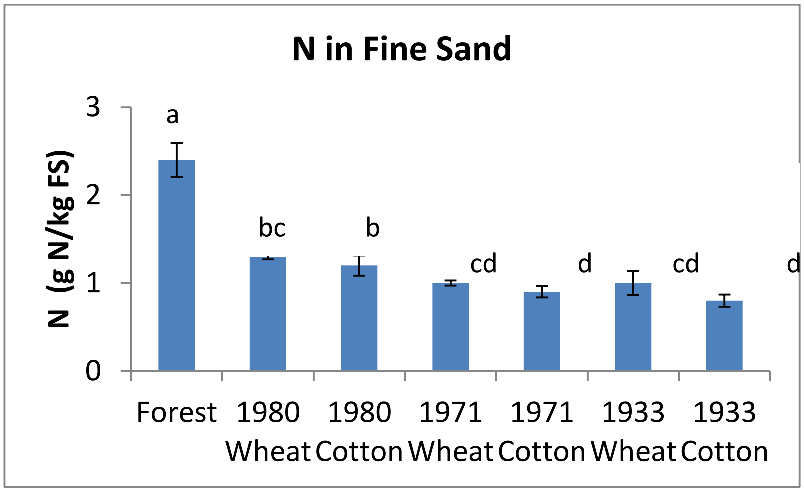


Figure 30. *Nitrogen concentrations of the Fine Sand fraction, obtained in the studied plots (w for wheat, c for cotton), the comparison among the treatments and the standard deviations (different letters indicate significant differences between categories, p<0.05).*

The amounts of N in sand-size fractions are related to the amount of residue that is incorporated into the soil, and not to soil characteristics such as texture (Hassink, 1997; Caravaca et al., 1999). Organic N shows lower rates of mineralization in the sand fraction than in the other soil fractions (Buschiazzo et al., 2001).

Solomon et al. (2000) observed rapid increase of C and N on coarse and fine sand fractions during the first 3 years of cultivation after woodland clearing. In our work land use and the amount of years since deforestation were important factors affecting N contents in the fine sand fraction, with land use having the greatest effect. We observed that N was lost throughout the years since deforestation but there is a tendency of reaching an equilibrium state after 35-70 years. Hence, total N values in crop soils deforested in 1933 and 1971 were similar (0.90 and 0.94g of N kg^{-1} of soil, respectively), whereas total N values in crop soils deforested in 1980 were significantly higher (1.20 g N kg^{-1} of soil). The 1933 deforestation which had the lowest total N values of the three year categories, holded lower values by 62.5% compared to the forest values.

For CT practices, the least contents of TN were reported in the sand fractions (Chivenge et al., 2003). In minimum-tillage sites, the increased SOC and N storage did not occur as POM, as reported for NT, but as mineral-associated OM (Jacobs et al.,

2009) and was significantly affected. In our study, differences in total N soil concentrations between the two management/crop types (wheat and cotton plots) compared were not significant (Figure 29). Higher (although not significant) values occured in the wheat fields, as can be explained due to the greater N inputs by fertilization (as SOC contents in fine sand did not play a role, being nearly equal). The fertilization rates as are presented in the Materials and Methods chapter, were 111.88 kg ha^{-1} for the wheat plots compared to the 98.75 kg ha^{-1} for the cotton plots, when both plants seem to absorb similar amounts of N throughout the year (Kumbhra et al., 2007; Kumbhra et al., 2008, see previous chapter). The 1933 cotton plots had 71.6% lower TN values than the forest plots, which were the lowest values for any subcategory.

3.2.2.3 Nitrogen in the silt and clay fractions

Referring to silt, the N contents in the forest plots were always significantly higher than any agricultural treatment (Figures 31 to 34). The most recent deforestation (1980) seemed to withhold significantly more N within the silt fraction than the other two deforestation categories (1933 and 1971) which had similar values (Figure 32). The cotton plots (referring to management type) had slightly higher values than the wheat plots, but not significantly (Figure 33). Only land use as a factor played a significant role.

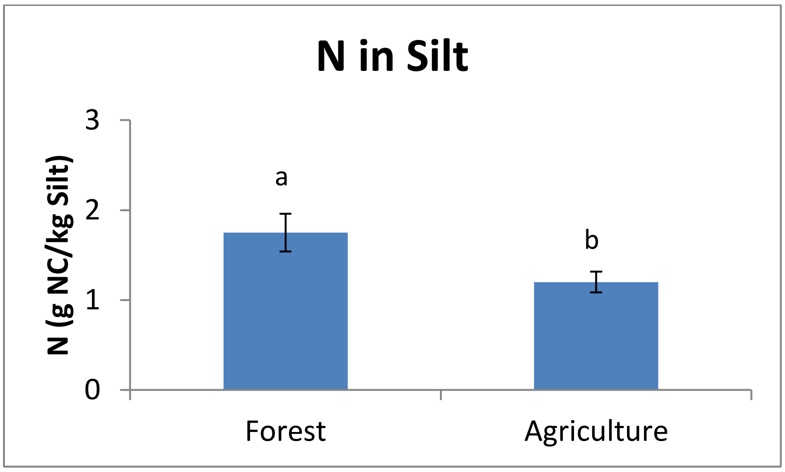


Figure 31. *Effect of deforestation and crop establishment on Nitrogen concentrations of the Silt fraction for topsoil horizons (0-15 cm), and the standard deviations. Different letters indicate significant differences between forest and agriculture (p<0.05).*

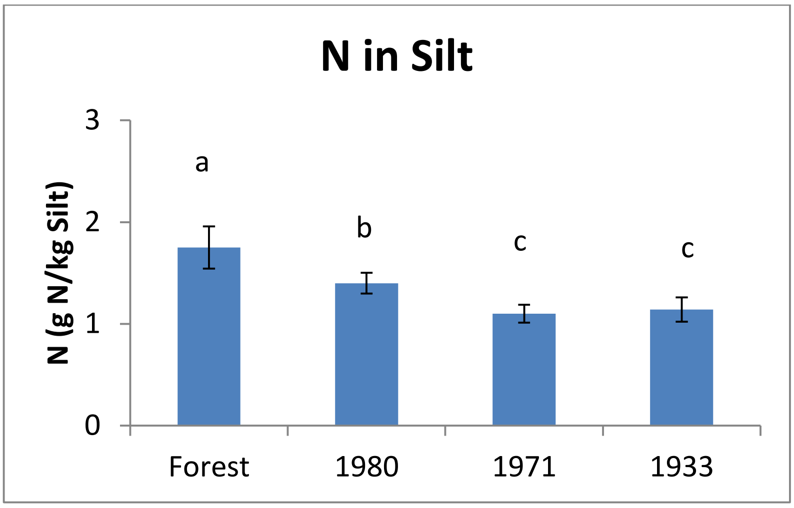


Figure 32. *Effect of time since deforestations and subsequent cultivation on Nitrogen concentrations of the Silt fraction, and the standard deviations. Different letters indicate significant differences between deforested plots on 1933, 1971, 1980, and forest plots ($p<0.05$).*

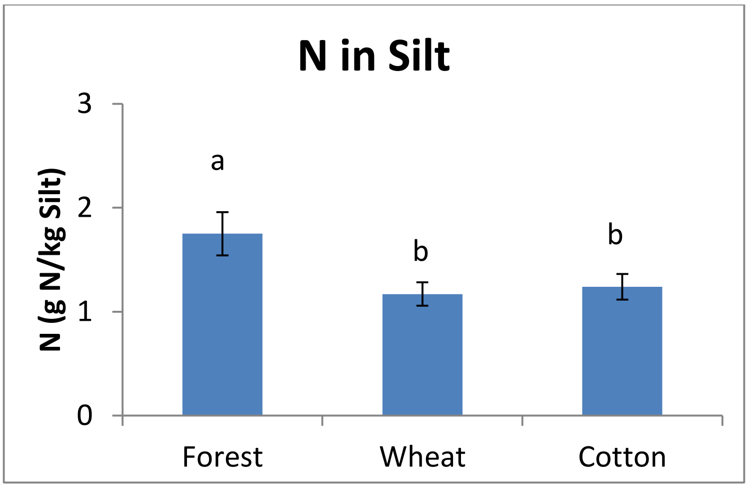


Figure 33. *Type of vegetation effects on Nitrogen concentrations, [g N kg^{-1} of Silt fraction], and the standard deviations. Different letters indicate significant differences between wheat, cotton, and forest plots ($p<0.05$).*

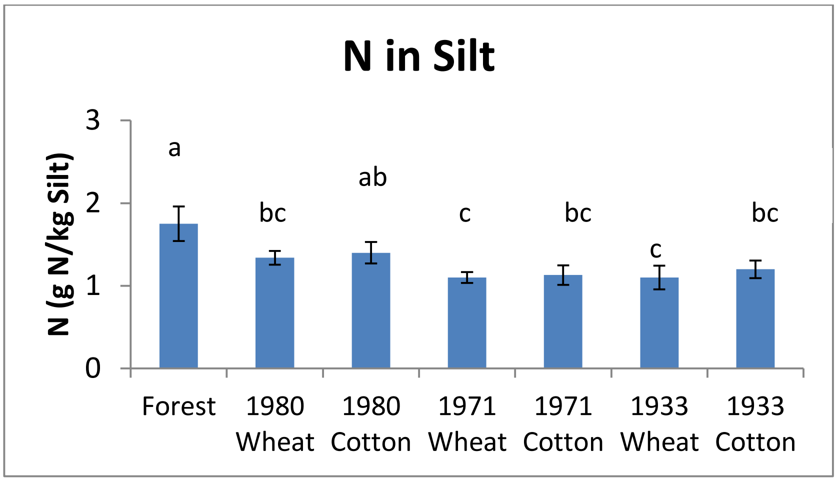


Figure 34. *Nitrogen concentrations of the Silt fraction, obtained in the studied plots, the comparison among the treatments (different letters indicate significant differences between categories, p<0.05) and the standard deviations.*

For clay, none of the studied factors played any significant roles (Figures 35 to 38). The 1980 year category had the highest value (even from forest) but it differed significantly only with the 1971 year category, although marginally (p=0.0516), whereas the forest value was not significantly higher than the 1971 year category for a p value of 0.0476 (Figure 36). Management seemed to play a role, as the N values of the wheat and cotton plots varied significantly, and forest varied significantly with the cotton plots, but when the observations refer to the subcategory level, management played a significant role only for the 1971 subcategories (Figures 37 and 38).

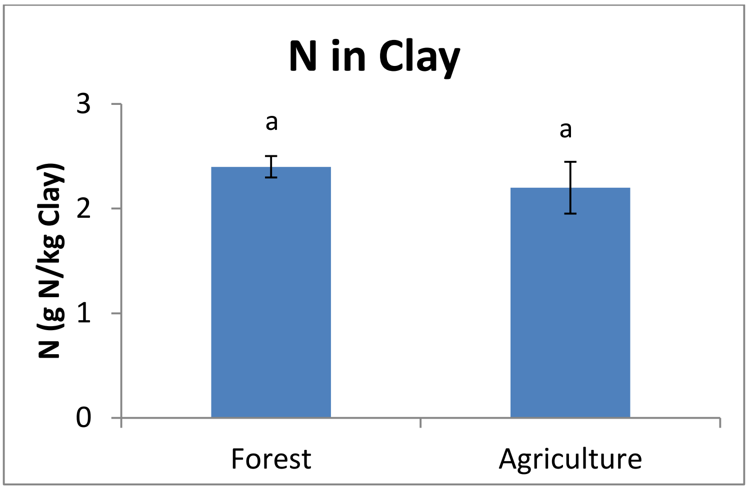


Figure 35. *Effect of deforestation and crop establishment on Nitrogen concentrations of the Clay fraction for topsoil horizons (0-15 cm), and the standard deviations. Different letters indicate significant differences between forest and agriculture (crop) ($p<0.05$).*

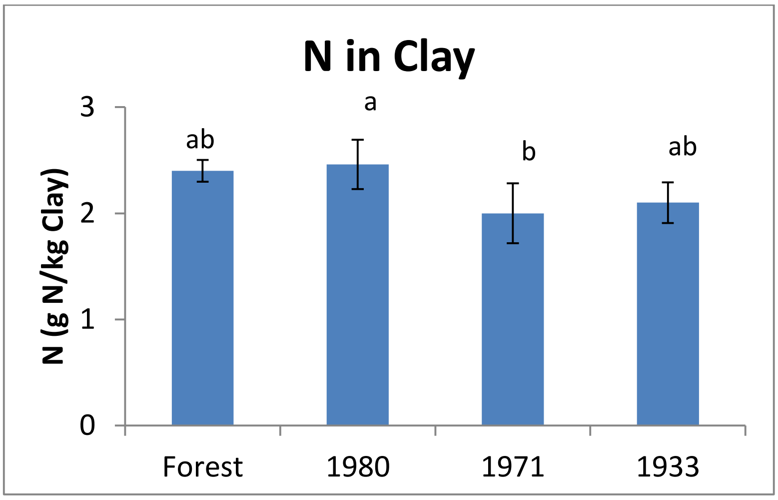


Figure 36. *Effect of time since deforestations and subsequent cultivation on Nitrogen concentrations of the Clay fraction, and the standard deviations. Different letters indicate significant differences between deforested plots on 1933, 1971, 1980, and forest plots ($p<0.05$).*

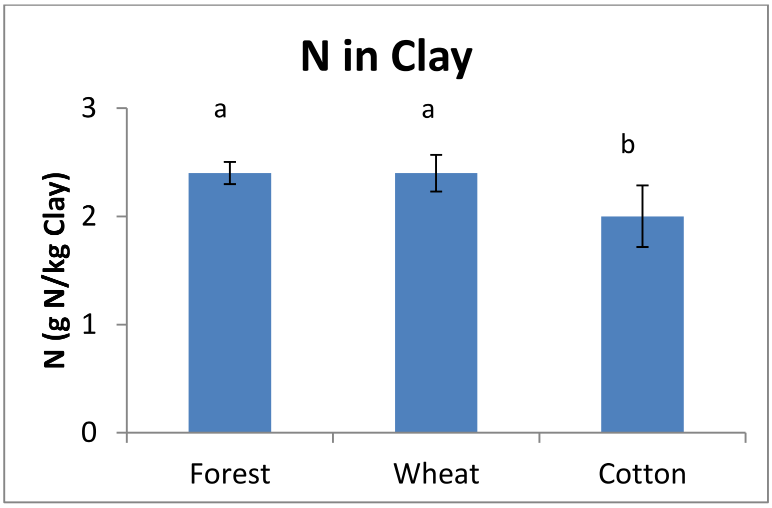


Figure 37. *Type of vegetation effects on Nitrogen concentrations in the Clay fraction [g N kg^{-1} of clay], and the standard deviations. Different letters indicate significant differences between wheat, cotton, and forest plots (p<0.05).*

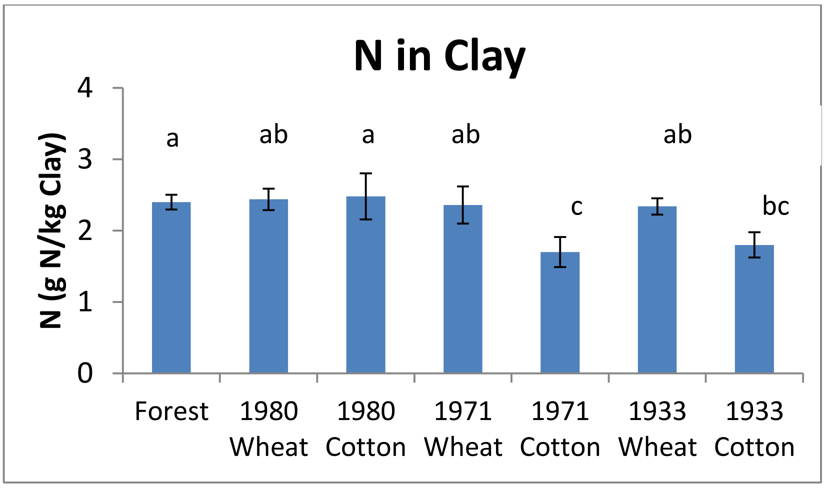


Figure 38. *Nitrogen concentrations of the Clay fraction, obtained in the studied plots (w for wheat, c for cotton), the comparison among the treatments and the standard deviations (different letters indicate significant differences between categories, p<0.05).*

In every case of our study, for every land use type, all the categories, subcategories and management practices, the silt and clay contents were higher than in the fine sand fraction (except for the forest fine sand fraction, in which N contents were marginally higher than in the clay fraction -0.238% compared to 0.236%- due to accumulation of fresh organic material as discussed above), with the clay contents being always higher (all the significances are presented in Table 33). The contents of SOC and N in the silt and clay fractions in the study of Caravaca et al. (1999) and Lehmann et al. (2001) were higher than in the sand fraction confirming that the SOM associated with silt and clay is physically better protected against microbial decomposition, hence contributing to higher amounts of SOC and N to the soil samples (Glaser et al., 2000).

The N contents between the fractions depend significantly on land use and time (amount of time since deforestation) as factors; on the other hand, management, time*management and time*management*fraction as factors did not affect significantly the differences in SOC contents between the fractions (Table 33). For the forest values, the trends are discussed above. Agriculture as a land use factor, affected the N contents in each fraction significantly, i.e. the amounts of N between those present in clay and in silt, the amounts of N between those present in clay and in fine sand, as well as the amounts of N between those present in silt and in fine sand differed significantly. The tendency though between forest sites and agricultural plots is not the same; for the forest plots, N contents in fine sand and in clay were significantly greater than N in silt, fine sand and clay values being similar. This is expected, as the majority of the N amounts were present in sand, as fresh material, and clay on the other hand had the capacity to preserve N, thus resulting in silt having the lower values. In the agricultural plots the N amounts in fine sand were much less than those in the forest, and significantly lower than the amounts withheld in silt. The clay fraction in this case acted as a protector of N, having the significantly greater values. Overall, land use changes play very important roles in N contents.

In the age level (time as a factor), all the inter-comparisons were significant. As years passed by (from the most recent to the earlier deforestation), the differences in the amounts between the silt and clay values increased. The N losses were less pronounced in clay, as this fraction obtained increased capacity of SOC holding. Specifically, the values between agricultural and forest clay N contents were similar.

Management as a factor did not play a significant role overall, but the wheat and cotton plots followed the same tendency as agriculture as a whole. Generally, all the N contents in fine sand of the cotton plots were significantly lower than the silt and/or clay values, but when it comes to the wheat management practices differences between the fine sand and silt values were not significant. Cultivating the land for many years has resulted in the depletion of N in the fine sand fraction; the results of this continuous cultivation were more pronounced in the conventionally tilled cotton plots. Reduced tillage practices led to a more mild loss of N in the fine sand fraction compared to the N contents in silt. Concerning time*management*fraction as a factor, differences were significant, except between the amounts of N in fine sand and silt in the wheat subcategories. The reasons of these indifferences are presented above. The amount of N was mostly present and protected in the clay fraction. This can also be shown by their correlation (N and clay) which is strong and positive (p value is less than 0.05, Table 27). The capacity of silt to protect and withhold N is significantly lower than the respective capacity of clay, whereas referring to SOC values the opposite trend was observed. Nitrogen compounds seemed to require stronger charges in the soil particles, as clay can present, hence silt overall is not that capable of withholding N as SOC.

Our results showed that land use change from forest to crop caused a significant decrease on total N in the silt fraction (we can see a significant decrease also in the fine sand fraction) but no significant effect was observed in the clay fraction, indicating that the SOM bound to clay is more stable than that found in silt and sand particle size fractions. This may be attributed to physical and chemical stabilization of the SOM against biodegradation by the clay. Generally, differences in land use could be due to the relatively lower depletion of SOC and N from clay-sized separates of the cultivated fields compared to the forest plots. Furthermore, organic substances interact with clay to form organo-mineral complexes and microaggregates, which make the organic matter less accessible to decomposers. Caravaca et al. (1999) and Caravaca et al. (2004) reported higher percentages of clay-N and silt-N under natural vegetation than at cultivated sites, because forest soil was not tilled or exposed to erosion. The amounts of N between these two fractions were similar, although higher in clay. The percentage of clay-associated N on forest soils was lower than cultivated sites whereas the percentage of silt-associated N was higher (Caravaca et al., 1999).

Saroa and Lal (2003) mentioned significant positive correlations between TN (as well as SOC) and clay, with the clay fraction accounting for 62-77% of the TN concentration in the whole soil. They explained this observation by attributing to clay particles the ability to bind the labile fractions, leading to the formation of passive SOC pools with slow turnover times due to physical protection by clay minerals.Time transcurred since deforestation showed significant effect in total N concentrations of silt fraction, showed significant higher values in plot deforested in 1980 than in 1971 and

1933 deforested plots, however N contents in the clay fraction of our work imply the capacity of this fraction to preserve N. The soils in all the plots reached their capacity to preserve N (especially in the forest and the wheat plots), although depending on the amount of years since deforestation few N amounts were lost. Again there was a tendency of reaching an equilibrium state after 35-70 years after deforestation. Solomon et al. (2000) mentioned that contents of C and N for the silt and clay fractions were found in the order: Forest > 3 years of cultivation > 15 years of cultivation, in agreement with the trend of our case.

Our results showed that contents of N in the silt fraction referring to the management differences follow the same trend with those of SOC the silt fraction. It is the only fraction at which the cotton fields have higher amounts (not significantly though) of N than the amounts in the wheat fields. The same analysis could be presented in this case, although N fertilization was higher in the wheat fields. Nitrogen in silt is not withheld so strongly as in the clay fraction, hence irrigation techniques play a more important role than fertilization, resulting in higher levels of N as NH_4^+ forms rather than NO_3^-. The more frequent fertilization of cotton could also aid in this direction. Hassink (1997) reported that low water inputs (in his study he referred especially to precipitation) could lead to low inputs of N.

Nitrogen contents in the clay fraction of our work imply the capacity of this fraction to preserve N. The soils in all the plots reached their capacity to preserve N (especially in the forest and the wheat plots), although depending on the amount of years since deforestation few N amounts are lost. There is no pattern of this capacity, but the wheat fields seemed to have significant higher values, especially those of the most recent deforestation's plots. These differences depend only in the values of the 1933 and (especially of the) 1971 wheat plots, as the most recent deforestation had slightly higher values at the cotton plots. The bigger amounts of N fertilization in the wheat plots resulted in larger withholding capacities of the clay particles. For the 1980 category this is not a fact as values were practically similar. Big amounts (more than 25 years) of time affected the N withholding capacity in the wheat fields. It is the only case of the results of this study where forest values were not the highest (compared to both 1980 wheat and cotton subcategories). On the other hand, Six et al. (2002) reported significant differences in the light fraction of soils with different management practices, but no differences in the clay fraction.

Table 33. *ANOVA significances for N contents among fractions (FS: Fine Sand, Silt and Clay) for each factor*.

Factor		FS *versus* Silt	FS *versus* Clay	Silt *versus* Clay
Land Use				
	Forest	***	ns	***
	Agriculture	***	***	***
Time since deforestations				
	1933	**	***	***
	1971	*	***	***
	1980	*	***	***
Management				
	Wheat	ns	***	***
	Cotton	***	***	***
Time*Management				
	1933wheat	ns	***	***
	1933cotton	***	***	***
	1971wheat	ns	***	***
	1971cotton	*	***	**
	1980wheat	ns	***	***
	1980cotton	*	***	***

Note: *Significance levels: *** (p<0.001),** (p<0.01), * (p<0.05), and ns: not significant.*

3.3 THE C/N RATIO IN SOIL

3.3.1 C/N in whole soil

Overall, forest values were significantly higher than agricultural use, but not than the values of the 1971 cotton subcategory and the two subcategories of the 1980 deforestation (Figures 39 to 42). Year categories between them did not vary significantly, except when comparing the values from oldest and from the most recent deforestations (Figure 40). In terms of management, no significant differences between the two management types were observed (Figure 41), with the wheat plots having slightly lower values. Between the management subcategories, only the cotton plots of the 1933 and 1980 year categories had significantly different values (Figure 42).

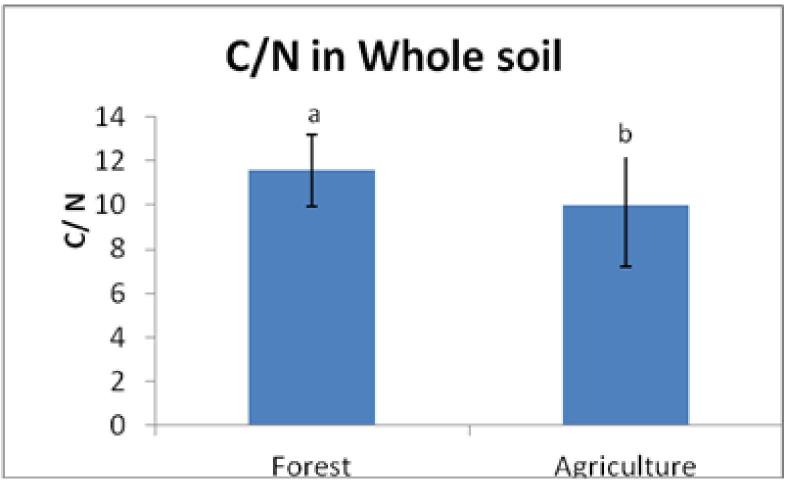


Figure 39. *Effect of deforestation and crop establishment on Carbon to Nitrogen ratio for whole soil from topsoil horizons (0-15 cm), and the standard deviations. Different letters indicate significant differences between forest and agriculture ($p<0.05$).*

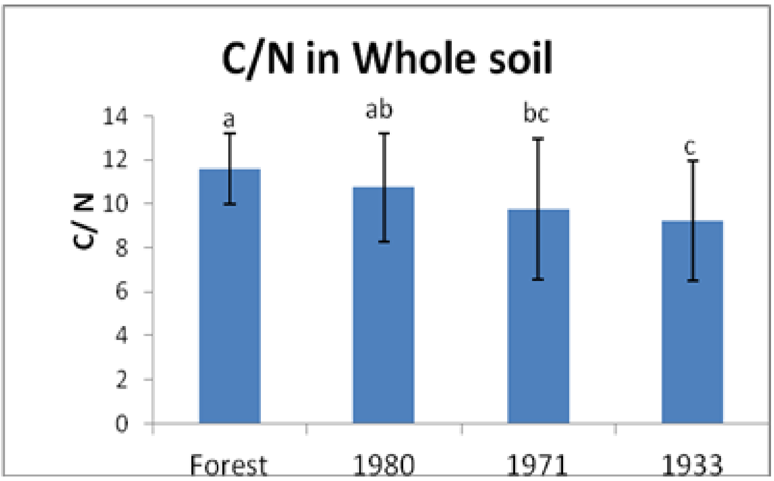


Figure 40. *Effect of time since deforestations and subsequent cultivation on Carbon to Nitrogen ratio for whole soil from topsoil horizons (0-15 cm), and the standard deviations. Different letters indicate significant differences between deforested plots on 1933, 1971, 1980, and forest plots (p<0.05).*

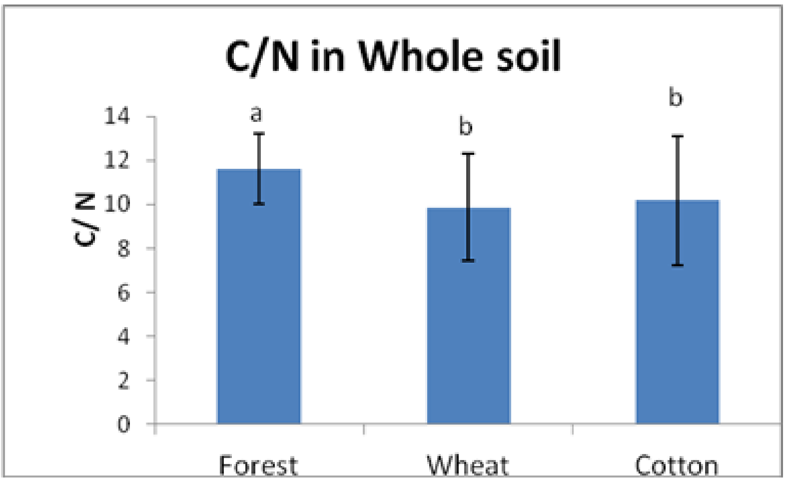


Figure 41. *Type of management effects on whole soil Carbon to Nitrogen ratio, and the standard deviations. Different letters indicate significant differences between wheat, cotton and forest plots (p<0.05).*

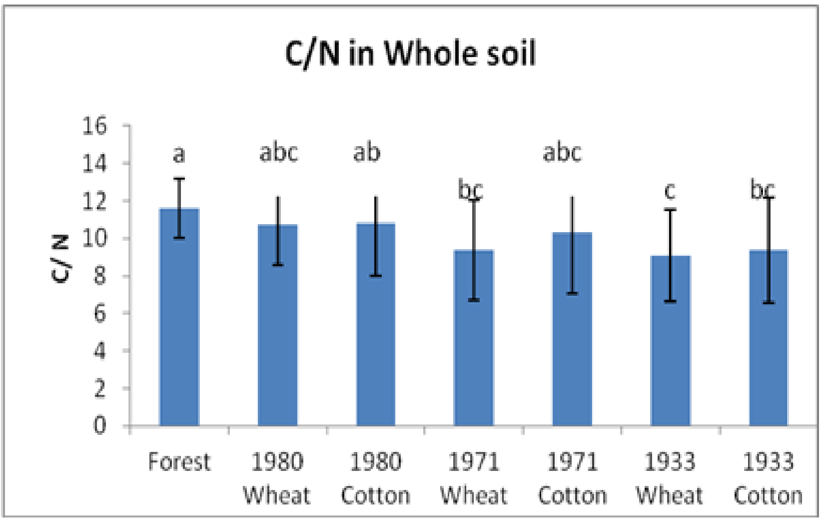


Figure 42. *Whole soil Carbon to Nitrogen ratio obtained in the studied plots, the comparison among the treatments and the standard deviations (different letters indicate significant differences between categories, p<0.05).*

In our study, C/N values ranged from 9.1 for the 1933 wheat subcategory plots to 11.6 for the forest plots, with a mean value for crop soils of 10.01 (Tables 28 and 30). The differences between the forest values and those from the agricultural sites were significant, agreeing with the remarks of Wright and Hons (2004).

The C to N ratio informs about the level of humification and the degree of decomposition of the organic matter (He et al., 2009): low ratios indicate more humified organic material, whereas high ratios less decomposed organic matter (Caravaca et al., 1999). High values of the ratio could indicate low quality litter (Lehmann et al., 2001) and strong influence by plant residues (Breulman, 2011), whereas higher quality residues have a lower C/N ratio (Wright and Hons, 2004). Higher forest values are due to the increased presence of fresh organic material (POM contents) compared to agricultural sites, whereas the highest SOM decomposition rates as a result of cultivation practices, and enhanced N rates as a result of fertilization, result in lower values at the agricultural plots. Witter and Kanal (1998) and Lehmann et al. (2001) argued that agricultural land use and especially fertilization lowers C/N rates. Tillage enhances SOM decomposition lowering the C/N ratio of cultivated soils (Chivenge et al., 2003). Upon cultivation, N declines slightly greater than C, resulting to wider values under cultivation than under non cultivated soils (Saggar et al., 2001). Chivenge et al. (2003) mentioned values of 13.6 for cultivated sites, 14.7 for weedy fallow and 11.5 for mulch ripping. In all the secondary forest stands of their study, the ratio varied 10-11 (Jiménez et al., 2008). Agricultural sites had generally lower ratios in their study, with mean values of 10 compared to 15.1 for forested soils (Caravaca et al., 1999, 2004). Despite the result of N inputs during N-fertilization, it is accepted that the accumulation of N is independent of the carbon input rates (Caravaca et al., 1999). Our results are near the values mentioned by Ashargie et al. (2007), who mentioned values near 12 under forest, narrowing to 9 under cultivation, because the effect of cultivation was more pronounced on SOC than N. The type of forest we have in our area of study (*Quercus pubescens* species) could probably attribute to the relatively slightly lower C/N values obtained in the forest. The studies of Caravaca et al. (1999, 2004) refer to pine trees in Spain, Jiménez et al. (2008) ran their search in a tropical forest of Costa Rica, whereas Ashargie et al. (2007) studied a mixed tropical forest of Podocarpus falcatus in Ethiopia.

On the other hand, the higher C/N ratios of cultivated soils that were observed at the study of Buschiazzo et al. (2001) were ascribed to the greater mineralization rates of organic N vs Organic C that the authors obtained in their research. The authors generally reported small changes in the ratio due to cultivation. Furthermore, the ratio was not affected by tillage in the study of Jacobs et al. (2009). Solomon et al. (2002) reported not significant differences between the different land use systems of their work, whereas Leifeld and Kögel-Knabner (2005) argued that it is not distinguishable for mineral fractions of the different land-use patterns.

The amount of time of deforestation seemed to play an important role in the ratio values (Table 30 and Figure 40), resulting in lower values of the ratio as more time elapsed from deforestation.

Soils under NT regimes typically contain more labile undecomposed SOM resulting to lower ratios (Balota et al., 2003; Wright and Hons, 2004). The ratio was reported higher under CT treatment than under NT by Dalal et al. (1991); under NT values of 10.7 and under CT values of 12.2 were mentioned by Wright and Hons (2004). Our values for RT (wheat plots) and CT (cotton plots) were 9.87 and 10.18 respectively. These values (although not significantly different) were expected due to higher SOC amounts compared to N amounts in the wheat than in the cotton plots. On the other hand, Islam and Weil (2000) mentioned that the ratio did not vary among the sites of their study and Wright and Hons (2004) that it is generally not affected by cropping sequence.

Differences in the ratio may be due to differences in management history, and also to the presence of inert charcoal in the soil, which is produced by the burning of the vegetation (Hassink, 1994).

To the subcategories level (time*management as a factor), no significant differences between subcategories of the same deforestation were observed (Figure 42). Hence, the factor time*management was not significant.

3.3.2 C/N in Fractions

Forests (being rich in POM, and in fresh material correlated to fine sand) had generally high values (Six et al., 2001, 2002). In every case of our study, in every forest fraction of the forest soils, the ratio was significantly much greater than any value of the agricultural plots.

Referring to the fine sand fraction, all the year categories varied significantly with each other, but management had not an important influence on the C/N ratio (Figures 44 and 46). The wheat and cotton subcategories within year category never varied significantly (Figure 45). The C/N ratio in fine sand followed the same tendency than SOC in this fraction. The ratio in the forests was significantly higher in the fine sand fraction compared to the silt and clay fractions (Table 30).

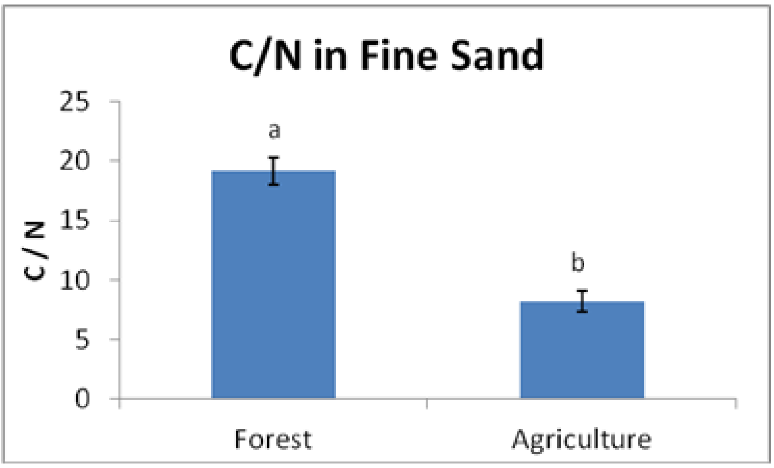


Figure 43. *Effect of deforestation and crop establishment on the Carbon to Nitrogen ratio of the Fine Sand fraction for topsoil horizons (0-15 cm), and the standard deviations. Different letters indicate significant differences between forest and agriculture (crop) (p<0.05).*

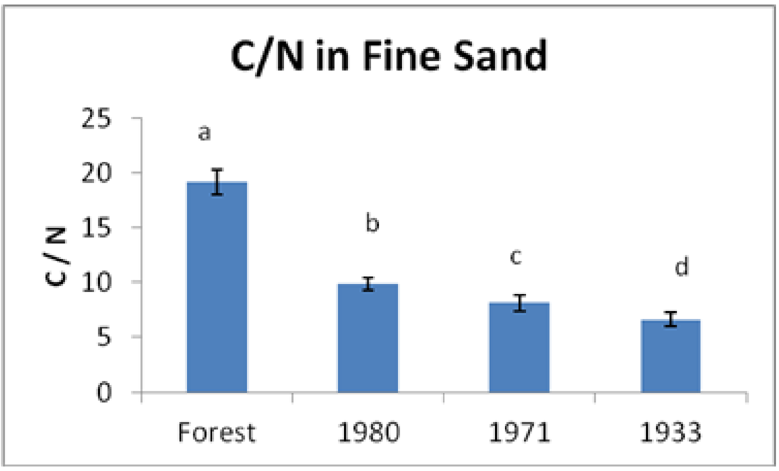


Figure 44. *Effect of time since deforestations and subsequent cultivation on the Carbon to Nitrogen ratio of the Fine Sand fraction, and the standard deviations. Different letters indicate significant differences between deforested plots on 1933, 1971, 1980, and forest plots (p<0.05).*

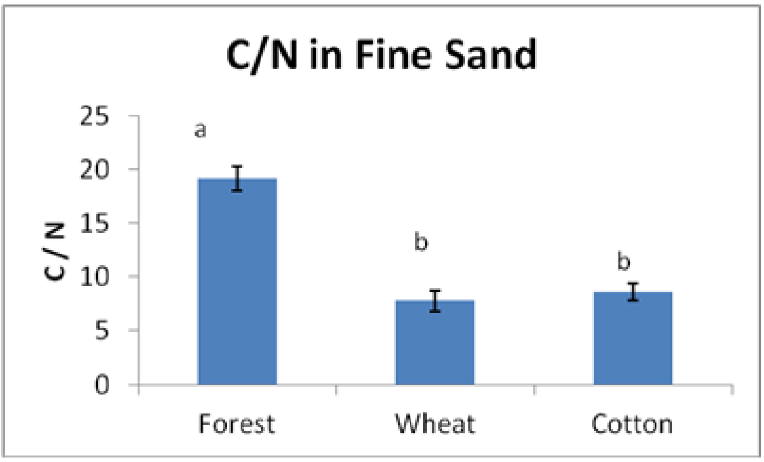


Figure 45. *Type of management effects on the Carbon to Nitrogen ratio of the Fine Sand fraction, and the standard deviations. Different letters indicate significant differences between wheat (NIR), cotton (IR), and forest plots ($p<0.05$).*

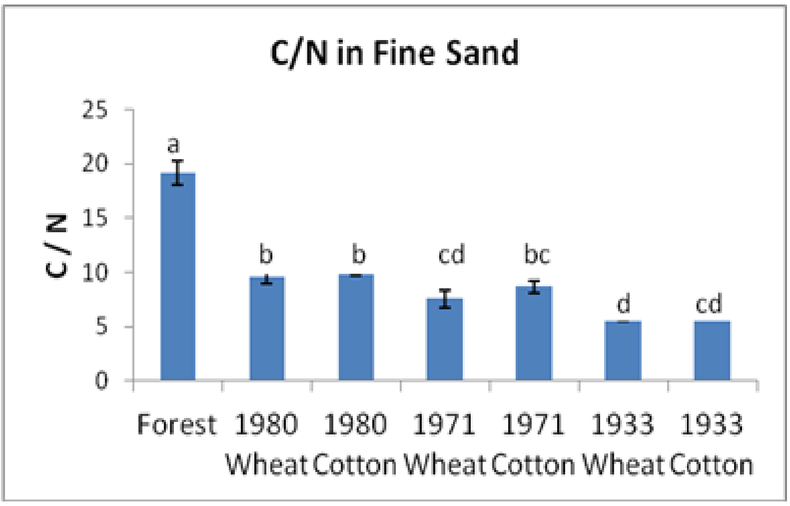


Figure 46. *The Carbon to Nitrogen ratio of the Fine Sand fraction, obtained in the studied plots, the comparison among the treatments and the standard deviations (different letters indicate significant differences between categories, $p<0.05$).*

For the silt fraction, forest plots holded the larger values of the C/N ratio compared to all the agricultural plots (with significant differences) (Figures 47 to 50). The 1980 year category had a significantly higher value than the 1933 category, but a non-significantly higher value than the 1971 category (Figure 48). Management played a slightly important role, as excluding the 1933 wheat subcategory which had the significantly lower value, all the other wheat and cotton subcategories did not vary significantly among each other (Figure 50). Due to the 1933 wheat subcategory, wheat and cotton management types varied significantly (Figure 49).

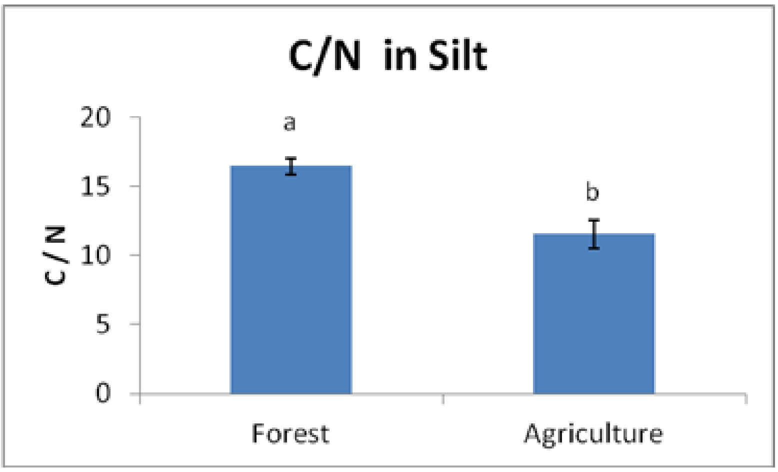


Figure 47. *Effect of deforestation and crop establishment on the Carbon to Nitrogen ratio of the Silt fraction for topsoil horizons (0-15 cm), and the standard deviations. Different letters indicate significant differences between forest and agriculture (crop) (p<0.05).*

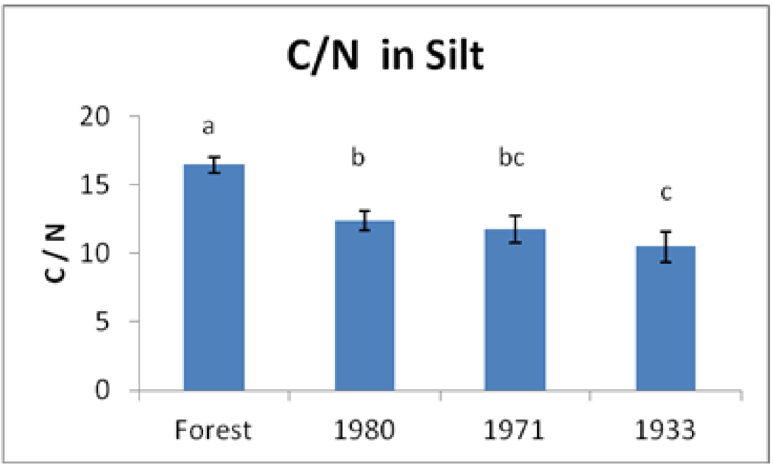


Figure 48. *Effect of time since deforestations and subsequent cultivation on the Carbon to Nitrogen ratio of the Silt fraction, and the standard deviations. Different letters indicate significant differences between deforested plots on 1933, 1971, 1980, and forest plots (p<0.05).*

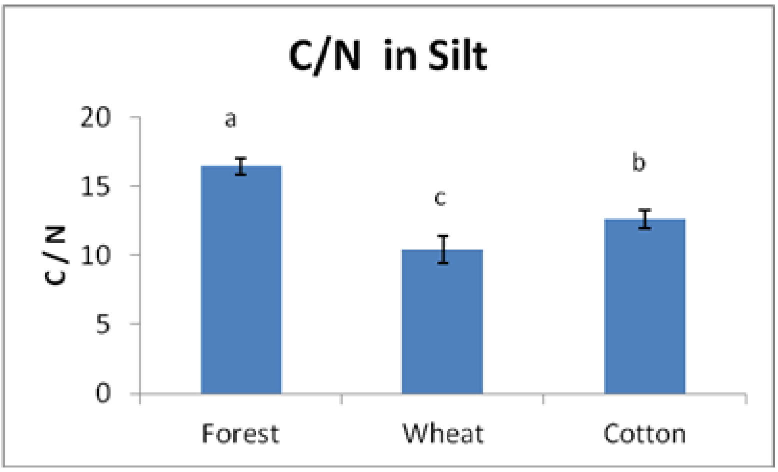


Figure 49. *Type of manatgement effects on the Carbon to Nitrogen ratio of the Silt fraction, and the standard deviations. Different letters indicate significant differences between wheat, cotton, and forest plots ($p<0.05$).*

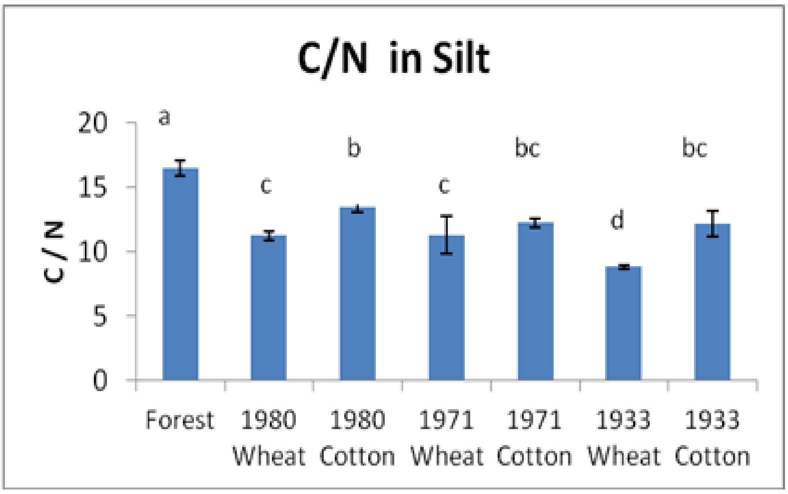


Figure 50. *The Carbon to Nitrogen ratio of the Silt fraction, obtained in the studied plots, the comparison among the treatments and the standard deviations (different letters indicate significant differences between categories, $p<0.05$).*

Except for the forest plots which holded the significantly higher values, no other factor played a role in the clay C/N values (Figures 51 to 54). The 1933 year category referring to use as a factor and the cotton management type had slightly higher values than the rest sites (Figures 52 and 53).

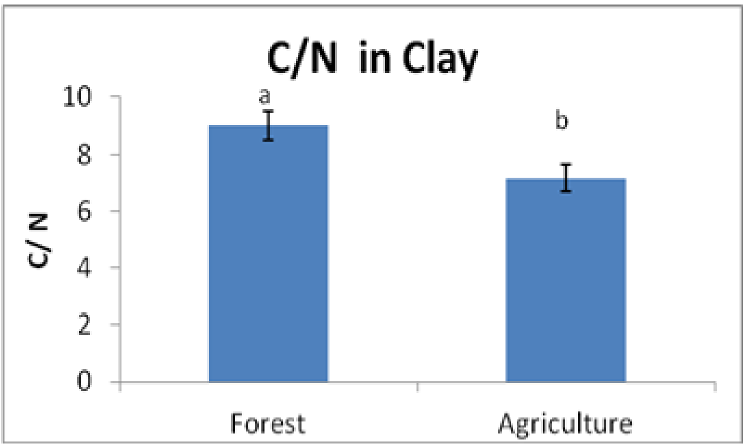


Figure 51. *Effect of deforestation and crop establishment on the Carbon to Nitrogen ratio of the Clay fraction for topsoil horizons (0-15 cm), and the standard deviations. Different letters indicate significant differences between forest and agriculture (crop) (p<0.05).*

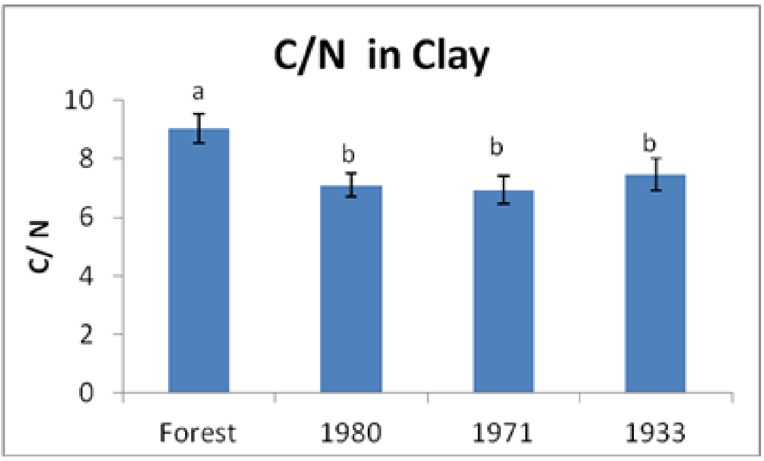


Figure 52. *Effect of time since deforestations and subsequent cultivation on the Carbon to Nitrogen ratio of the Clay fraction, and the standard deviations. Different letters indicate significant differences between deforested plots on 1933, 1971, 1980, and forest plots (p<0.05).*

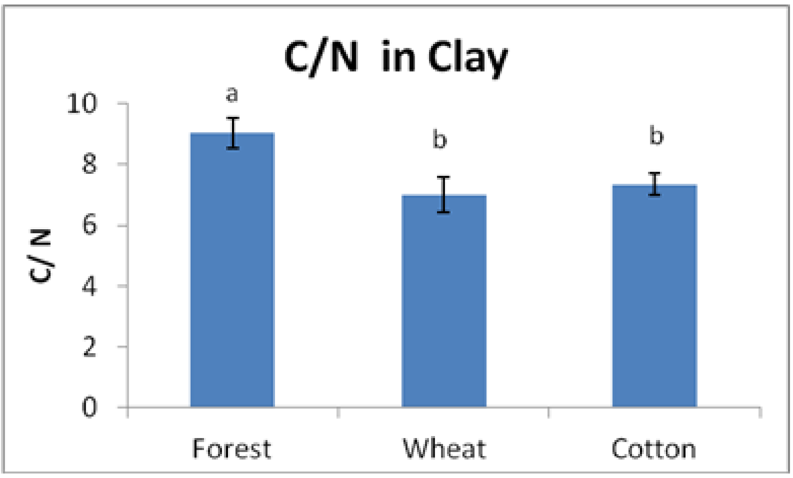


Figure 53. *Type of management effects on the Carbon to Nitrogen ratio of the Clay fraction, and the standard deviations. Different letters indicate significant differences between wheat, cotton, and forest plots (p<0.05).*

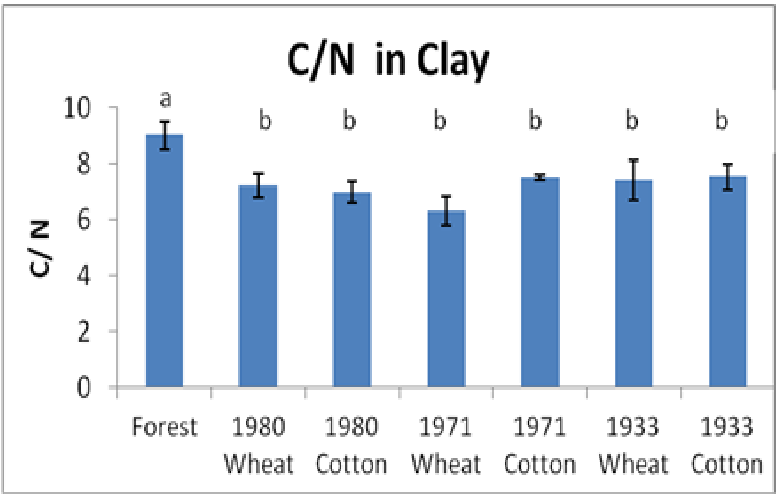


Figure 54. *The Carbon to Nitrogen ratio of the Clay fraction, obtained in the studied plots, the comparison among the treatments and the standard deviations (different letters indicate significant differences between categories, p<0.05).*

The amount of time since deforestation played a higher role in the order: sand>silt>clay. The stabilization of organic matter in clay results in a steady content throughout the years, thus with no significant differences (Figure 52). For fine sand the differences were significant (Figure 44), for every year category (following the same tendencies that were observed for SOC), whereas for silt only between the oldest and newest deforestation (Figure 48), suggesting that only big amounts of time transcurred since land use change may have significant effects in the values of the C to N ratio in the silt fraction.

The cotton fields had always greater C/N ratios for all size fractions than wheat soils, but only for silt the differences were significant (Figures 45, 49, 53). The higher values of the C/N ratio in cotton soils could also be attributed to greater N values for the wheat fields, as discussed in the N chapter. Furthermore, Beare et al. (1994) reported lower ratio values for surface samples of NT than CT. In NT and RT regimes there is considerable biological activity near the surface, along with the activities of fungi, roots, soil fauna, probably helping to incorporate POM within macroaggregates and to increase their structural stability.

The subcategories generally followed the above trends, but the differences of the values for the time*management factor were not significant (Table 31, Figures 46, 50, 54).

Generally the most stable and consistent values of the C/N ratio were observed in the clay fraction. This fact shows the clay capacity to preserve SOC and N contents; soils after years of deforestation could reach an equilibrium and this is evident in the ratio values of the clay fraction. The potential of agricultural soils to withhold C and N in the clay fractions is similar.

In forest soils we can see an significant decrease of the values of C/N ratio with the decrease of the fraction size, 19.2 for fine sand, 16.4 for silt and 9.0 for clay, however for crop soils the ratio for the silt fraction (11.5) was significant higher than in the other two fractions, followed by the value for fine sand (8.2) and finally clay (7.2) (Tables 30 and 34). Chan (2001) indicated that SOM in the <53µm fraction (referring to the silt and clay fractions) with ratios close to 10 is mostly made up of more humified materials. The C to N ratio declined as particle size decreased (Christensen, 1992; Caravaca et al., 1999; Guggenberger and Zech, 1999; Lehmann et al., 2001; Schmidt and Kögel-Knabner, 2002; Caravaca et al., 2004; He et al., 2009), indicating an increasing degree of humification or decomposition. Saroa and Lal (2003) argued these differences to be significant due to greater microbial alteration of SOM in fine fractions; in mulch treated soils, Saroa and Lal (2003) also observed significant differences among fractions in the order: sand>silt>clay. These relations of the values (significant or not) appear to be a general trend. Solomon et al. (2002) mentioned values from 22.4 for sand to 8.8 for clay, reflecting a progressive SOM humification with decreasing particle diameter. A significant interaction between site, management, and aggregate size existed for C/N ratios in their study (Buschiazzo et al., 2001). Lower values of C/N in clay than in silt were also reported by Christensen (2001), with sand fractions having the highest values. Generally, higher C/N ratios are attributable to incompletely humified organic material.

In our case the above general trend implies only for the forest plots and for the sand and clay fraction in most of the agricultural plots. The ratio values for the silt fractions in all the agricultural plots, referring to the amount of years since deforestation (time as a factor) and to the management factor as well as to the time*management factor, were higher than the values of the C/N ratio for the other size fractions (Table 30), and it is not easy to explain why this occured. This significance was found for all the interrelations except for the 1980 wheat plots. The higher C/N ratio obtained for the silt fraction for both forest and cultivated soils suggests the presence of less decomposed SOM, while as Caravaca et al. (1999) suggested, the SOM associated with the clay fraction can be considered to be more humified. As for the silt and clay fractions the narrowest C/N ratios suggest the most humified state and potentially the largest degree of microbial origin (He et al., 2009). The only year category that had fine sand values lower than those in clay, is the 1933 category, with both its subcategories (not significantly though). This is due to the small amounts of SOC in the 1933 plots, which are mostly applicable to the sand fraction rather to clay (which is the stable fraction in terms of and N withholding capacity). The 1971 year category had significant differences between the fine sand and clay values, but not for the subcategories, rendering amount of years as a more significant factor than management for these plots. The last insignificance occured for the fine sand and silt fraction of the 1980 wheat subcategory. Increased values of both SOC and N in these fractions resulted in a ratio with no significant differences, although silt had still higher values. Generally the low SOC amounts in the fine sand fractions of our agricultural soils resulted in very low amounts of the ratio of this fraction for these plots.

Our results showed that significant differences in the C/N ratios with land use change, time transcurred since deforestation and type of management were mostly pronounced in the sand-sized separates, while they were minor in the other fractions (Table 30); Guggenberger and Zech (1999) showed similar findings. Fine sand presented the most distinctive differences among forest and agricultural plots. It is the fraction which showed the greatest reduction from forest (19.16) converting to agriculture (8.21), whereas the smallest reduction can be found in the clay values, 9.01 compared to 7.16 respectively. Hassink (1994) mentioned quite similar values, in sandy soils 10-20 and in loams and clays 8-12 (his results do not refer to fractions but to type of soils). Caravaca et al. (1999) reported significantly different values for silt and clay (13.6 compared to 8.5 respectively).

Overall, the C/N ratio did not seem to be a reliable soil quality indicator. Some trends are observed as discussed above, but they were not consistent. In agreement with these conclusions are the remarks made by Christensen (1992) and Solomon et al. (2000), who both mentioned that the changes of the SOM contents induced by different land use systems were not consistently reflected by the C/N ratios. Thus, the C/N ratio of SOM must be considered as less informative indicator of SOM quality than the C and N contents alone.

Table 34. *ANOVA significances for C/N values among fractions (FS: Fine Sand, Silt and Clay) for each factor.*

Factor	**FS *versus* Silt**	**FS *versus* Clay**	**Silt *versus* Clay**
Land Use			
Forest	**	***	***
Agriculture	***	***	***
Time since deforestations			
1933	***	ns	***
1971	***	*	***
1980	***	***	***
Management			
Wheat	***	ns	***
Cotton	***	**	***
Time*Management			
1933wheat	**	ns	ns
1933cotton	***	ns	***
1971wheat	***	ns	***
1971cotton	***	ns	***
1980wheat	ns	**	***
1980cotton	***	***	***

Note: *Significance levels: *** (p<0.001),** (p<0.01), * (p<0.05), and ns: not significant.*

CONCLUSIONS

- Deforestation and further establishment of agricultural use leads to decreases in SOC and levels of microbiological properties, suggesting a deterioration of soil quality. Intense management techniques practiced in Greece the earlier years of the 20th century resulted in a great loss of SOM in the surface mineral soil of the study area by more than 70%. Deforestation increases the MBC/SOC ratio at both treatments, due to agricultural management. Forest soils have less stressed conditions for the microbial community as the significantly lower values of the metabolic quotient (qCO_2) suggest.

- Despite the fact that a relatively large amount of time had elapsed since deforestation and subsequent crop establishment in the three compared deforestation stages (1933, 1971 and 1980), the amounts of SOC, MBC and SR were significantly higher in soils deforested in the most recent stage (1980). The amount of 25 years since the latest deforestation (in 1980) seems to be a critical value of years, soils after that deteriorate fast (as shown in the values of the properties of the 1971 deforestation) until equilibrium (with the values of the 1933 deforestation).

- Reduced tillage practices (as in the wheat fields) could be considered more sustainable actions compared to conventional tillage management techniques (of the cotton plots) towards soil health, as they improve the conditions of the soil to protect SOC and enhance the microbial community and activity, compared to conventional tillage management techniques (of the cotton plots).

- Overall, SOC, MBC, SR and qCO_2 could be considered as reliable indicators of the changes occurring in the soil. Especially the metabolic quotient is a very good indicator referring to land use changes and type of management of the agricultural sites. The MBC/SOC ratio is not a solid indicator for land use changes, as many authors mentioned different trends of values from forest and agricultural sites. The interpretation of SOC values should be made with great care referring to time transcurred since land use change, since great losses are observed during the 25 first years of deforestation but after that the changes are minimal, suggesting a route towards equilibrium.

- Land use changes have significant effects in SOC and N contained in the soil particle-size fractions. The amount of POM, and C and N in the fine sand fraction present the most distinctive differences (losses, due to loss of fresh organic material) among forest (significantly higher values) and agricultural plots. When land use regimes changes, large amounts of POM are lost. After that, the amount of POM cannot be used as an indicator for the agricultural plots, only for monitoring land use changes.

- The amount of SOC and N in the clay fraction should not be considered as a sensitive indicator in order to monitor changes in land use regimes, as the contents remain rather stable depending on the nature of land use.

- The stabilization of organic matter in clay results in a steady content throughout the years, thus with no significant differences. Clay protects both SOC and N contents. Soil organic carbon and total N contents are significantly greater for all the agricultural plots for the clay fraction compared to the other fractions.

- As years pass by (from the most recent to the earlier deforestation), the differences in the amounts between the silt and clay values increase. The N losses are less pronounced in clay, as this fraction obtains increased capacity of SOC holding. Specifically, the values between agricultural and forest clay N contents are similar. Especially the soils of the most recent deforestation (1980) have the highest SOC values, suggesting a reduction of the amounts of SOC withheld in the silt and clay fraction with time; this shows a tendency of reduced capacity of these fractions to hold SOC equally through time.

- The amount of time since deforestation plays a higher role in the order: sand>silt>clay and is adversely associated with the values of SOC and N in the particles, except for clay where after 25 years of cultivation the capacity of this fraction to preserve SOM seems to have reached an equilibrium. Soil organic matter bound in the clay particles are well protected, hence values in clay are always higher in this particle-size fraction referring to agricultural fields.

- While SOC in the fractionated samples is affected mainly (not taking into account the land use factor) by the amount of years since deforestations (the time factor), N is significantly correlated to the management practices of the agricultural fields, where differences in the amounts of N fertilizer application play the leading role. The effect of cultivation is more pronounced on C than N leading to lower values of the C/N ratio. Overall the ratio is not a reliable indicator as it does not present consistent trends. Significant differences of the ratio were mostly pronounced at the fine sand fraction.

- Irrespective of the management that follows, the most significant factor is the land use change for the majority of the studied properties either in the whole soil or in the soil particle fractions. This is also concluded from the comparisons between wheat and cotton fields, where many of the differences are not significant. Then, our hypotheses that assumed the management factor to have the leading role in the interpretation of the differences obtained between the values of our work, finally were not correct.

BIBLIOGRAPHY

BIBLIOGRAPHY

Agele S, Ewulo B., Oyewusi I., 2005. Effects of Some Soil Management Systems on Soil Physical Properties, Microbial Biomass and Nutrient Distribution Under Rainfed Maize Production in a Humid Rainforest Alfisol. Nutrient Cycling in Agroecosystems 72, 121-134.

Alef K., Nannipieri P., 1995. Methods in applied soil microbiology and biochemistry. Academic Press, Harcourt Brace and Company, London.

Al-Kaisi M.,M., Yin X., 2005. Tillage and Crop Residue Effects on Soil Carbon and Carbon Dioxide Emission in Corn–Soybean Rotations. Journal of Environmental Quality 34, 437–445.

Alvarez C.R., Alvarez R., 2000. Short-term effects of tillage systems on active soil microbial biomass. Biology and Fertility of Soils 31, 157–161.

Alvarez C.R., Alvarez R., Grigera M.S., Lavado R.S., 1998. Associations between organic matter fractions and the active soil microbial biomass. Soil Biology & Biochemistry 30, 767–773.

Alvarez R., Santanatoglia O.J, García R., 1995. Effect of temperature on soil microbial biomass and its metabolic quotient in situ under different tillage systems. Biology and Fertility of Soils 19, 227-230.

Anderson T.H., 2003. Microbial eco-physiological indicators to assess soil quality. Agriculture, Ecosystems and Environment 98, 285–293.

Anderson T.H., Domsch K.H., 1989. Ratios of microbial biomass carbon to total organic-C in arable soils. Soil Biology and Biochemistry 21, 471–479.

Anderson T.H., Domsch K.H., 1990. Application of eco-physiological quotients (qCO2 and qD) on microbial biomasses from soils of different cropping histories. Soil Biology and Biochemistry 22, 251–255.

Anderson T.H., Domsch K.H., 1993. The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. Soil Biology and Biochemistry 25, 393-395.

Angers D.A., Pesant A., Vigneux J., 1992. Early cropping-induced changes in soil aggregation, organic matter and microbial biomass. Soil Science Society of America Journal 56, 115-119.

Ashagrie Y., Zech W., Guggenberger G., Mamo T., 2007. Soil aggregation, and total and particulate organic matter following conversion of native forests to continuous cultivation in Ethiopia. Soil and Tillage Research 94, 101–108.

Balota E.L., Colozzi-Filho A., Andrade D.S., Dick R.P., 2003. Microbial biomass in soils under different tillage and crop rotation systems. Biologyh and Fertility of Soils 38, 15–20.

Bastida F., Moreno J.L., Hernández T., García C., 2006. Microbiological activity in a soil 15 years after its devegetation. Soil Biology and Biochemistry 38, 2503–2507.

Bauhus J., Pare D., Cote L., 1998. Effects of tree species, stand age and soil type on soil microbial biomass and its activity in a southern boreal forest. Soil Biology and Biochemistry 30, 1077-1089.

Beare M.H., Hendrix P.F., Coleman D.C., 1994. Water-stable aggregates and organic matter fractions in conventional and no-tillage soils. Soil Science Society of America Journal 58, 777-786.

Bending G.D., Turner M.K., Rayns F., Marx M.C., Wood M., 2004. Microbial and biochemical soil quality indicators and their potential for differentiating areas under contrasting agricultural management regimes. Soil Biology and Biochemistry 36, 1785-1792.

Blagodatskaya E.V., Bogomolova I.N., Pampura T.V., Blagodatsky S.A., 2006. Sensitivity of eco-physiological indicators of soil quality with respect to agricultural and industrial impact. Geophysical Research Abstracts, Vol. 8, 06025.

Borken W., Xu Y.-J., Davidson E.A., Beese F., 2002. Site and temporal variation of soil respiration in European beech, Norway spruce and Scots pine forests. Global Change Biology 8, 1205-1216.

Bouma T.J., Bryla D.R., 2000. On the assessment of root and soil respiration for soils of different textures: interactions with soil moisture contents and soil CO_2 concentrations. Plant and Soil 227, 215–221.

Brady N.C., Weil R.R., 2002. *The nature and properties of soils.* 13th Edition, Prentice Hall International, 960pp.

Bremer E., Ellert B.H., Janzen H.H., 1995. Total and light-fraction carbon dynamics during four decades after cropping changes. Soil Science Society of America Journal 59, 1398-1403.

Breulmann M., 2011. *Functional soil organic matter pools and soil organic carbon stocks in grasslands: an ecosystem perspective.* Doctoral dissertation, Helmholtz Centre for Environmental Research, UFZ.

Buchmann N., 2000. Biotic and abiotic factors controlling soil respiration rates in *Picea abies* stands. Soil Biology and Biochemistry 32, 1625-1635.

Buschiazzo D.E., Hevia G.G., Hepper E.N. Urioste A., Bono A.A., Babinec F., 2001. Organic C, N and P in size fractions of virgin and cultivated soils of the semi-arid pampa of Argentina. Journal of Arid Environments 48, 501–508.

Calderón F.J., Jackson L.E., 2002. Rototillage, Disking, and Subsequent Irrigation: Effects on Soil Nitrogen Dynamics, Microbial Biomass, and Carbon Dioxide Efflux. Journal of Environmental Quality 31, 752–758.

Campbell C.A., Biederbeck V.O., McConkey B.G., Curtin D., Zentner R.P., 1999. Soil quality effect of tillage and fallow frequency. Soil organic matter quality as influenced by tillage and fallow frequency in a silt loam in southwestern Saskatchewan. Soil Biology and Biochemistry 31, 1–7.

Caravaca F., Lax A., Albaladejo J., 1999. Organic matter, nutrient contents and cation exchange capacity in fine fractions from semiarid calcareous soils. Geoderma 93, 161–176.

Caravaca F., Lax A., Albaladejo J., 2004. Aggregate stability and carbon characteristics of particle-size fractions in cultivated and forested soils of semiarid Spain. Soil and Tillage Research 78, 83–90.

Cardelli R., Marchini F., Saviozzi A., 2012. Soil organic matter characteristics, biochemical activity and antioxidant capacity in mediterranean land use systems. Soil and Tillage Research 120, 8-14.

Carpenter-Boggs L., Stahl P.D., Lindstrom M.J., Schumacher T.E., 2003. Soil microbial properties under permanent grass, conventional tillage, and no-till management in South Dakota. Soil and Tillage Research 71, 15–23.

Carter M.R., 1986, Microbial biomass as an index for tillage induced changes in soil biological properties. Soil and Tillage Research 7, 29–40.

Carter M.R., 1991. The influence of tillage on the proportion of organic carbon and nitrogen in the microbial biomass of medium-textured soils in a humid climate. Biology and Fertility of Soils 11, 135 – 139.

Chan K.Y., 2001. Soil particulate organic carbon under different land use and management. Soil Use and Management 17, 217-221.

Chander K., Goyal S., Mundra M.C., Kapoor K.K., 1997. Organic matter, microbial biomass and enzyme activity of soils under different crop rotations in the tropics. Biology and Fertility of Soils 24, 306–310.

Chen H., Hou R., Gong Y., Li H., Fan M., Kuzyakov Y., 2009. Effects of 11 years of conservation tillage on soil organic matter fractions in wheat monoculture in Loess Plateau of China. Soil and Tillage Research 106, 85-94.

Chivenge P.P., Murwira H.K., Giller K.E., 2003. Tillage effects on soil organic carbon and nitrogen distribution in particle size fractions of a red clay soil profile in Zimbabwe. *In:* Bationo, A. (ed.). Managing nutrient cycles to sustain soil fertility in sub-Saharan Africa. Academy Science Publishers (ASP); Centro Internacional de Agricultura Tropical (CIAT), Nairobi, Kenya. p. 113-126.

Christensen B.T., 1985. Carbon and Nitrogen in Particle Size Fractions Isolated from Danish Arable Soils by Ultrasonic Dispersion and Gravity-Sedimentation. Acta Agriculturae Scandinavica 35, 175-187.

Christensen B.T., 1992. Physical fractionation of soil and organic matter in primary particle size and density separates. Advances in Soil Science, 20, 1-90.

Christensen B.T., 2001. Physical fractionation of soil and structural and functional complexity in organic matter turnover. European Journal of Soil Science 52, 345-353.

Clapp C.E., Hayes M.H.B., 1999. Characterization of humic substances isolated from clay- and silt-sized fractions of a corn residue-amended agricultural soil. Soil Science 164, 899–913.

Conant R.T., Paustian K., Elliott E.T., 2001. Grassland management and conversion into grassland: effects on soil carbon. Ecological Applications 11:343–355.

Costantini A., Cosentino D., Segat A., 1996. Influence of tillage systems on biological properties of a Typic Argiudoll soil under continuous maize in central Argentina. Soil and Tillage Research 38, 265-271.

Dalal R.C., 1998. Soil microbial biomass-what do the numbers really mean? Australian Journal of Experimental Agriculture 38, 649–665.

Dalal R.C., Henderson P.A., Glasby J.M., 1991. Organic matter and microbial biomass in a vertisol after 20yr of zero-tillage. Soil Biology and Biochemistry 23, 435-441.

Dalal R.C., Mayer R.R., 1986. Long-term trends in fertility of soils under continuous cultivation and cereal cropping in Southern Queensland. III. Distribution and kinetics of soil organic carbon in particle size fractions. Australian Journal of Soil Research 24, 293–300.

Davidson E.A., Belk E., Boone R.D., 1998. Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. Global Change Biology 4, 217–227.

Davidson E., Verchot L., Henrique Cattânio J., Ackerman I.L., Carvalho J.E.M., 2000. Effects of soil water content on soil respiration in forests and cattle pastures of eastern Amazonia Biogeochemistry 48: 53–69, 2000.

Dilly O., Munch J.C., 1998. Ratios between estimates of microbial biomass content and microbial activity in soils. Biology and Fertility of Soils 27, 374–379.

Dinesh R., Chaudhuri S.G., Ganeshamurthy A.N., Pramanik S.C., 2004. Biochemical properties of soils of undisturbed and disturbed mangrove forests of South Andaman (India). Wetlands Ecology and Management 12, 309–320.

Dinesh R., Ghoshal Chaudhuri S., Ganeshamurthy A.N., Chanchal Dey, 2003. Changes in soil microbial indices and their relationships following deforestation and cultivation in wet tropical forests. Applied Soil Ecology 24, 17-26.

Doran J.W., 1980. Soil microbial and biochemical changes associated with reduced tillage. Soil Science Society of America Journal 44, 764-771.

Doran J.W., 1987. Microbial biomass and mineralizable nitrogen distribution in non-tillage and plowed soils. Biology and Fertility of Soils 5, 68 -75.

Duxbury J. M., Smith M. S., Doran J. W., Jordan C., Szott L., Vance E., 1989. Soil organic matter as a source and a sink of plant nutrients. Dynamics of soil organic matter in tropical ecosystems, 2, 33-67.

Eleftheriadis, A., 2005. Irrigation and Drainage: Theory and Practice. Technological Institute of Kavala, Branch of Drama, Department of Landscape Architecture, Drama, 126p. (*In Greek*)

Emmerling C., Udelhoven T., Schröder D., 2001. Response of soil microbial biomass and activity to agricultural de-intensification over a 10 year period. Soil Biology and Biochemistry, 33, 2105–2114.

Entry A., Mitchell C.C., Backman C.B., 1996. Influence of management practices on soil organic matter, microbial biomass and cotton yield in Alabama's "Old Rotation". Biology & Fertility of Soils 23, 353 – 358.

FAO, 2007. Methods of analysis for soils of arid and semi-arid regions. Rome, 50 pp.

Fauci M.F., Dick R.P., 1994. Microbial biomass as an indicator of soil quality: effects of long-term management and recent soil amendments. In : Doran et al. (Eds), Defining soil quality for a sustainable environment Soil Science Society of America special publication no.35, Madison ,WI, USA, pp. 229-234.

Fließbach A., Oberholzer H.R., Gunst L., Mäder P., 2007. Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agriculture, Ecosystems and Environment 118, 273-284.

Fließbach A., Martens R., Reber H.H., 1994. Soil microbial biomass and microbial activity in soils treated with metal contaminated sewage sludge. Soil Biology and Biochemistry 26, 1201-1205.

Follett R.F., Schimel D.S., 1989. Effect of tillage practices on microbial biomass dynamics. Soil Science Society of America Journal 53, 1091-1096.

Franchini J.C., Crispino C.C., Souza R.A., Torres E., Hungria M., 2007. Microbiological parameters as indicators of soil quality under various soil management and crop rotation systems in southern Brazil. Soil and Tillage Research 92, 18–29.

Frank A.B., Liebig M.A., Hanson J.D., 2002. Soil carbon dioxide fluxes in northern semiarid grasslands. Soil Biology and Biochemistry 34, 1235–1241.

Frank A.B., Liebig M.A., Tanaka D.L., 2006. Management effects on soil CO_2 efflux in northern semiarid grassland and cropland. Soil and Tillage Research 89, 78-85.

Franzluebbers A.J, Arshad M.A., 1996. Soil organic matter pools with conventional and zero tillage in a cold, semiarid climate. Soil and Tillage Research 39, 1-11.

Franzluebbers A.J., Hons F.M., Zuberer D.A., 1994. Long-term changes in soil carbon and nitrogen pools in wheat management systems. Soil Science Society of America Journal 58, 1639-1645.

Franzluebbers A.J., Haney R.L., Honeycutt C.W., Arshad M.A., Schomberg H.H., Hons F.M., 2001. Climatic influences on active fractions of soil organic matter. Soil Biology and Biochemistry 33, 1103-1111.

Garcia C., Hernandez T., Roldan A., Martin A., 2002. Effect of plant cover decline on chemical and microbiological parameters under Mediterranean climate. Soil Biology and Biochemistry 34, 635-642.

Ghani A., Dexter M., Perrott K.W., 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilization, grazing and cultivation. Soil Biology and Biochemistry 35, 1231-1243.

Glaser B., Turrión M.B., Solomon D., Ni A., Zech W., 2000. Soil organic matter quantity and quality in mountain soils of the Alay Range, Kyrgyzia, affected by land use change. Biology and Fertility of Soils 31, 407–413.

Gómez-Acata, E.S., Valencia-Becerril I., Valenzuela-Encinas C., Velásquez-Rodríguez A.S., Navarro-Noya1 Y.E.,, Montoya-Ciriaco N., Suárez-Arriaga M,.C., Rojas-Valdez A., Reyes-Reyes B.G., Luna-Guido M., Dendooven L., 2016. Deforestation and cultivation with maize (*zea mays l.)* has a profound effect on the bacterial community structure in soil. Land Degradation and Development 27, 1122–1130.

Gömöryová E., Strelcová K., Skvarenina J., san Gömöry D., 2013. Responses of soil microorganisms and water content in forest floor horizons to environmental factors. European Journal of Soil Biology 55, 71-76.

Grandy A.S., Robertson G.P., 2007. Land-Use Intensity Effects on Soil Organic Carbon Accumulation Rates and Mechanisms. Ecosystems 10, 58–73.

Guggenberger G., Zech W., 1999. Soil organic matter composition under primary forest, pasture, and secondary forest succession, Region Huetar Norte, Costa Rica. Forest Ecology and Management 124, 93-104.

Guo L.B., Gifford R.M., 2002. Soil carbon stocks and land use change: a meta analysis. Global Change Biology 8, 345-360.

Hajabbasi M.A., Jalalian A., Karimzadeh H.R., 1997. Deforestation effects on soil physical and chemical properties, Lordegan, Iran. Plant and Soil 190, 301-308.

Haney R.L., Franzluebbers A.J., Jin V.L., Johnson M.-V., Haney E.B., White M.J., Harmel R.D., 2012. Soil Organic C:N vs. Water-Extractable Organic C:N. Open Journal of Soil Science 2, 269-274.

Harris J.A., 2003. Measurements of the soil microbial community for estimating the success of restoration. European Journal of Soil Science 54, 801–808.

Hassink J., 1994. Effects of soil texture and grassland management on soil organic C and N and rates of C and N mineralization. Soil Biology and Biochemistry 26, 1221-1231.

Hassink J., 1997. The capacity of soils to preserve organic C and N by their association with clay and silt particles. Plant and Soil 191, 77–87.

He Y., Chen C., Xu Z., Williams D., Xu J., 2009. Assessing management impacts on soil organic matter quality in subtropical Australian forests using physical and chemical fractionation as well as 13C NMR Spectroscopy. Soil Biology and Biochemistry 41, 640–650.

Hendrix P.F., Han C.R., Groffman P.M., 1988. Soil respiration in conventional and no-tillage agroecosystems under different winter cover crop rotations. *Soil and Tillage Research*, *12*, 135-148.

HNMS (Hellenic national meteorological service), 2006. Climatic and meteorological data for Greece. Available at: http://www.hnms.gr

Howard D.M., Howard P.J.A., 1993. Relationships between CO_2 evolution, moisture content and temperature for a range of soil types. Soil Biology and Biochemistry 25, 1537-1546.

Huang Z., Xu Z., Chen C., 2008. Effect of mulching on labile soil organic matter pools, microbial community functional diversity and nitrogen transformations in two hardwood plantations of subtropical Australia. Applied Soil Ecology 40, 229–239.

Insam H., 1990. Are the soil microbial biomass and basal respiration governed by the climatic regime? Soil Biology and Biochemistry 22, 515-532.

Insam H., Domsch K.H., 1988. Relationship between soil organic carbon and microbial biomass on chronosequences of reclamation sites. Microbial Ecology 15, 177-188.

Insam H., Mitchell C.C., Dormaar J.F., 1991. Relationship of soil microbial biomass and activity with fertilization practice and crop yield of three Ultisols. Soil Biology and Biochemistry 23, 459-464.

Islam K.R., Weil R.R., 2000. Land use effects on soil quality in a tropical forest ecosystem of Bangladesh. Agriculture, Ecosystems and Environment 79, 9-16.

Jacobs A., Helfrich M., Hanisch S., Quendt U., Rauber R., Ludwig B., 2010. Effect of conventional and minimum tillage on physical and biochemical stabilization of soil organic matter. Biology and Fertility of Soils 46,671–680.

Jacobs A., Rauber R., Ludwig B., 2009. Impact of reduced tillage on carbon and nitrogen storage of two Haplic Luvisols after 40 years. Soil and Tillage Research 102, 158–164.

Jiménez J.J., Lal R., Russo R.O., Leblanc H.A., 2008. The soil organic carbon in particle-size separates under different regrowth forest stands of north eastern Costa Rica. Ecological Engineering 34, 300–310.

Jinbo Z., Changchun S., Shenmin W., 2007. Dynamics of soil organic carbon and its fractions after abandonment of cultivated wetlands in northeast China. Soil and Tillage Research 96, 350–360.

Joergensen R.G., Castillo X., 2001. Interrelationships between microbial and soil properties in young volcanic ash soil of Nicaragua. Soil Biology and Biochemistry 33, 1581–1589.

Joergensen R.G., Scheu S., 1999. Depth gradients of microbial and chemical properties in moder soils under beech and spruce. Pedobiologia 43, 134-144.

Johnson D.B., Williamson J.C., 1994. Conservation of mineral nitrogen in restored soils at opencast coal mine sites: I. Results from field studies of nitrogen transformations following restoration**.** European Journal of Soil Science 45, 311-317.

Kaffka S., Koepf H.H., 1989. A Case Study on the Nutrient Regime in Sustainable Farming. Biological Agriculture and Horticulture 6, 89-106.

Karlen D.L., Wollenhaupt N.C., Erbach D.C., Berry E.C., Swan J.B., Eash N.S., Jordahl J.L., 1994. Crop residue effects on soil quality following 10 years of no-till corn. Soil and Tillage Research 31, 149-167.

Klopatek J.M., 2002. Belowground carbon pools and processes in different age stands of Douglas-fir. Tree Physiology 22, 197–204.

Klopatek J.M., 2002. Belowground carbon pools and processes in different age stands of Douglas-fir. Tree Physiology 22, 197–204.

Kucuker M.A., Guney M., Volkan Oral H., Copty N.K., Onay T.T., 2015. Impact of deforestation on soil carbon stock and its spatial distribution in the Western Black Sea Region of Turkey. Journal of Environmental Management147, 227-235.

Kumbhar A.M., Buriro U.A., Oad F.C., chachar Q.I., Kumhar M.B., Jamro G.H., 2007. Yield and N-uptake of wheat (*Triticum Aestivum L.*) under different fertility levels and crop sequence. Pakistan Journal of Botany 39, 2027-2034.

Kumbhar A.M., Buriro U.A., Junejos S., Oad F.C., Jamro G.H., Kumbhar B.A., Kumbhar S.A., 2008. Impact of different nitrogen levels on cotton growth, yield and N-uptake planted in legume rotation. Pakistan Journal of Botany 40, 767-778.

La Scala N., Bolonhezi D., Pereira G.T., 2006. Short-term soil CO_2 emission after conventional and reduced tillage of a no-till sugar cane area in southern Brazil. Soil and Tillage Research 91, 244-248.

Lal R., 1997. Residue management, conservation tillage and soil restoration for mitigating greenhouse effect by CO_2- enrichment. Soil and Tillage Research 43, 81-107.

Lal R., Mahboubi A.A, Fausey N.R., 1994. Long-term tillage and rotation effects on properties of a central Ohio soil. Soil Science Society of America Journal 58, 517-522.

Lantz A., Lal R., Kimble J., 2001. Land use effects on soil carbon pools in two major land resource areas of Ohio, USA. In: Stott D.E., Mohtar, R.H., Steinhardt G.C. (Eds), Sustaining the global form. 10th International Soil Conservation Organization Meeting held 24-29 May, 1999, at Purdue University and the USDA-ARS National Soil Erosion Research Laboratory, pp.499-502.

Lee K.-H., Jose S., 2003a. Soil respiration, fine root production, and microbial biomass in cottonwood and loblolly pine plantations along a nitrogen fertilization gradient. Forest Ecology and Management 185, 263–273.

Lee K.-H., Jose S., 2003b. Soil respiration and microbial biomass in a pecan – cotton alley cropping system in Southern USA Agroforestry Systems 58: 45–54, 2003.

Lehmann J., da Silva Cravo M., Zech W., 2001. Organic matter stabilization in a Xanthic Ferralsol of the central Amazon as affected by single trees: chemical characterization of density, aggregate, and particle size fractions. Geoderma 99, 147–168.

Leifeld J., Kögel-Knabner I., 2005. Soil organic matter fractions as early indicators for carbon stock changes under different land-use? Geoderma 124, 143–155.

Lemenih M., Itanna F., 2004. Soil carbon stocks and turnovers in various vegetation types and arable lands along an elevation gradient in southern Ethiopia. Geoderma 123, 1777-188.

Li X.-G., Li F.-M., Zed R., Zhan Z.-Y., Singh B., 2007. Soil physical properties and their relations to organic carbon pools as affected by land use in an alpine pastureland. Geoderma 139, 98-105.

Liang Q., Chen H., Gong Y., Fan M., Yang H., Lal R., Kuzyakov Y., 2012. Effects of 15 years of manure and inorganic fertilizers on soil organic carbon fractions in a wheat-maize system in the North China Plain. Nutrient Cycling in Agroecosystems 92, 21–33.

Liu H.S., Li L.H., Han X.G., Huang J.H., Sun J.X., Wang H.Y., 2006. Respiratory substrate availability plays a crucial role in the response of soil respiration to environmental factors. Applied Soil Ecology 32, 284–292.

Llorente M., Lafuente F., Ruipérez C., Turrión M.B., 2008. Uso de parámetros microbiológicos edáficos como indicadores del efecto del uso del suelo en el páramo calizo castellano-leonés. Cuadernos de la *Sociedad Española de Ciencias Forestales* 25, 273–280.

Llorente M., Turrión M.B., 2009. Microbiological parameters as indicators of soil organic carbon dynamics in relation to different land use management. European Journal of Forest Research 129, 73-81.

Lohila A., Aurela M., Regina K., Laurila T., 2003. Soil and total ecosystem respiration in agricultural fields: effect of soil and crop type. Plant and Soil 251, 303–317.

López-Garrido R., Deurer M., Madejón E., Murillo J.M., Moreno F., 2012. Tillage influence on biophysical soil properties: The example of a long-term tillage experiment under Mediterranean rainfed conditions in South Spain. Soil and Tillage Research 118, 52–60.

Madejón E., Murillo J.M., Moreno F., López M.V., Arrue J.L., Alvaro-Fuentes J., Cantero C., 2009. Effect of long-term conservation tillage on soil biochemical properties in Mediterranean Spanish areas. Soil and Tillage Research 105, 55-62.

Mann L.K., 1986. Changes in soil carbon storage after cultivation. Soil Science 142, 279-288.

Mao D.M., Min Y.W., Yu L.L., Martens R., Insam H., 1992. Effect of afforestation on microbial biomass and activity in soils of tropical china. Soil Biology and Biochemistry 24, 865-872.

Marinari S., Mancinelli R., Campiglia E., Grego S., 2006. Chemical and biological indicators of soil quality in organic and conventional farming systems in Central Italy. Ecological Indicators 6, 701–711.

Mataix-Solera J., Zornoza R., Lloret E., Guerrero C., García-Orenes F., Mataix-Beneyto J., Navarro J., Gómez I., 2006. Soil organic carbon, microbial biomass and basal respiration 11 years after a forest fire in a Mediterranean environment. Geophysical Research Abstracts 8, 01249, European Geosciences Union.

Melero S., López-Garrido R., Murillo J. M., Moreno F., 2009. Conservation tillage: Short-and long-term effects on soil carbon fractions and enzymatic activities under Mediterranean conditions. Soil and Tillage Research 104, 292-298.

McGill W.B., Cannon K.R., Robertson J.A., Cook F.D., 1986. Dynamics of soil microbial biomass and water soluble organic C in Breton L after 50 years of cropping to two rotations. Canadian Journal of Soil Science 66, 1-19.

Minetos D., Polyzos S., 2007. Forest land use changes in Greece: An ordinal regression analysis at the regional level. Conference proceedings: 47th Congress of ERSA, Paris. Discussion Paper Series 13, 165-192.

Mohr D., 2004. Soil degradation in simple oak coppice forests of the AHR-Eifel. Implications for forest management derived from soil ecological studies. Cuvillier Verlag, Göttingen. 146p.

Moscatelli M.C., Lagomassino A., Marinari S., De Angelis P., Grego S., 2005. Soil microbial indices as bioindicators of environmental changes in a poplar plantation. Ecological Indicators 5, 171–179.

Nael M., Khademi H., Hajabbasi M.A., 2004. Response of soil quality indicators and their spatial variability to land degradation in central Iran. Applied Soil Ecology 27, 221–232.

Nelson P.N., Dictor M.C., Soulas G., 1994. Availability of organic carbon in soluble and particle-size fractions from a soil profile. Soil biology and Biochemistry 26, 1549-1555.

Nogueira M.A., Albino U.B., Brandão-Junior O., Braun G., Cruz M.F., Dias B.A., Duarte R.T.D., Gioppo N.M.R., Menna P., Orlandi J.M., Raimam M.P., Rampazo L.G.L., Santos M.A., Silva M.E.Z., Vieira F.P., Torezan J.M.D., Hungria M., Andrade G., 2006. Promising indicators for assessment of agroecosystems alteration among natural, reforested and agricultural land use in southern Brazil. Agriculture, Ecosystems and Environment 115, 237–247.

North P.F., 1976. Towards an absolute measurement of soil structural stability using ultrasound. Journal of Soil Science 27, 451-459.

Oorts K., Venalauwe B., Recous S., Merckx R., 2005. Redistribution of particulate organic matter during ultrasonic dispersion of highly weathered soils. European Journal of Soil Science 56, 77-91.

Panayiotopoulos K.P., 1984. Soil Physics. Yahoudi – Yapouli publications, Thessaloniki, Greece. (*In Greek*)

Pascual J.A., Ross M., Hernández T., García C., 2001. Effect of long-term monoculture on microbiological and biochemical properties in semiarid soils. Communications in Soil Science and Plant Analysis 32, 537-552.

Paustian K., Elliott E.T., Six J., Hunt H.W., 2000. Management options for reducing CO_2 emissions from agricultural soils. Biogeochemistry 48, 147-163.

Perucci P., Bonciarelli U., Santilocchi R., Bianchi A.A., 1997. Effect of rotation, nitrogen fertilization and management of crop residues on some chemical, microbiological and biochemical properties of soil. Biology & Fertility of Soils_24, 311-316.

Pinzari F., Trinchera A., Benedetti A., Sequi P., 1999. Use of biochemical indices in the mediterranean environment: comparison among soils under different forest vegetation. Journal of Microbiological Methods 36, 21–28.

Poeplau C., Don A., 2013. Sensitivity of soil organic carbon stocks and fractions to different land-use changes across Europe. Geoderma 192, 189-201.

Pompili L., Mellina A.S., Benedetti A., 2006. Microbial Indicators for evaluating soil quality in differently managed soils. Geophysical Research Abstracts, Vol. 8, European Geosciences Union.

Poppe L.J., Fredericks J.J., Hathaway J.C., 1988. A computer program to calculate centrifugation parameters for sedimentation analyses. Computers and Geosciences 14, 541–545.

Powlson D.S., Brookes P.C., Christensen B.T., 1987. Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. Soil Biology and Biochemistry 19, 159–164.

Puget P., Chenu C., Balesdent J., 2000. Dynamics of soil organic matter associated with particle-size fractions of water-stable aggregates. European Journal of Soil Science 51, 595–605.

Quiroga A.R., Buschiazzo D.E., Peinemann N., 1996. Soil organic matter particle size fractions in soils of the semiarid Argentinian pampas. Soil Science 161, 104-108.

Raich J.W., 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus 44, 81-99.

Raich J.W., Tufekcioglou A., 2000.Vegetation and soil respiration: Correlations and controls. Biogeochemistry 48, 71–90.

Rampazzo N., Mentler A., 2001. Influence of different agricultural land use on soil properties along the Austrian-Hungarian border. Austrian Journal of Agricultural Research 52, 89-115.

Rasmussen P.E., Parton W.J., 1994. Long-term effects of residue management in wheat-fallow: I. Inputs, yield, and soil organic matter. Soil Science Society of America Journal 58, 523-530.

Ratnayake R.R, Seneviratne G., Kulasooriya S.A., 2011. The effect of cultivation on organic carbon content in the clay mineral fraction of soils. International Journal of Soil Science 6, 217-223.

Reicosky D.C., Kemper W.D., Langdale G.W., Douglas C.L.Jr, Rasmussen P.E., 1995. Soil organic matter changes resulting from tillage and biomass production. Journal of Soil & Water Conservation 50, 253-261.

Reicosky D.C., 1997. Tillage-induced CO2 emission from soil. Nutrient Cycling in Agroecosystems 49, 273–285.

Roldán A., Salinas-García J.R., Alguacil M.M., Caravaca F., 2007. Soil sustainability indicators following conservation tillage practices under subtropical maize and bean crops. Soil and Tillage Research 93, 273-282.

Romanyà J., Rovira P., 2011. Man appraisal of soil organic C content in Mediterranean agricultural soils. Soil Use and Management 27, 321-332.

Rudrappa L., Purakayastha T.J., Singh D., Bhadraray S., 2006. Long-term manuring and fertilization effects on soil organic carbon pools in a Typic Haplustept of semi-arid sub-tropical India. Soil and Tillage Research 88, 180–192.

Ruiz Benito P., Cuevas J.A., Bravo de la Parra R., Prieto F., García del Barrio J.M., Zavala M.A., 2010. Land use change in a Mediterranean metropolitan region and its periphery: assessment of conservation policies through CORINE Land Cover data and Markov models. Forest Systems 19, 315-328.

Saggar S., Yeates G.W., Shepherd T.G., 2001. Cultivation effects on soil biological properties, microfauna and organic matter dynamics in Eutric Gleysol and Gleyic Luvisol soils in New Zealand. Soil and Tillage Research 58, 55-68.

Saha S.K., Ramachandran Nair P.K., Nair V.D., Mohan Kumar B., 2010. Carbon storage in relation to soil size-fractions under tropical tree-based land-use systems. Plant and Soil 328, 433-446.

Sahani U., Behera N., 2001. Impact of deforestation on soil physicochemical characteristics, microbial biomass and microbial activity of tropical soil. Land Degradation and Development 12, 93-105.

Santruckova H., Straskraba M., 1991. On the relationship between specific respiration activity and microbial biomass in soils. Soil Biology and Biochemistry 23, 525-332.

Saroa G.S., Lal R., 2003. Soil restorative effects of mulching on aggregation and carbon sequestration in a Miamian soil in central Ohio. Land Degradation and Development 14, 481-493.

SAS Institute Inc., 2010. *SAS/STATTM User's Guide, Release 9.1*. Cary, N.C, USA

Sato A., Seto M., 1999. Relationship between rate of carbon dioxide evolution, microbial biomass carbon, and amount of dissolved organic carbon as affected by temperature and water content of a forest and an arable soil. Communications in Soil Science and Plant Analysis_ 30, 2593-2605.

Schmidt M.W., Kögel-Knabner I., 2002. Organic matter in particle-size fractions from A and B horizons of a Haplic Alisol. European Journal of Soil Science 53, 383-391.

Serrano J.M., Shahidian S., Marques da Silva J., Carvalho M., 2016. Monitoring of soil organic carbon over 10 years in a Mediterranean silvo-pastoral system: potential evaluation for differential management. Precision Agriculture 17, 274-295.

Shahbazi F., Aliasgharzad N., Ebrahimzad S.A., Najafi N., 2013. Geostatistical analysis for predicting soil biological maps under different scenarios of land use. European Journal of Soil Biology 55, 20-27.

Sigstad E.E., Bejas M.A., Amoroso M.J., García C.I., 2002. Effect of deforestation on soil microbial activity. A worm-composite can improve soil quality? A microcalorimetric analysis at 25ºC. Thermochimica Acta 394, 171-178.

Six J., Conant R.T., Paul E.A., Paustian K., 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant and Soil 241, 155-176.

Six J., Guggenberger G., Paustian K., Haumaier L., Elliott E. T., Zech, W., 2001. Sources and composition of soil organic matter fractions between and within soil aggregates. European Journal of Soil Science, 52, 607-618.

Sleutel S., De Neve S., Singier B., Hofman G., 2006. Organic C levels in intensively managed arable soils – long-term regional trends and characterization of fractions. Soil Use and Management 22, 188–196.

Soil Survey Division Staff, 1993. *Soil survey manual*. United States Department of Agriculture (USDA). pp. 63–65.

Soil Survey Staff, 1999. *Soil taxonomy: A basic system of soil classification for making and interpreting soil surveys.* 2nd edition. Natural Resources Conservation Service. U.S. Department of Agriculture Handbook 436.

Soil Survey Staff, 2010. Keys to Soil Taxonomy, 11th ed. USDA-Natural Resources Conservation Service, Washington, DC.

Solomon D., Fritzsche F., Tekalign M., Lehmann J., Zech W., 2002. Soil Organic Matter Composition in the Subhumid Ethiopian Highlands as Influenced by Deforestation and Agricultural Management. Soil Science Society of America Journal 66, 68–82.

Solomon D., Lehmann J., Zech W., 2000. Land use effects on soil organic matter properties of chromic Luvisols in the semiarid tropics: Carbon, nitrogen, lignin and carbohydrates. Agriculture, Ecosystems and Environment 78, 203–213.

Sparling G.P., 1992. Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. Australian Journal of Soil Research 30, 195-207.

Spedding T.A., Hamel C., Mehuys G.R., Madramootoo C.A., 2004. Soil microbial dynamics in maize-growing soil under different tillage and residue management systems. Soil Biology and Biochemistry 36, 499–512.

Steiner C., Teixeira W. G., Lehmann J., Nehls T., de Macêdo J. L.V., Blum W. E., Zech W., 2007. Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. Plant and soil, 291, 275-290.

Tan Z., Lal R., 2005. Carbon sequestration potential estimates with changes in land use and tillage practice in Ohio, USA. Agriculture, Ecosystems and Environment 111, 140–152.

Tiessen H., Moir J.O., 1993. Total and organic carbon. In: Carter, M.R. (Ed.), Soil Sampling and Methods of Analysis. Lewis Publishers, pp. 187 - 199.

Tufekcioglu A., Raich J.W., Isenhart T.M., Schultz R.C., 2001. Soil respiration within riparian buffers and adjacent crop fields. Plant and Soil 229, 117–124.

Turrión M.B., Lafuente F., Mulas R., López O., Ruipérez C., Pando V., 2012. Effects on soil organic matter mineralization and microbiological properties of applying compost to burned and unburned soils. Journal of environmental management 95, 245-249.

USDA, 1972. Soil survey laboratory methods and procedures for collecting soil samples. Washington, DC, USA: U.S. Gov. Print. Office.

Vance E.D., Brookes P.C., Jenkinson D.S., 1987. An extraction method for measuring soil microbial biomass carbon. Soil Biology and Biochemistry 19, 703-704.

Veldkamp E., 1994. Organic carbon turnover in three tropical soils under pasture after deforestation. Soil Science Society of America Journal 58, 175-180.

Vittori Antisari L., Dell'Abate M.T., Buscarolli A., Cherardi M., 2010. Role of soil organic matter characteristics in a pedological survey: "Bosco Frattona" natural reserve (Site of Community Importance, Italy) case study. Geoderma. 156, 302-315.

von Lutzow M., Leifeld J., Kainz M., Kögel-Knabner I., Munch J.C., 2002. Indications for soil organic matter quality in soils under different management. Geoderma 105, 243–258.

Wagai R., Brye K.R., Gower S.T., Norman J.M., Bundy L.G., 1998. Land use and environmental factors influencing soil surface CO_2 flux and Microbial biomass in natural and managed ecosystems in southern Wisconsin. Soil Biology and Biochemistry 30, 1501-1509.

Wander M.M., Hedrick D.S., Kaufman D., Traina S.J., Stinner B.R., Kehrmeyer S.R., White D.C., 1995. The functional significance of the microbial biomass in organic and conventionally managed soils. Plant and Soil 170, 87-97.

Wang W.J., Dalal R.C., Moody P.W., Smith C.J., 2003. Relationships of soil respiration to microbial biomass, substrate availability and clay content. Soil Biology and Biochemistry 35, 273–284.

Wardle D.A., Ghani A., 1995. A critique of the microbial metabolic quotient (qCO_2) as a bioindicator of disturbance and ecosystem development. Soil Biology and Biochemistry 27, 1601-1610.

Wardle D.A., Yeates G.W., Nicholson K.S., Bonner K.I., Watson R.N., 1999. Response of soil microbial biomass dynamics, activity and plant litter decomposition to agricultural intensification over a seven-year period. Soil Biology & Biochemistry 31, 1707–1720.

Witter E., Kanal A., 1998. Characteristics of the soil microbial biomass in soils from a long-term field experiment with different levels of C input. Applied Soil Ecology 10, 37-49.

Wright A.L., Hons F.M., 2004. Soil Aggregation and Carbon and Nitrogen Storage under Soybean Cropping Sequences. Soil Science Society of America Journal 68, 507–513.

Wu J., Joergensen R.G., Pommering B., Chaussod R., Brookes P.C., 1990. Measurement of soil microbial biomass C – an automated procedure. Soil Biology and Biochemistry 22, 1167–1169.

Xu X., Inubushi K., Sakamoto K., 2006. Effect of vegetations and temperature on microbial biomass carbon and metabolic quotients of temperate volcanic forest soils. Geoderma 136, 310–319.

Yang J.C., Huang J.H., Pan Q.M., Tang J.W., Han X.G., 2004. Long-term impacts of land-use change on dynamics of tropical soil carbon and nitrogen pools. Journal of Environmental Science (China) 16, 256-261.

Zhao L., Sun Y., Zhang X., Yang X., Drury C.F., 2006. Soil organic carbon in clay and silt sized particles in Chinese mollisols: Relationship to the predicted capacity. Geoderma 132, 315–323.

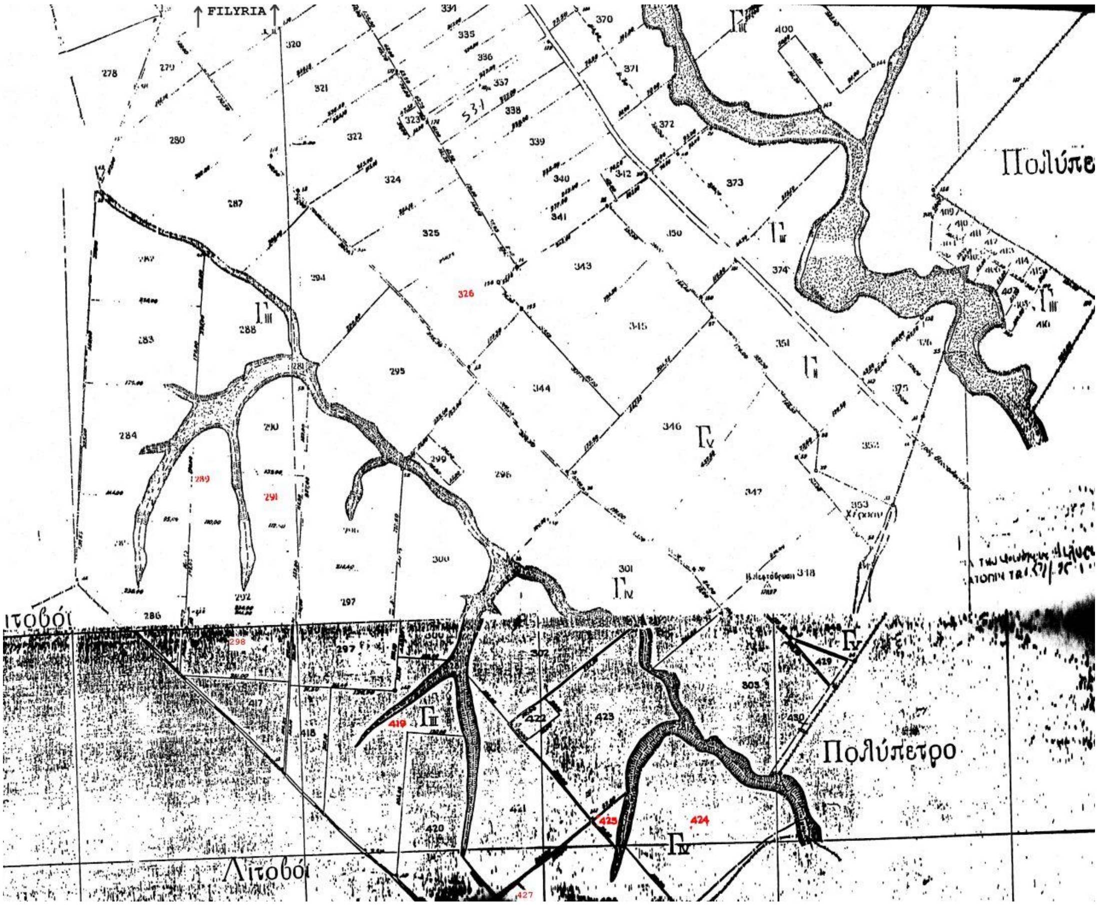
Zhong Y., Yan W., Shangguan Z., 2015. Soil carbon and nitrogen fractions in the soil profile and their response to long-term nitrogen fertilization in a wheat field. Catena 135, 38-46.

Zinn Y.L., Resck D.V.S., da Silva J.E., 2002. Soil organic carbon as affected by afforestation with *Eucalyptus* and *Pinus* in the Cerrado region of Brazil. Forest Ecology and Management 166, 285-294.

APPENDIX

1. **Maps of the deforestations of the area**
2. **Photos of the fields**
3. **Published articles derived from the thesis**

1. **Maps of the deforestations of the area**



Map 1. The 1933 deforestation

Map 2. The 1971 deforestation

Map 3. The 1980 deforestation

2. Photos of all the fields *(except for the selected ones presented in the Materials and Methods chapter)*

Wheat fields derived from the 1933 deforestation.

Cotton fields derived from the 1933 deforestation.

Cotton fields derived from the 1933 deforestation.

Wheat fields derived from the 1971 deforestation.

Wheat fields derived from the 1971 deforestation.

Wheat fields derived from the 1971 deforestation.

Cotton fields derived from the 1971 deforestation.

Cotton fields derived from the 1971 deforestation.

Wheat fields derived from the 1980 deforestation.

Wheat fields derived from the 1980 deforestation.

Wheat fields derived from the 1980 deforestation.

Cotton fields derived from the 1980 deforestation.

Cotton fields derived from the 1980 deforestation.

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Original article

Soil microbiological properties affected by land use, management, and time since deforestations and crop establishment

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ABSTRACT

Deforestation is a common practice worldwide in order to gain agricultural land. In Filyria, Kilkis, Greece, three major deforestations took place in 1933, 1971 and 1980. Cultivation effects, referring to soil microbial properties are studied, in deforested fields, using the adjacent remaining oak forest as reference. The soils are cultivated with cotton (conventional tillage and irrigation, alternation with wheat every two years) or wheat (reduced tillage and no irrigation). The estimation and interpretation of the microbial properties were based on the analyses of soil organic carbon (SOC), microbial biomass carbon (MBC), the MBC/SOC ratio, potential soil respiration (SR), and the metabolic quotient (qCO_2). The forest ecosystem appears to provide better conditions for microbial growth and activity, having significantly greater SOC (31.8 g C kg^{-1} soil in forest versus 9.6 g C kg^{-1} soil in crop plots), MBC (1080 mg C kg^{-1} soil in forest versus 492 mg C kg^{-1} soil in crop plots) and SR (4.78 mg C CO_2 kg^{-1} soil d^{-1} in forest versus 2.99 mg C CO_2 kg^{-1} soil d^{-1} in crop plots), and significantly lower qCO_2 rates than the crop plots although its organic matter quality results to a lower MBC/SOC ratio. The number of years since deforestation played a major role for most of the parameters analyzed, although after many years the equilibrium reached by the ecosystem appeared to moderate the differences. Disturbance through cultivation decreases soil quality. Reduced tillage without irrigation of the wheat crops leads to more suitable conditions for the microbial populations (as expressed by the microbiological properties) than conventional tillage with irrigation of the cotton crops.

1. Introduction

Losses of soil organic carbon (SOC) often occur when converting forest to cultivated land, mainly due to lower inputs of organic matter, reduced physical protection of SOC as a result of tillage and changes in soil temperature and moisture regime which accelerate decomposition rates [29]. Soil tillage involves the physical disturbance of the upper soil layers, breaking down soil aggregates, thus influencing C stability in the soil [24]. Conservation tillage techniques seem to increase soil organic matter (SOM) in the upper layer, thus increasing the micro-aggregation and aggregate stability. These practices therefore could promote an enhancement of C sink at a global scale. Changes in SOC content occur slowly and do not always provide adequate information of changes in soil quality that may occur [6]. It is therefore important to identify SOM fractions more sensitive to a change of land use or management which can be applied as early indicators of the dynamics of the soil C. Soil microbiological properties have been reported as a reliable tool in order to estimate early changes in the dynamics and distribution of soil microbial processes in different land use systems [16]. Microbial biomass carbon (MBC), potential soil respiration rate (SR), metabolic quotient (qCO_2, ratio of respired C to biomass C), ratio of microbial biomass C to total organic C (MBC/SOC), are variables that have been suggested as indicators for assessing soil management effects on soil quality [1]. The microbial quotient (MBC/SOC) proved to be a reliable soil microbial parameter for describing changes in man-made ecosystems and more sensitive than its single components, MBC and SOC [2]. The metabolic quotient indicates the intensity of microbial metabolism and reflects the maintenance energy requirements [1] or generally stress by different factors [4].

Euro-Mediterranean regions are currently threatened by global changes [25]. Minetos and Polyzos [21] carried out a regional analysis of forest land use changes in Greece during the last

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decades. They observed that the prefecture of Kilkis, where the present study is located, showed a high rate of depletion of their forest site. There is a scarcity of studies addressing land use change dynamics and its effect on SOC and microbial properties in these semiarid regions.

The aim of this paper is to determine the changes in terms of soil microbiological properties that resulted from three stages of deforestation which occurred 25, 34 and 72 years before the experiment, in Northern Greece, by measuring and assessing SOC, MBC, MBC/SOC ratio, SR and qCO_2. The big amount of time that passed since the first deforestation, the availability of data from three different deforestation dates (1933, 1971 and 1980) and the remaining undisturbed forest, offer an interesting opportunity of studying these changes, along with assessing factors such as land use (forestry vs agriculture) and type of crop (wheat vs cotton). We have hypothesized that the effects on SOC and microbiological properties are similar in terms of amount of time since deforestation, but vary according to the subsequent type of crop installed.

2. Materials and methods

2.1. Study area

The study area is located in Filyria, prefecture of Kilkis, North Greece (40°54′11.69″ N–40°53′41.9″ N, and 22°28′47.97″E–22°29′37.10″E). The altitude ranges from 145 to 195 m. The climate is temperate Mediterranean with mean annual temperature of 15.0 °C, (absolute maximum 40.4 °C, absolute minimum −17.4 °C) and mean annual precipitation of 506 mm. Soils have developed from limestone, and are classified as Xeralfs according to Soil Taxonomy. Soil A horizons have clay loam textures, with percentages of sand, silt and clay of 30, 36, and 34%, respectively for the forest topsoil mineral horizon, and 26, 38, and 36%, respectively for the Ap horizon at agricultural land. Mean pH was between 7.7 and 8.0 and concentrations of $CaCO_3$ (method described below) were 1–7% at the forest sites and 1–14% at the cultivated plots. There are no differences between the plots of the study area in terms of basic soil properties. The vegetation is forest of *Quercus* species (especially *Quercus pubescens*). Socio-economic policies resulted to deforestation of the area at 3 stages (1933, 1971, 1980) leaving only 80 ha of natural forest undisturbed. These fields (2–9 ha mean extension) are cultivated with wheat, cotton and cherry trees. Mean slope of the area is 2%.

2.2. Management practices

The agricultural plots selected for this study are cultivated with cotton or wheat. For wheat, management practices include plowing up to 20–30 cm, fertilization with 20–10–0 (applying 300–400 kg ha^{-1}), and NH_4NO_3 (100–150 kg ha^{-1}). No irrigation is applied. Cotton management includes deep plowing up to 30–40 cm in September, followed by surface plowing in March. Fertilization with 11–15–15 or 12–12–17 + micronutrients (250–300 kg ha^{-1}) is applied once per period and NH_4NO_3 (100 kg ha^{-1}) twice per period. In terms of irrigation, every 10–15 days at summer 300–400 m^3 of water per hectare is applied by sprinklers, meaning that the whole soil surface of the area is watered. Cotton fields are alterned with wheat every two years (two consecutive years of cotton crops, one year of wheat). Wheat fields are cultivated solely with wheat. Management practices are carried out with the same principles since 1933. History data were collected from the Greek Forestry Service, the National Agricultural Service and from interviews with farmers managing the land since 1946.

Practice of irrigation and tillage are the main differences among the two indicated crop types, hence for this study the cultivated plots were divided to wheat crops and cotton crops. This division to subcategories relates to reduced tillage practices for the wheat plots (no deep plowing) that are not irrigated, and conventional tillage practices for the cotton plots that are irrigated.

2.3. Experimental design and sampling

Three sampling categories referring to deforestation year (1933, 1971, and 1980) in the cultivated fields and one category referring to the natural remaining forest were considered. For each deforestation year category, two crop subcategories were considered (wheat and cotton), and from each crop subcategory, 12 plots were sampled. In adjacent areas, twelve plots of remaining undisturbed forest (*Q. pubescens*) were selected. A composite sample was taken in each plot (72 cultivated plots and 12 forest plots). The composite sample was obtained by mixing fifteen random subsamples from the 0–15 cm mineral layer. Soil samples were collected at the end of November 2005, after harvest.

2.4. Total organic C analyses

Soil organic carbon was calculated from the total carbon measurement subtracting C from carbonates. Total carbon was measured by dry combustion on a LECO 2000 C/N/H analyzer. Soil total carbonates were determined by elimination with acid previously titrated with 0.5 M NaOH [8].

2.5. Microbiological properties

The fumigation–extraction method [30] was used in order to determine MBC. Microbial biomass C was calculated as a difference in C content in fumigated and non-fumigated sample (EC) using k_{EC} coefficient (MBC = EC:k_{EC}). The value k_{EC} = 0.45 was used to calculate microbial biomass C [32].

Potential soil respiration was determined in closed jars and under laboratory-controlled conditions following the Isermeyer method modified by Llorente et al. [17]. Soil samples were wetted to 75% of water holding capacity and incubated in 1 L jars at 29 °C for 3 days.

The metabolic quotient represents the potential soil respiration per unit microbial biomass, and was calculated as reported by Anderson and Domsch [3]. The microbial quotient (MBC/SOC) represented the fraction of MBC with respect to the SOC [3].

2.6. Statistical analysis

All data were subjected to ANOVA and when significant differences were detected ($p < 0.05$), Tukey's test was performed to allow separation of means. A factorial ANOVA with a control, forest, was carried out in order to determine the effect of deforestation, time since deforestation and type of crop. The Eq. (1) shows the factorial analysis of variance used for this model:

$$Y_{jkl(i)} = \mu + \alpha_i + \beta_{j(i)} + \gamma_{k(i)} + \beta_{\gamma jk(i)} + \varepsilon \boldsymbol{ijl(i)} \quad (1)$$

Where, $Y_{jkl(i)}$: analyzed soil property, μ: overall mean, α_i: main effect of land use, with two levels (crop and forest), $\beta_{j(i)}$: main effect of time elapsed since deforestation with four levels (deforested in 1933, 1971 and 1980 for crops, and remaining forest), $\gamma_{k(i)}$: main effect of type of crop with three levels (wheat, cotton for crop plots, and forest), $\beta\gamma_{jk(i)}$: interaction between time and type of crop, and ε_{ijk}: experimental error. The last two factors (time and type of crop) and their interaction were nested into the land use factor. The normality, independence and homoscedasticity of the residuals were checked. Correlations between studied soil properties and the time since deforestation were established applying regression analysis. All statistics were calculated with the use of the Statistica 7 software package.

3. Results

As can be seen in Table 1, the land use factor (cultivated sites versus forest) was significant ($p < 0.001$) for SOC, MBC, MBC/SOC ratio, SR and qCO_2. Forest soils showed significant higher values of SOC, MBC and SR than cultivated soils, however the MBC/SOC ratio and qCO_2 was significantly higher under crop than under forest (Fig. 1).

The double interaction (time * type of crop) was not significant for all the studied properties. As the interaction was not significant, we can examine only the main effects. Significantly more properties were affected by time after deforestation (deforested in 1933, 1971, and 1980) and subsequent cultivation than by type of crop (Table 1).

3.1. Soil organic carbon

The value of SOC in the forest sites was more than three times higher than in the crop sites (Fig. 1). The results shown in Table 1 indicate that the amount of time since deforestation was adversely associated with SOC. As can be seen in Fig. 2, the soils under forest had significant higher values of SOC than the deforested soils, also deforested in 1980 had significantly more SOC than the soils deforested in 1971 and 1933, but no significant differences were observed between the latter categories, 1971 and 1933.

During the first 30 years after deforestation and subsequent cultivation, SOC decreased with a rate of 0.71 g C kg^{-1} soil y^{-1}, the equation obtained for SOC versus number of years since deforestation (t) was: $SOC = -0.71t + 31.6$; with an r^2 of 0.996.

On the other hand, management showed a significant effect on SOC ($p < 0.05$) as can be seen in Table 1. Significantly higher SOC concentrations were found in the topsoil horizon of the wheat plots than of the cotton plots (Fig 3).

3.2. Soil microbial biomass carbon

Forest soils had a significantly higher value of MBC than the cultivated soils (Fig. 1). In forest soils the MBC was more than two times higher than in cultivated plots.

The ANOVA results for MBC showed significant differences for time and type of crop factors but not for their interaction (Table 1). This microbial property was more sensitive than SOC regarding type of crop. The same behavior/pattern was observed for both types of crop regardless of the deforestation date.

The highest values of MBC for cultivated plots were found in the 1980 category and the lowest ones in plots deforested in 1971. There were significant differences among the three year categories and with the soil under forest (Fig. 2).

In terms of management, conventional tillage practices and irrigation applied in the cotton plots caused a significant decrease on MBC in comparison with the values observed for the wheat plots where no irrigation and reduced tillage practices were used (Fig. 3).

3.3. Soil microbial biomass carbon to soil organic carbon ratio (MBC/SOC)

Forest soils had a significant lower microbial quotient than crop soils (Table 1). No significant effects of type of crop and of the interaction time * type of crop were observed for the MBC/SOC ratio (Table 1), whereas the amount of time since deforestation and subsequent crop establishment affects this ratio more than the type of crop.

There was a significantly good correlation between the number of years since deforestation and the microbial quotient, ($MBC/SOC = 0.36t + 40.6$; $r^2 = 0.774$). The y-intercepts (40.6 g MBC kg^{-1} SOC) are very close to the microbial quotient for forest soil (36.7 g MBC kg^{-1} SOC, Table 1), which could be considered with 0 years since deforestation.

3.4. Potential soil respiration (SR)

Soil under forests had significantly higher SR values than the crop soils (Fig. 1). The 1980 category had the highest (significantly different) values among the year categories (Fig. 2). The amount of time since deforestation plays a more significant role comparing to crop type for this property. No significant differences were found for type of crop factor and for the interaction time * type of crop (Table 1).

3.5. Soil respiration to soil microbial biomass carbon ratio (qCO_2)

The performed ANOVAs for qCO_2 were significant for land use and type of crop (Table 1). Significant lower qCO_2 values were observed in forest soils than in cultivated soils, and in wheat soils than in cotton soils. No differences were observed among years from deforestation and subsequent cultivation.

Table 1
Mean values of soil organic carbon (SOC), soil microbial biomass carbon (MBC), MBC to SOC ratio (MBC/SOC), potential soil respiration (SR), and metabolic quotient (qCO_2), for topsoil horizons (0–15 cm) of forest, wheat and cotton crops, and their standard errors. ANOVA significance levels of the effects of land use, time since deforestation, type of crop and the interaction between time and type of crop.

Treatment	SOC [g C kg^{-1} soil]	MBC [mg C-MBC kg^{-1} soil]	MBC/SOC [g C-MBC kg^{-1} SOC]	SR [mg C-CO_2 kg^{-1} soil d^{-1}]	qCO_2 [mg C-CO_2 kg^{-1} MBC d^{-1}]
Forest	31.8 ± 1.4	1080 ± 28	37 ± 5	4.8 ± 0.2	4.5 ± 0.4
1933 wheat	8.5 ± 0.6	508 ± 16	63 ± 4	2.9 ± 0.1	5.7 ± 0.3
1933 cotton	7.3 ± 0.6	458 ± 14	67 ± 5	3.0 ± 0.1	6.4 ± 0.3
1971 wheat	8.6 ± 0.7	470 ± 14	59 ± 6	2.7 ± 0.2	5.8 ± 0.4
1971 cotton	7.6 ± 0.7	382 ± 10	57 ± 7	2.5 ± 0.1	6.8 ± 0.4
1980 wheat	14.0 ± 1.0	622 ± 29	46 ± 3	3.7 ± 0.2	6.1 ± 0.4
1980 cotton	11.9 ± 0.8	509 ± 72	44 ± 3	3.3 ± 0.2	6.5 ± 0.4
Factor					
Land use	***	***	***	***	***
Time	***	***	***	***	ns
Type of crop	*	***	ns	ns	*
Time * type of crop	ns	ns	ns	ns	ns

Note: significance levels: *** ($p < 0.001$), ** ($p < 0.01$), * ($p < 0.05$), and ns: not significant; $n = 12$ for each group.

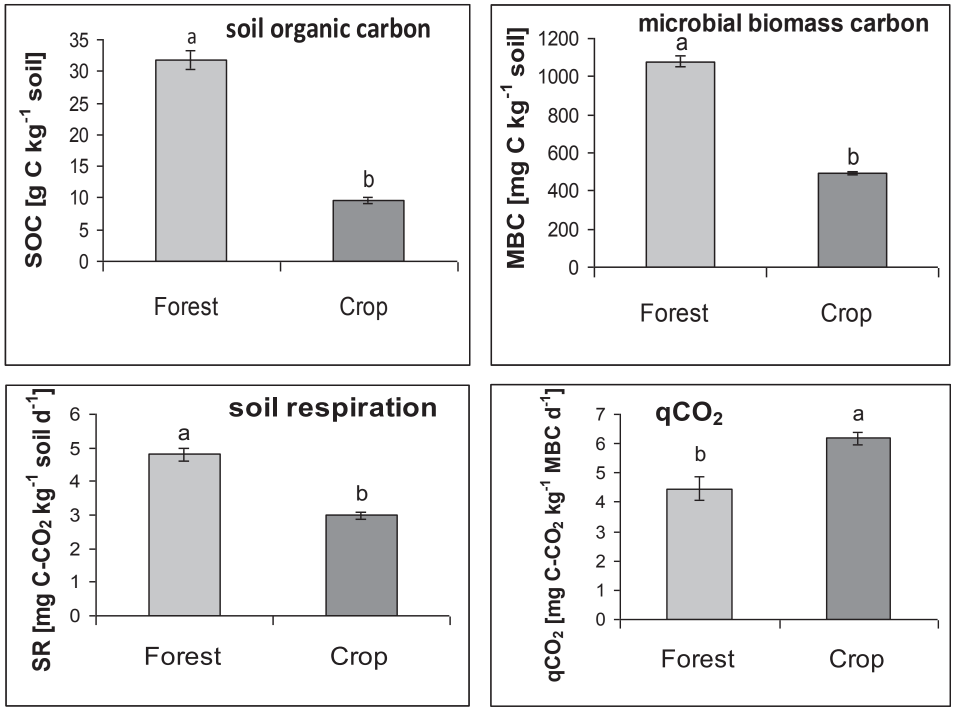

Fig. 1. Effect of deforestation and crop establishment on soil organic carbon (SOC), microbial biomass carbon (MBC), potential soil respiration (SR) and metabolic quotient (qCO_2), for topsoil horizons (0–15 cm). Different letters indicate significant differences between forest and crop ($p < 0.05$).

4. Discussion

4.1. Land use change effect: forest versus cultivation

In terms of land use, forest sites had significantly higher SOC concentrations than the cultivated sites in the present study. In many studies, forest soils were nearly always found to contain more SOC than deforested areas or agricultural soils [18].

The concentrations of MBC in the cultivated soils obtained in this study (382–622 mg kg^{-1}) fall well within the range observed in cultivated calcareous soils under Mediterranean conditions [19] who obtained mean MBC values between 470 and 624 mg kg^{-1} for traditional tillage and between 694 and 805 mg kg^{-1} for conservation tillage. Vittori Antisari et al. [31] found values of MBC between 853 and 1491 mg kg^{-1} for soil profile epipedons of typic Calciustepts under *Quercus petraea* L in Italy. The values obtained in our study for forest topsoil horizons are within this range (Table 1). Our results showed that deforestation followed by crop establishment caused a decrease by half of the MBC pool. In our study MBC trends follow changes in SOC contents, with which they are

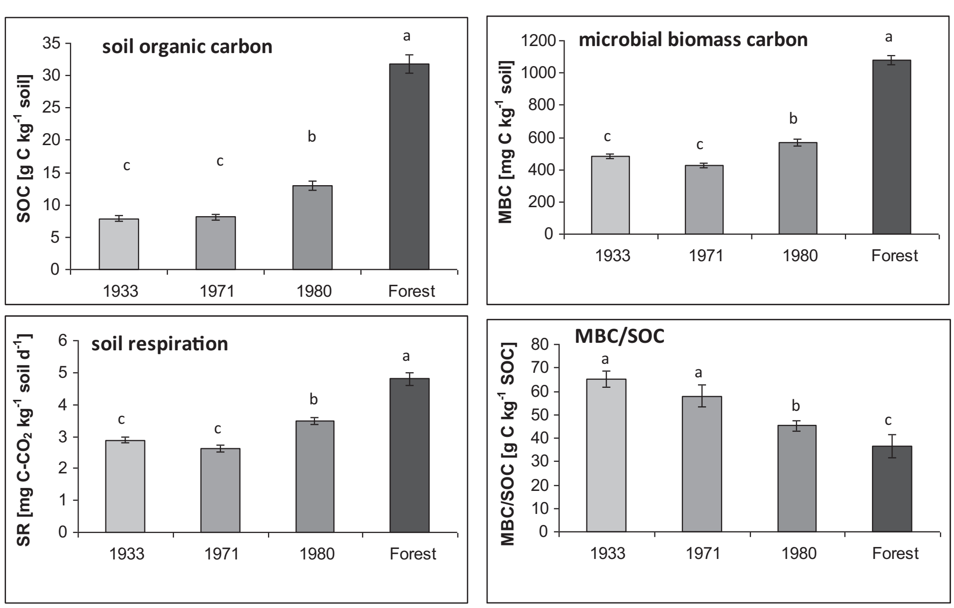

Fig. 2. Effect of time since deforestations and subsequent cultivation on soil organic carbon (SOC), microbial biomass carbon (MBC), potential soil respiration (SR) and the MBC to SOC ratio. Different letters indicate significant differences between deforested plots on 1933, 1971, 1980, and forest plots ($p < 0.05$).

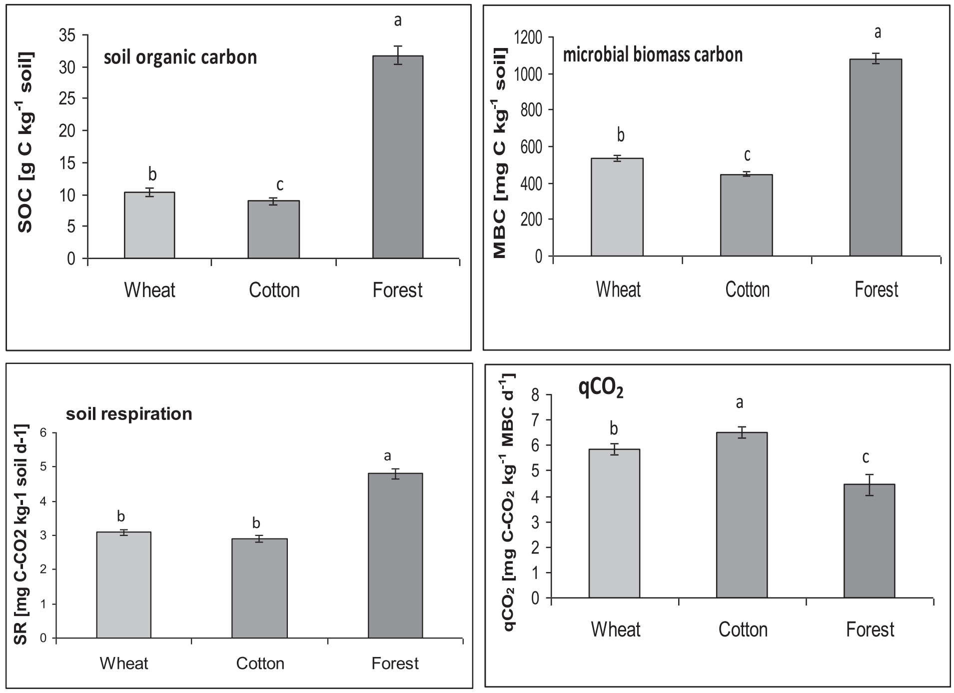

Fig. 3. Type of vegetation effects on soil organic carbon, SOC [g C-SOC kg^{-1} soil], microbial biomass carbon, MBC [mg C-MBC kg^{-1} soil] soil respiration, SR [mg C-CO_2 kg^{-1} soil d^{-1}] and metabolic quotient, qCO_2 [mg C-CO_2 kg^{-1} MBC d^{-1}]. Different letters indicate significant differences between wheat, cotton, and forest plots ($p < 0.05$).

positively correlated [27]. Sahani and Behera [26] found 83% less MBC at deforested sites, compared to values under forest. Lack of a proper soil environment and poor SOM level are the factors for such a decline in biomass size in the deforested site.

The values of the MBC/SOC ratio in the studied soils varied from 3.7 to 6.7%; most of the researchers mention values from 0.5 to 7%. There are contradictory results reported by bibliography about the effect of land use change from forest to crop in the MBC/SOC ratio. Anderson and Domsch [2] argued that the ratio will increase for a time if organic matter input to a soil increases, and will decrease otherwise. Sahani and Behera [26] ascribed the low MBC/SOC ratio that was found generally at deforested sites and especially at deforested barren sites of their experiment, to less microbial immobilization of nutrients. Nogueira et al. [23] did not detect significant effects on the MBC/SOC ratio by different sites (fallow, wheat, eucalyptus forest). On the other hand, Moscatelli et al. [22] and Fließbach et al. [10] stated that fertilization increases the MBC/SOC ratio, which seems to be strongly affected by the soil nutritional status. These authors indicated that the microbial pool is strongly dependent on N and probably suffers from a competition with plants for this element. According to this affirmation the nutritional improvement caused by fertilization could explain the significant higher values of the MBC/SOC ratio observed in our study for cultivated plots in comparison with the ratio values of forest plots.

The SR values obtained in this study are similar to those reported by Flieβbach et al. [9] for crop soils. According to Frank et al. [12], higher SOC and MBC lead to higher SR, and lowest values of SR corresponded to sites with lowest MBC. Clearance of vegetation has a negative effect on the development of microbial activity, as revealed by the significant lower values of SR [4,26]. Hence, as expected, changes in land use strongly affected potential soil respiration, as our results showed that forest rates in terms of SR were significantly higher than the corresponding agricultural rates.

In the present study, forest soils showed significant lower qCO_2 values than the cultivated soils; deforestation followed by crop establishment caused an increase of 40% in the qCO_2 values. Moscatelli et al. [22] indicated that the efficiency of soil microbial populations in acquiring or utilizing SOC and the intensity of C mineralization are measured by qCO_2. Low values are related with more efficient utilization of C by the microbial community, and accordingly forest soils showed a more efficient microbial community than crop soils.

4.2. Type of crop effect: wheat versus cotton crops

The main differences between the two crop types compared in this work are: a) the irrigation practice carried out in the cotton plots versus no irrigation of the wheat plots, b) the plowing practices, with those in the wheat plots to be considered as reduced tillage, and those in the cotton plots to be considered as conventional tillage practices, and c) the N fertilizer applications, which are higher in the cotton plots than in the wheat plots.

Our results showed that the type of crop played an important role on the SOC amount, obtaining significantly lower values of this property in the cotton compared to the wheat plots ($p < 0.05$, Fig. 2). Madejón et al. [20] found significant higher SOC values under reduced tillage plots than conventional tillage plots at the surface layer (0–5 cm). Tillage promotes SOM decomposition through crop residue incorporation into the soil and physical breakdown of residues.

Wheat and cotton crops of this study showed significant differences ($p < 0.001$) in terms of MBC that can be attributed to tillage and irrigation practices, such as reduced tillage practices at wheat plots compared to cotton/wheat rotation plots, enhanced application of N fertilizer which promotes MBC enrichment, increased amount of crop residues (that has as consequence a significant increase in SOC) at wheat plots, and differences in hydrological soil regimes caused by irrigation. Moscatelli et al. [22] found that N fertilization enhanced MBC levels, lowering energetic maintenance requirements, whereas Guo and Gifford [15] stated that although fertilization can increase biomass production, it may also enhance decomposition. Madejón et al. [20] reported that reduced tillage accumulated crop residues and SOC, which are substrates for soil microorganisms near the surface resulting to an increase in MBC and various soil processes in the surface soil.

Many authors indicate that when soil management changes, MBC responds more quickly than SOM, which is relatively slow to change [7,22], hence the MBC/SOC ratio is a good indicator of these changes. However, our results showed that both variables were separately sensitive to management changes, but not the MBC to SOC ratio. Franzluebbers et al. [13] stated that the MBC to SOC ratio increases when cropping intensity increases.

Land use, management practices and environmental conditions influence SR processes [12], however in our study potential SR was not significantly affected by crop type (Table 1). The water factor (irrigation) does not seem to enhance substantially SR [14], although it increases due to increased biological activity.

Franzluebbers et al. [13] found qCO_2 to be greater in wheat crops without N fertilization than rotated wheat or not cultivated land, but not under conventional cultivated crops. Moscatelli et al. [22] reported qCO_2 to be strongly affected by the nutritional status of the soil. Metabolic quotient was found lower in zero tilled than conventionally tilled plots, indicating greater accumulation of potentially more active and decomposable in field-disturbed cultivated soil, which therefore may actually have a slower *in situ* decomposition rate than soil under zero tillage [11]. The wheat plots, considered in our study as reduced tillage, have significant lower values than the cotton plots, on which conventional tillage is practiced. The latter fact agrees with the above discussion.

4.3. Effect of time since deforestation and subsequent cultivation

Sigstad et al. [28] stated that SOC after 15 years since deforestation with continuous cultivation is almost completely worn out. Our results showed that practicing agriculture for many years after deforestation leads to a further decrease of SOC levels, more specifically after 25, 34 and 72 years of cultivation the SOC decrease was 60%, 74% and 77% respectively. The most recent year category (1980) showed significantly higher amounts of SOC than the other two categories (1971 and 1933). Much of this loss in SOC can be attributed to soil tillage, which induces SOC loss by acceleration of organic C oxidation. Another tillage-related factor that contributes to soil C losses is soil aggregate disruption, which exposes once-protected organic matter to decomposition [24].

The significantly highest amounts of MBC and SR were found in the most recent crop plots, as expected. For the MBC/SOC ratio the effect of the time since deforestation followed by subsequent cultivation was also significant. Higher MBC to SOC ratios were observed in the older cultivated plots (1933 category). Fließbach et al. [10] found SR not to differ between farming systems. Campbell et al. [5] found that SR activity was significantly affected from tillage practices only after 12 years. Soils will tend towards a state of equilibrium if both environment and agricultural practices remain constant over long periods [2]. The MBC, SR and MBC to SOC ratio indicate that at least in the 1933 and 1971 plots this new equilibrium has been achieved.

On the other hand the number of years since deforestation and subsequent cultivation did not show significant differences in qCO_2 values.

5. Conclusions

Deforestation and further establishment of agricultural use leads to decreases in SOC and levels of microbiological properties, suggesting a deterioration of soil quality. Despite the fact that a relatively large amount of time had elapsed since deforestation and subsequent crop establishment in the three compared deforestation stages (1933, 1971 and 1980), the amounts of SOC, MBC and SR were significantly higher in soils deforested in the most recent stage (1980).

The type of crop affected the studied soil properties. Cotton plots (considered as conventional management) had significantly lower SOC and MBC values and microbiological efficiency levels than the wheat plots (considered as reduced management).

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References

[1] T.H. Anderson, Microbial eco-physiological indicators to assess soil quality, Agric. Ecosyst. Environ. 98 (2003) 285–293.
[2] T.H. Anderson, K.H. Domsch, Ratios of microbial biomass carbon to total organic-C in arable soils, Soil Biol. Biochem. 21 (1989) 471–479.
[3] T.H. Anderson, K.H. Domsch, The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils, Soil Biol. Biochem. 25 (1993) 393–395.
[4] F. Bastida, J.L. Moreno, T. Hernández, C. García, Microbiological activity in a soil 15 years after its devegetation, Soil Biol. Biochem. 38 (2006) 2503–2507.
[5] C.A. Campbell, V.O. Biederbeck, B.G. McConkey, D. Curtin, R.P. Zentner, Soil quality effect of tillage and fallow frequency. Soil organic matter quality as influenced by tillage and fallow frequency in a silt loam in southwestern Saskatchewan, Soil Biol. Biochem. 31 (1999) 1–7.
[6] R. Cardelli, F. Marchini, A. Saviozzi, Soil organic matter characteristics, biochemical activity and antioxidant capacity in mediterranean land use systems, Soil Tillage Res. 120 (2012) 8–14.
[7] H. Chen, R. Hou, Y. Gong, H. Li, M. Fan, Y. Kuzyakov, Effects of 11 years of conservation tillage on soil organic matter fractions in wheat monoculture in Loess Plateau of China, Soil Tillage Res. 106 (2009) 85–94.
[8] FAO, Methods of Analysis for Soils of Arid and Semi-arid Regions, 2007. Rome, 50 pp.
[9] A. Flieβbach, R. Martens, H.H. Reber, Soil microbial biomass and microbial activity in soils treated with metal contaminated sewage sludge, Soil Biol. Biochem. 26 (1994) 1201–1205.
[10] A. Fließbach, H.R. Oberholzer, L. Gunst, P. Mäder, Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming, Agric. Ecosyst. Environ. 118 (2007) 273–284.
[11] J.C. Franchini, C.C. Crispino, R.A. Souza, E. Torres, M. Hungria, Microbiological parameters as indicators of soil quality under various tillage and crop-rotation systems in Southern Brazil, Soil Tillage Res. 92 (2007) 18–29.
[12] A.B. Frank, M.A. Liebig, D.L. Tanaka, Management effects on soil CO_2 efflux in northern semiarid grassland and cropland, Soil Tillage Res. 89 (2006) 78–85.
[13] A.J. Franzluebbers, F.M. Hons, D.A. Zuberer, Long-term changes in soil carbon and nitrogen pools in wheat management systems, Soil Sci. Soc. Am. J. 58 (1994) 1639–1645.
[14] E. Gömöryová, K. St relcová, J. Skvarenina, D. San Gömöry, Responses of soil microorganisms and water content in forest floor horizons to environmental factors, Eur. J. Soil Biol. 55 (2013) 71–76.
[15] L.B. Guo, R.M. Gifford, Soil carbon stocks and land use change: a meta analysis, Global Change Biol. 8 (2002) 345–360.
[16] Q. Liang, H. Chen, Y. Gong, M. Fan, H. Yang, R. Lal, Y. Kuzyakov, Effects of 15 years of manure and inorganic fertilizers on soil organic carbon fractions in a wheat-maize system in the North China Plain, Nutr. Cycl. Agroecosyst. 92 (2012) 21–33.
[17] M. Llorente, F. Lafuente, C. Ruipérez, M.B. Turrión, Uso de parámetros microbiológicos edáficos como indicadores del efecto del uso del suelo en el páramo calizo castellano-leonés, Cuad. Soc. Esp. Cienc. For. 25 (2008) 273–280.
[18] M. Llorente, M.B. Turrión, Microbiological parameters as indicators of soil organic carbon dynamics in relation to different land use management, Eur. J. Res. 129 (2010) 73–81.
[19] R. López-Garrido, M. Deurer, E. Madejón, J.M. Murillo, F. Moreno, Tillage influence on biophysical soil properties: the example of a long-term tillage experiment under Mediterranean rainfed conditions in South Spain, Soil Tillage Res. 118 (2012) 52–60.
[20] E. Madejón, J.M. Murillo, F. Moreno, M.V. López, J.L. Arrue, J. Alvaro-Fuentes, C. Cantero, Effect of long-term conservation tillage on soil biochemical properties in Mediterranean Spanish areas, Soil Tillage Res. 105 (2009) 55–62.

[21] D. Minetos, S. Polyzos, Forest land use changes in Greece: an ordinal regression analysis at the regional level, Discuss. Pap. Ser. 13 (2007) 165–192.
[22] M.C. Moscatelli, A. Lagomassino, S. Marinari, P. De Angelis, S. Grego, Soil microbial indices as bioindicators of environmental changes in a poplar plantation, Ecol. Indic. 5 (2005) 171–179.
[23] M.A. Nogueira, U.B. Albino, O. Brandão-Junior, G. Braun, M.F. Cruz, Promising indicators for assessment of agroecosystems alteration among natural, reforested and agricultural land use in southern Brazil, Agric. Ecosyst. Environ. 115 (2006) 237–247.
[24] K. Paustian, E.T. Elliott, J. Six, H.W. Hunt, Management options for reducing CO_2 emissions from agricultural soils, Biogeochemistry 48 (2000) 147–163.
[25] P. Ruiz Benito, J.A. Cuevas, R. Bravo de la Parra, F. Prieto, J.M. García del Barrio, M.A. Zavala, Land use change in a Mediterranean metropolitan region and its periphery: assessment of conservation policies through CORINE Land Cover data and Markov models, For. Syst. 19 (2010) 315–328.
[26] U. Sahani, N. Behera, Impact of deforestation on soil physicochemical characteristics, microbial biomass and microbial activity of tropical soil, Land Degrad. Dev. 12 (2001) 93–105.
[27] F. Shahbazi, N. Aliasgharzad, S.A. Ebrahimzad, N. Najafi, Geostatistical analysis for predicting soil biological maps under different scenarios of land use, Eur. J. Soil Biol. 55 (2013) 20–27.
[28] E.E. Sigstad, M.A. Bejas, M.J. Amoroso, C.I. García, Effect of deforestation on soil microbial activity. A worm-composite can improve soil quality? A microcalorimetric analysis at 25 °C, Thermochim. Acta 394 (2002) 171–178.
[29] Z. Tan, R. Lal, Carbon sequestration potential estimates with changes in land use and tillage practice in Ohio, USA, Agric. Ecosyst. Environ. 111 (2005) 140–152.
[30] E.D. Vance, P.C. Brookes, D.S. Jenkinson, An extraction method for measuring soil microbial biomass carbon, Soil Biol. Biochem. 19 (1987) 703–704.
[31] L. Vittori Antisari, M.T. Dell'Abate, A. Buscarolli, M. Cherardi, Role of soil organic matter characteristics in a pedological survey: "Bosco Frattona" natural reserve (Site of Community Importance, Italy) case study, Geoderma 156 (2010) 302–315.
[32] J. Wu, R.G. Joergensen, B. Pommering, R. Chaussod, P.C. Brookes, Measurement of soil microbial biomass C – an automated procedure, Soil Biol. Biochem. 22 (1990) 1167–1169.