Eur J Forest Res (2010) 129:73–81
DOI 10.1007/s10342-008-0249-z

ORIGINAL PAPER

Microbiological parameters as indicators of soil organic carbon dynamics in relation to different land use management

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Received: 22 December 2007 / Revised: 13 May 2008 / Accepted: 17 July 2008 / Published online: 4 February 2009

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

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

Communicated by A. Merino and A. Rubio.

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Introduction

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

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

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

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

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

interest for improving our understanding of SOM balance (Riffaldi et al. 1996).

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

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

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

More specifically, the aims of the study were: (a) to assess the sensitivity of the parameters MBC, $C_{K_2SO_4}$, and $C–CO_{2(21d)}$, to changes in land use; (b) to study the effects of land use, tree cover and depth on microbial C mineralization activity; (c) to assess the effect of land use, tree cover and depth on microbial qCO_2.

Methods

Site description

The study was carried out in calcareous moor soils in the region of Castilla y León (north western Spain), UTM: 30T 384465 E 4639001 N. The mean annual rainfall in the region is below 400 mm under a xeric moisture regime, and the mean annual temperature is approximately 12.3°C. The altitude of the moor varies between 800 and 900 m, with low slopes (<7%). The soils are Inceptisol Xerepts, which are quite homogeneous but differ in their land-use history. The native vegetation in the area is the holm-oak wood (*Quercus ilex* subsp *ballota*). In the nineteenth century most of the forest was converted into agricultural land but since the 1950s reforestation with *Pinus halepensis* has been carried out on abandoned agricultural land.

Sampling procedures

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

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

Physical and chemical characterization

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

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

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

Soil respiration and microbial biomass

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

In order to measure microbial respiration, 50 g of moist soil (at 70% of water holding capacity) sample were placed

Table 1 Characteristics of the plots studied

Vegetation	*Quercus ilex*	*Pinus halepensis*
Altitude (m.a.s.l.)[a]	879	885
Basal area (m^2 ha^{-1}	13	50
Density (tree ha^{-1})	3,200	1,184

[a] Meters above sea level

in 0.5 l stoppered glass jars and incubated at 28°C. The CO_2 evolved was collected, after 3, 5, 7, 9, 14, 21, 28, 35, 42, 49, 59, 69, 79, 89, and 98 days of incubation, in 10 ml 0.5 M NaOH and determined by titration with 0.5 M HCl (Alef 1995).

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

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

Statistical analyses

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

Results and discussion

Physical and chemical characterization

The main physical and chemical properties are shown in Tables 2 and 3. The soils studied were similar in texture, and classified as sandy-clay-loam, according to the USDA classification, and contained large amount of coarse fragments (mineral fragments >2 mm). The soils studied also showed a moderately alkaline soil reaction (7.9–8.2), with an electrical conductivity lower than 5 dS/m and a high content of carbonates (15–39%), which showed a

Table 2 Main physical properties of soils sampled under different land use and at different depths, and the results of the analysis of variance

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

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

CL Cropped land, *QFOC* native *Quercus* forest outside tree cover, *QFTC* native *Quercus* forest under tree cover, *PP Pinus* plantation, *Coarse* mineral fragments >2 mm, *HC* soil holding, *BD* bulk density

Table 3 Main chemical properties of soils sampled under different land use and different depths, and the results of the analysis of variance

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

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

CL Cropped land, *QFOC* native *Quercus* forest outside tree cover, *QFTC* native *Quercus* forest under tree cover, *PP Pinus* plantation, *Coarse* mineral fragments >2 mm, *HC* soil holding, *BD* bulk density

significant tendency ($p < 0.05$) to increase with depth. The soil N content, organic C, and C/N ratio were higher in the upper soil layers (Table 3), probably because of the higher input of fresh organic matter at the soil surface. Furthermore, the organic matter content and N availability varied with the land use. The cropped land displayed the lowest content of organic C. This is explained by the low input of organic matter and the losses by tillage. Soil tillage induces soil C loss by acceleration of organic C oxidation, which results in the release of large amounts of CO_2 to the atmosphere (La Scala et al. 2008; Prior et al. 2000; Ellert and Janzen 1999). Another tillage-related factor that contributes to soil C losses is soil aggregate disruption, which exposes once-protected organic matter to decomposition (Grandy and Robertson 2007; De Gryze et al. 2006).

C pools

MBC and $C–CO_{2(21d)}$ were considered as labile fractions of organic matter and were tested as sensitive indicators of land use change. All the profiles showed a decrease in labile C pools with increasing depth (Table 4). The soils under tree cover (pine or holm-oak) presented the highest labile C pools and a sharper depth gradient.

MBC ranged from 11 to 59 μg g^{-1}, and $C_{K_2SO_4}$ from 10 to 23 μg g^{-1} (Table 4). These values are low in comparison with those reported by other authors for non-calcareous soils (Lagomarsino et al. 2006), but are consistent with the values reported by other authors for calcareous soils (García et al. 1997).

Many studies of SOC and land use change use measurements of MBC as early indicators of changes in SOM (Powlson et al. 1987; García-Gil et al. 2000; Palma et al. 2000). However, in the calcareous soils studied, measurements of $C_{K_2SO_4}$ and $C–CO_{2(21d)}$ appeared to be at least as sensitive as MBC measurements (Table 4).

Microbial activity and C mineralization

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

Table 4 Microbial biomass carbon (MBC), mineralized C accumulated in 21 days of incubation ($C–CO_{2(21d)}$), K_2SO_4 extractable C ($C_{K_2SO_4}$) and metabolic quotient (qCO_2) of the soils sampled under different land use and at different depths, and results of analysis of variance

	MBC (μg C g^{-1})	$C–CO_{2(21d)}$ (mg $C–CO_2$ g^{-1})	$C_{K_2SO_4}$ (μg C g^{-1})	qCO_2 (mg $C–CO_2$ g^{-1} C 21 $days^{-1}$)
CL				
0–10 cm	35.6 ± 4.1 ab	0.55 ± 0.01 bcd	13.7 ± 4.4 ab	22.3 ± 1.7
10–20 cm	19.8 ± 3.4 de	0.34 ± 0.06 cde	12.7 ± 2.9 ab	21.9 ± 4.1
20–30 cm	11.1 ± 4.2 e	0.23 ± 0.05 e	10.8 ± 4.6 b	28.4 ± 11.7
QFOC				
0–10 cm	43.3 ± 3.5 ab	0.52 ± 0.04 bc	16.6 ± 1.9 ab	19.1 ± 2.5
10–20 cm	17.0 ± 2.0 de	0.43 ± 0.06 cd	10.8 ± 1.3 b	38.9 ± 9.7
20–30 cm	12.1 ± 3.7 e	0.37 ± 0.06 de	9.9 ± 0.9 b	44.2 ± 13.9
CFTC				
0–10 cm	41.2 ± 8.8 ab	1.07 ± 0.05 a	23.32 ± 2.4 a	39.8 ± 14.1
10–20 cm	24.7 ± 4.8 cde	0.71 ± 0.05 b	13.21 ± 4.2 ab	38.6 ± 8.5
20–30 cm	23.7 ± 2.6 de	0.45 ± 0.02 de	11.46 ± 4.6 ab	26.9 ± 2.6
PP				
0–10 cm	58.9 ± 6.6 a	1.04 ± 0.03 a	23.29 ± 9.9 a	27.7 ± 5.9
10–20 cm	30.4 ± 5.8 bcd	0.72 ± 0.02 b	16.62 ± 4.6 ab	34.2 ± 7.2
20–30 cm	15.3 ± 5.3 cde	0.43 ± 0.05 de	16.90 ± 3.9 ab	40.4 ± 15.6
Analysis of variance				
Land use	n.s.	*	**	n.s.
Depth	***	***	**	n.s.
Land use × depth	***	***	***	n.s.

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

CL Cropped land, *QFOC* native *Quercus* forest outside tree cover, *QFTC* native *Quercus* forest under tree cover, *PP Pinus* plantation

The cumulative mineralized C presented a curvilinear relationship over time, and showed a larger initial release of CO_2 followed-after 21 days incubation- by a slower linear increase throughout the remaining 98 day incubation period (Fig. 1).

The microbial activity decreased clearly with increasing depth in all the profiles studied, in response to the decreasing labile C pools (Agnelli et al. 2004). The soil respiration was higher under than outside tree cover (Table 5). Several studies have shown that tree canopy cover reduces oscillations in soil temperature (Kang et al. 2000) and evaporative water losses (Aguilera et al. 1999) and this influences biological processes in the soil, such as the rate of SOM decomposition of SOM (Paul et al. 2004). However, there were no significant differences between pine and holm-oak cover for C mineralization kinetic parameters (C_o, C_ok) (Table 5). Thus, for these calcareous soils, the effect of tree cover on soil mineralization appears to be independent of the tree species (pine or holm-oak).

In the soils studied the C_o ranged from 0.6 to 1.3 mg C g^{-1} of soil (Table 5), values that are low in comparison with those reported by Goberna et al. (2006) for Calcixerolls in the semiarid Mediterranean region and under different types of land use, or by Fernández et al. (1999), for Cambisols under pine forest located within a humid temperate zone, but consistent with those reported by Baldock and Skjemstad (2000), who revealed the role of carbonates in organic C stabilization against microbial attack.

C mineralization rates (k) ranged from 0.043 to 0.078 (Table 5), but no regular trends were observed with regard to these parameters. These values are similar to those reported by Lagomarsino et al. (2006) on loam soils within a Mediterranean region but lower than those reported by Fernández et al. (1999) for Cambisols within a humid temperate zone. The differences in C mineralization rates among different areas of study may be explained by differences in mean precipitation rates. Turrión et al. (2001) showed that mean annual precipitation affects microbial activity.

C_ok may be a more precise estimate than the individual parameters examined (Saviozzi et al. 1993). A regular depth-related pattern towards less degradable substrate was observed throughout all profiles, as indirectly indicated by

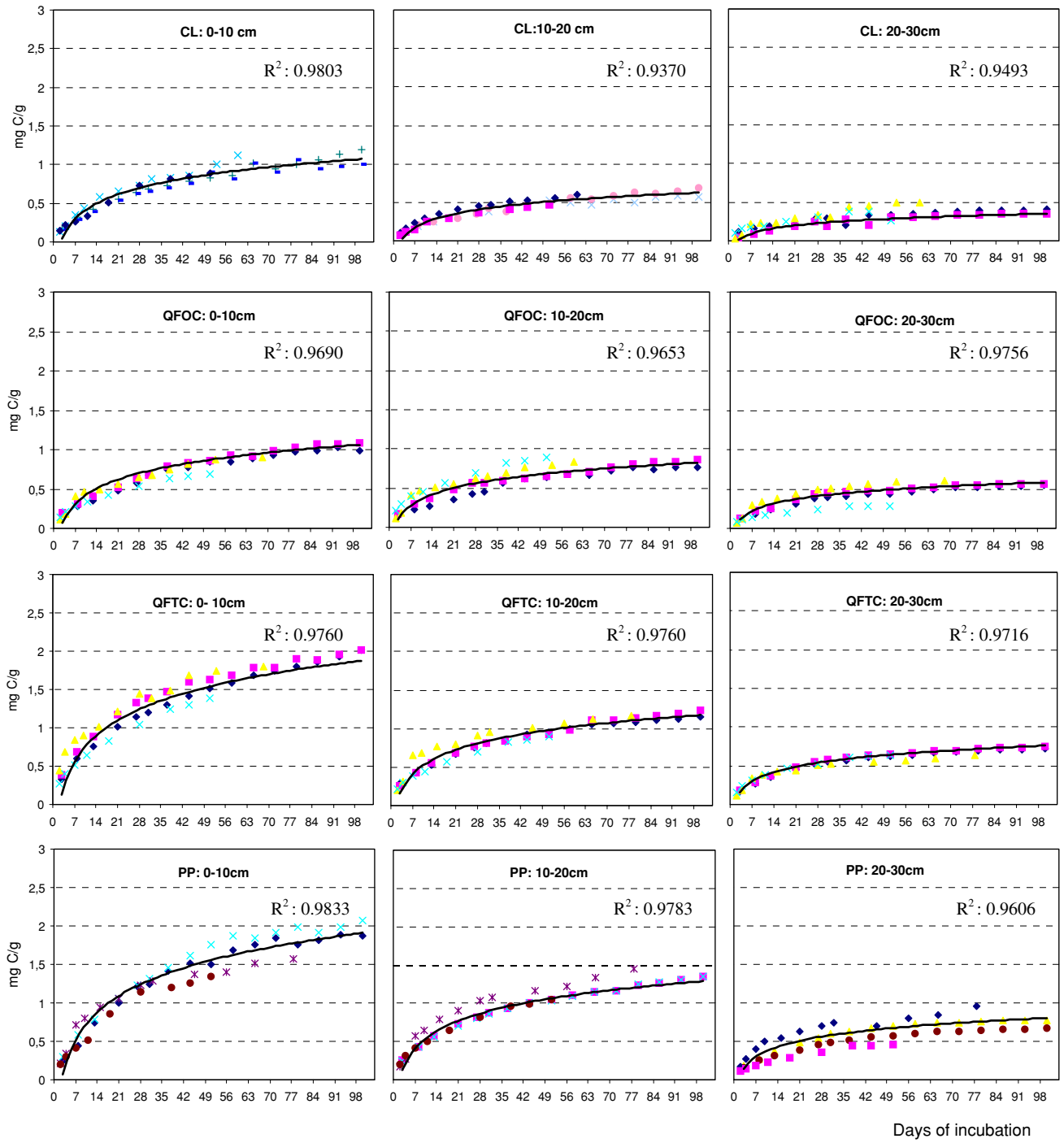

Fig. 1 Cumulative curves of the C mineralization during 98 days of incubation and the first-order equation ($C_m = C_o(1 - e^{-kt})$) fitted to the experimental curve. *CL* Cropped land; *QFOC* native *Quercus* forest outside of tree cover; *QFTC* native *Quercus* forest under tree cover; *PP Pinus*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

the decrease in the initial potential mineralization rate ($C_o k$) (Table 5). The deeper layers of the soil profile are enriched with progressively more recalcitrant products because microorganisms use easily degradable substrate (Dell′Abate et al. 2002).

Principal components analysis

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

Table 5 Parameters of microbial mineralization activity of soils sampled under different land use and at different depths, estimated according to the first-order equation and analysis of variance

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

Means and standard error for $n = 4$. Values of n.s., $p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ are reported. Values with the same letters are not significantly different ($p < 0.05$) for the interaction land use × depth

CL Cropped land, *QFOC* native *Quercus* forest outside tree cover, *QFTC* native *Quercus* forest under tree cover, *PP* *Pinus* plantation, *Coarse* mineral fragments >2 mm, *HC* soil holding, *BD* bulk density

The PCA carried out with 11 selected variables (C_o, C–$CO_{2(21d)}$, organic C content, total N content, WHC, percentage of clay, $C_o k$, $C_{K_2SO_4}$, C/N ratio, bulk density, qCO_2) identified two components (PC1 and PC2) that accounted for 80.3% of the total variance, with most of the variation explained by PC1 (67.5%) (Table 6).

PC1 was mainly related to depth and tree cover (Fig. 2). The most heavily weighted variables were those related to availability and lability of substrate at its positive extreme, and bulk density and clay content at the negative extreme. Differences in mineralization parameters may be attributed to differences in physical protection of SOM (Hassink 1997). The quantity of soil clay is believed to be one of the main factors that affect the capacity of soil to stabilize organic carbon. This stabilizing effect has been partly ascribed to adsorption of organic substances onto clays (Golchin et al. 1994).

The most heavily weighted variables in PC2 were qCO_2 at the positive extreme, and microbial biomass C at the negative extreme. PC2 was mainly associated with the interaction between land use and depth ($p < 0.001$) (Fig. 2). The metabolic quotient (qCO_2) is an index of microbial efficiency in the utilization of C resources (Anderson 2003; Moscatelli et al. 2005). Greater efficiency results in a low metabolic quotient (Xu et al. 2006). We found, in accordance with Paul and Clark (1989) and Margalef (1974), that micro-organisms were more efficient at utilizing C resources in soils in which less substrate was available.

Table 6 Principal components analysis

Variables	PC 1	PC 2
Co	0.975	0.132
C-$CO_{2\ (21d)}$	0.969	0.172
Organic C	0.953	−0.037
Total N	0.896	−0.192
WHC	0.850	−0.044
Clay	−0.845	−0.127
Cok	0.827	0.364
MBC	0.812	−0.481
$C_{K_2SO_4}$	0.780	−0.170
C/N	0.760	0.142
BD	−0.734	0.373
qCO_2	−0.064	0.935

Variable weightings in two principal components

Co potentially mineralizable C, *C-$CO_{2(21d)}$*, accumulated mineralized C in 21 days, *Organic C* organic C content, *Total N* total N content, *WHC* water holding capacity, *Clay* percentage of clay, *Cok* initial potential rate of C mineralization, *MBC* microbial biomass C; $C_{K_2SO_4}$, K_2SO_4 extractable C; C/N, C/N ratio; BD, bulk density; qCO_2, metabolic quotient

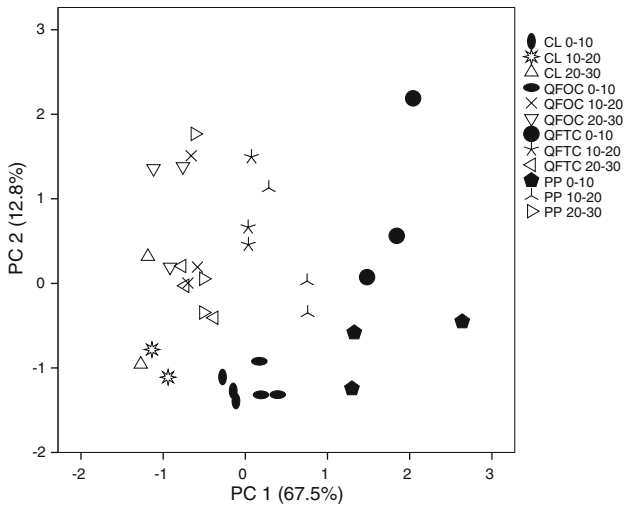

Fig. 2 Sample distribution based on PCA factor scores. The variation explained by each PC is given in *brackets*. *CL* Cropped land; *QFOC* native *Quercus* forest outside of tree cover; *QFTC* native *Quercus* forest under tree cover; *PP Pinus* plantation

Conclusions

In the calcareous soils under study, microbial biomass and activity clearly declined with increasing depth and showed low values in comparison with non-calcareous soils. The parameters $C_{K_2SO_4}$ and C-$CO_{2(21d)}$ appeared to be as sensitive indicators of land use changes as microbial biomass C.

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

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

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

Acknowledgments The authors thank Dr. Lafuente, Dr. Ruiperez, David Llorente, and Maria Varas for their assistance. This project was funded by the *Junta de Castilla y León*, Spain.

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