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Soil Biology & Biochemistry 34 (2002) 635–642

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Effect of plant cover decline on chemical and microbiological parameters under Mediterranean climate

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Received 13 October 2001; received in revised form 23 October 2001; accepted 19 November 2001

Abstract

The uses of many of the soils of Central Spain have changed and these changes have led frequently to the decline of natural plant cover. In this paper we report on the organic matter fractions and the microbial activity of soils developed from different substrates (limestone and granites) in relation to plant cover decline. Specific indicators of microbial activity (microbial biomass carbon (MBC), basal respiration, and some oxidoreductase and hydrolase activities) were measured. These indicators decreased in value in both limestone and acid soils where plant cover had declined (from climax, tree forest, to low bush); a similar effect was observed in the labile organic matter fractions (water soluble carbon (WSC), water soluble carbohydrates and polyphenolic compounds). For some measures of microbiological activity (e.g. MBC and soil respiration) the differences in acid soils were lower than in limestone soils. For example, MBC values in limestone soils ranged from 1426 ngC g^{-1} in tree forest to 498 ngC g^{-1} in low bush; while in acid soils, these values ranged from 428 ngC g^{-1} in tree forest to 265 ngC g^{-1} in low bush. Acid soils, unlike the limestone soils, showed no relationship between plant cover decline and hydrolase activities related to the N cycle (urease and protease). In general, soil microbial activity was negatively affected by plant cover degradation. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Organic matter fractions; Microbiological properties; Plant cover degradation; Enzyme activities; Mediterranean climate

1. Introduction

In many areas of Central Spain, changes in land use have led to the disappearance of holm oaks and the decline of plant cover in general. As degradation takes place some soil properties also change, particularly soil microbial activity. It is widely accepted that high levels of microbial activity are fundamental in maintaining soil quality. The microbial biomass is responsible for the nutrient cycling and the development and functionality of the soil system (Smith and Papendick, 1993). Microorganisms play a fundamental role in carrying out biogeochemical cycles in soil and in the formation of soil structure (Roldan et al., 1994a). Due to the complex dynamics of soil ecosystems, no single property is satisfactory for studying microbial activity. However, there is growing evidence that soil microbiological and biochemical parameters (microbial biomass carbon (MBC), enzyme activities and labile organic matter) may be used as early and sensitive indicators of soil ecological stress or restoration (Dick, 1997), and soil microbial activity in Mediterranean climates (Garcia et al., 1997a).

Previous studies on degraded soils in the Spanish Mediterranean area have clearly shown their very low physical quality (Roldan et al., 1994b). Among the many factors which are implicated in the control of soil biogeochemical cycles, microbiological and biochemical parameters have particular importance in semiarid areas, where degradation of the plant cover has a direct bearing on the scarcity of organic matter (Garcia et al., 1994), which is considered as the principal precursor of sustainability.

Several microbiological parameters have been used to describe the status and sustainable development of soil productivity in agricultural and natural ecosystems (Visser and Parkinson, 1992). Enzyme activity in the soil environment is considered to be a major factor contributing to overall soil microbial activity (Visser and Parkinson, 1992). Soil phosphatase activity has often been used in attempts to describe soil microbial activity (Garcia et al., 1994), while other methods include soil MBC and N, basal respiration and ATP content (Nannipieri et al., 1990). Several studies on the relationship between soil microbial activities and

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PII: S0038-0717(01)00225-5

Table 1
Characteristics of the sampling site

Soils	Locality	Altitude (m)	Soil type (FAO, 1998)	Vegetation (Peinado and Rivas-Martinez, 1987)	Plant cover (%)
L1	Torrelaguna	870	Calcaric Cambisol	Junipero oxycedri–Quercetum rotundifoliae, sbs Quercetosum faginae (tree forest)	85
L2	Torrelaguna	880	Rendzic Leptosol	Cephalantero rubrae–Quercetum faginae (tree forest, lower density than L1)	72
L3	Torrelaguna	900	Calcaric Cambisol	Rhamno lycioidis–Quercetum cocciferae (shrub forest)	64
L4	Torrelaguna	960	Eutric Cambisol	Cytiso scoparii–Retametum sphaerocarpae (high bush)	42
L5	Torrelaguna	800	Lithic Leptosol	Lino differentis–Salvietum lavandulifoliae (low bush)	44
L6	Torrelagu-na	850	Lithic Leptosol	Arrenathero baetici–Stipetum tenacissimae (sparse low bush)	30
G1	La Cabrera	1200	Distric Cambisol	Junipero oxycedri–Quercetum rotundifoliae (tree forest)	58
G2	La Cabrera	1050	Distric Cambisol	Cytiso scoparii–Genistetum floridae, facies Adenocarpus complicatus (high bush)	51
G3	La Cabrera	1060	Distric Cambisol	Cytiso scoparii–Genistetum floridae (sparse high bush)	38
G4	El Berrueco	1100	Distric Leptosol	Rosmarino officinalis–Cistetum ladaniferi (low bush)	42
G5	La Cabrera	1050	Chromic Alisol	Rosmarino officinalis–Cistetum ladaniferi (sparse low bush)	28

plant community succession have been carried out (Ross and Tate, 1993); these authors indicate that MBC and N decreased by about 50% from viz. red beech (*Nothofagus fusca*) to silver beech (*N. Menziesii*).

Field studies to ascertain microbial activity have been carried out on agricultural soils, and also, on abandoned agricultural soils (bare soil) (Garcia et al., 1994). However, microbial activity in relation to disturbed soils with different types of plant cover in Mediterranean climate, which is the objective of this paper, has not been studied. Such studies can provide a better understanding of the processes occurring in soil following the degradation of plant cover, and may be used as a tool for restoration programs.

2. Materials and methods

The study was conducted in the Sierra de Guadarrama, Madrid Province (Central Spain). The climate in this area is predominantly Mediterranean, with dry hot summers and cold winters. The annual mean temperature is 11 °C and total annual rainfall ranges from 700 to 900 mm, mostly concentrated in autumn and spring. It is possible to find a great variety of vegetation types suffering from different degrees of degradation, which is mainly attributed to traditional land uses (clearcuts and grazing). The area has undergone progressive depopulation and land uses are changing from agro-pastoral to other practices such as tourism and hunting. For this study, 11 sampling sites were selected. The soils of sites L1–L6 located near the village of Torrelaguna (UTM: 4.55 and 45.20) are developed from limestones and were chosen to cover a complete range of vegetation types from the climax (L1 and L2) to the most degraded (L6). Plant cover decline implies a change in species composition, as well as a decrease in biodiversity, biomass production and the percentage of soil cover. Sites G1–G5 are located between the villages of La Cabrera (UTM: 4.48 and 45.24) and El Berrueco (UTM: 4.53 and 45.27). The soils in this zone are developed from granitic materials, which implies (in this region) different vegetation types and less degradation than is observed in soils developed from limestone. Site locations, predominant soil types and vegetation types are given in Table 1.

2.1. Sampling procedure

Composite soil samples of the top 15 cm were randomly collected from six different places in each site. Sampling was carried out in spring because soil microbial activity in the Mediterranean climate is higher in this season (Garcia et al., 1997a). Each soil sample represented six 150 cm^3 subsamples, which were thoroughly mixed to obtain a composite sample. The samples were brought to the laboratory on the same day and kept in the refrigerator at 4 °C until they were analyzed.

Table 2
Chemical properties of soils developed on limestone (L) or granite (G) supporting different level of plant cover (for description of the sampling site and soil type, see Table 1. In parenthesis, standard deviation for $n = 6$, for LSD values, $p < 0.05$)

Soils	TOC ($g\ kg^{-1}$)	pH	EC ($dS\ m^{-1}$)
L1	41.7 (3.1)	7.41 (0.11)	257 (21)
L2	38.2 (2.6)	7.65 (0.09)	226 (18)
L3	37.6 (3.0)	7.46 (0.10)	299 (20)
L4	8.6 (0.6)	7.89 (0.10)	179 (16)
L5	15.1 (1.1)	7.89 (0.12)	149 (22)
L6	12.6 (1.1)	7.96 (0.15)	157 (19)
G1	14.2 (1.5)	5.56 (0.10)	62 (12)
G2	11.1 (0.9)	6.04 (0.16)	33 (8)
G3	8.9 (1.0)	6.21 (0.12)	22 (9)
G4	4.1 (0.6)	6.65 (0.20)	22 (10)
G5	6.2 (0.6)	6.44 (0.09)	23 (12)
LSD	± 2.1	± 0.12	± 29

2.2. Chemical analysis

Electrical conductivity (EC) was measured in a 1:5 (w/v) aqueous solution. Total N and total organic carbon (TOC) were determined by pre-treatment with HCl to eliminate carbonates followed by combustion at 1020 °C and measurement in a Carlo Erba Elemental Analyzer. Total P and K were determined in the nitric–perchloric digestion extract. Available P was extracted with sodium bicarbonate (Olsen et al., 1954) and was determined colorimetrically following the Murphy and Riley (1962) method. Extractable K was extracted with ammonium acetate (exchangeable K) and was determined by the flame photometry.

The following parameters were determined in an aqueous extract of soils (1:5 solid–liquid ratio), obtained after 2 h of mechanical shaking, centrifugation at 5000*g* and filtration through a 100 μm membrane: water soluble carbon (WSC) by oxidation with $K_2Cr_2O_7$ and measurement of absorbance at 590 nm (Sims and Haby, 1971); soluble carbohydrates by the method of Brink et al. (1960) and soluble polyphenol compounds by the Kuwatsuka and Shindo (1973) method.

2.3. Microbiological analysis

MBC was determined using a fumigation-extraction procedure (Vance et al., 1987). The 0.5 M K_2SO_4 extracted C was measured as indicated for WSC. Basal respiration was determined in 50 g dry soil placed in hermetically sealed flasks, moistened at 50% of its water holding capacity and incubated in the dark at 28 °C. CO_2 released was measured by IR CO_2 detector. The metabolic quotient (qCO_2) was calculated by dividing the C–CO_2 released from the sample in 1 h by the MBC content. The MBC to TOC ratio (MBC/TOC) was also calculated.

2.4. Enzymatic assays

Dehydrogenase activity. Dehydrogenase activity was determined by the Skujins' (1976) method modified by Garcia et al. (1997b). Soil (1 g) at 60% of its field water holding capacity was treated with 0.2 ml of 0.4% INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) in distilled water for 20 h at 22 °C in darkness. The INTF (iodo-nitrotetrazolium formazan) formed was extracted with 10 ml of a mixture of 1:1.5 ethylene/chloride acetone by shaking vigorously for 1 min and filtering through a Whatman No. 5 filter paper. Iodo-nitrotetrazolium formazan (NTF) was measured spectrophotometrically at 490 nm.

Urease activity. Two ml of pH 7 phosphate buffer and 0.5 ml of 6.4% urea were added to 0.5 g of soil and then the mixture was incubated at 30 °C for 90 min and the volume was made up to 10 ml with distilled water. The ammonium released after addition of 0.1 ml 10 M NaOH was measured using an ammonium selective electrode (CRISON micro pH 2002). A control without urea was used with each sample (Nannipieri et al., 1980).

Protease activity on N-α benzoyl-L-argininamide (protease–BAA). Two ml of phosphate buffer (pH 7) and 0.5 ml of 0.03 M *N*α-benzoyl-L-argininamide (BAA) substrate were added to 0.5 g of soil. The mixture was incubated at 37 °C for 90 min and then diluted to 10 ml with distilled water. The ammonium released was measured in the same way as for urease (Nannipieri et al., 1980).

Phosphatase activity. Two ml of 0.1 M maleate buffer (pH 6.5) and 0.5 ml of 0.115 M *p*-nitrophenyl phosphate (PNPP) were added to 0.5 g of soil and incubated at 37 °C for 90 min. The reaction was stopped by cooling to 2 °C for 15 min and then 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH were added to the soil mixture before centrifugation at 4000 rpm for 5 min. The *p*-nitrophenol (PNP) formed was determined in a spectrophotometer at 398 nm (Tabatabai and Bremner, 1969). Controls were made in the same way, although the substrate was added before the $CaCl_2$ and NaOH.

β-glucosidase activity. Two ml of 0.1 M maleate buffer (pH 6.5) and 0.5 ml of 50 m M *p*-nitrophenyl-β-D-glucopyranoside (PNG) were added to 0.5 g of soil. Then the same procedure reported for phosphatase assay was followed, except that the NaOH was substituted by Buffer Tris.

2.5. Statistical analysis

Statistical analysis (ANOVA analysis and test LSD for mean) was performed with the STATGRAPH program.

3. Results

3.1. Chemical parameters

All the limestone soils studied had a pH between 7 and 8 (Table 2), the highest values being shown by those supporting the most degraded vegetation (L4 high shrub, L5 and L6 low shrub). These latter soils also had the lowest EC values. The granitic soils studied showed acidic pH values (Table 2)

Table 3
Nitrogen and phosphorous contents in soils developed on limestone (L) or granite (G) supporting different level of plant cover (for description of sampling sites and soil types, see Table 1. In parenthesis, standard deviation for $n = 6$, for LSD values, $p < 0.05$)

Soils	Total N (g kg^{-1})	N–NH_4^+ (mg kg^{-1})	N–NO_3^- (mg kg^{-1})	Total P (mg kg^{-1})	Extr. P (mg kg^{-1})
L1	0.46 (0.10)	17.2 (3.1)	17.9 (3.1)	575 (38)	25 (4.2)
L2	0.28 (0.08)	9.4(0.6)	10.6 (2.2)	547 (43)	9 (2.3)
L3	0.43 (0.07)	14.3 (2.8)	12.3 (1.9)	677 (53)	7 (2.0)
L4	0.08 (0.01)	6.2 (1.1)	4.9 (1.0)	491 (26)	7 (1.2)
L5	0.16 (0.02)	7.2 (2.0)	5.8 (1.2)	603 (46)	9 (1.9)
L6	0.28 (0.03)	7.3 (1.4)	6.2 (1.4)	826 (59)	6 (1.1)
G1	0.09 (0.01)	10.4 (2.2)	18.5 (3.1)	603 (34)	6 (0.5)
G2	0.06 (0.01)	5.1(1.0)	6.6 (0.9)	399 (41)	5 (1.0)
G3	0.09 (0.02)	3.9 (1.0)	7.4 (1.7)	380 (28)	7 (0.7)
G4	0.04 (0.01)	1.8 (0.4)	11.5 (2.3)	427 (34)	6 (0.9)
G5	0.08 (0.01)	6.0 (1.5)	9.4 (1.9)	339 (31)	5 (0.4)
LSD	± 0.03	± 2.4	± 2.1	± 55	± 2

and their EC values were significantly lower than that of limestone soils.

As far as the soils formed on the limestone substrate is concerned, the TOC values were highest in the soils with a less degraded plant cover (L1 and L2 tree forest, and L3 shrub forest) (Table 2). TOC contents of the granitic soils (G1–G5) were much lower than those of the limestone soils regardless of the state of the plant cover (Table 2).

Total N behaved in a similar way as TOC. However, neither the available N fractions, nor the total and extractable P and K values showed any clear tendency, regardless of the type of substrate or state of plant cover (Table 3).

The water soluble organic C fraction is a labile part of the organic matter together with the light fraction of the organic matter and MBC. This fraction consists of a heterogeneous mixture of varying molecular weight components, such as mono and polysaccharides, polyphenols, proteins and low molecular weight organic acids. The water soluble organic C is markedly affected by the degradation of the plant cover caused by anthropogenic activity. Indeed, the greater the degradation, the lower the content of labile organic matter fractions (Table 4). This is particularly evident in limestone soils, where L1–L3, showed the highest values. In the case of granitic soils, too, the soils with the least degraded plant cover showed higher labile carbon fractions than the others.

Table 4
Labile carbon fractions of soils developed on limestone (L) or granite (G) supporting different level of plant cover (for description of sampling sites and soil type, see Table 1. In parenthesis, standard deviation for $n = 6$, for LSD values, $p < 0.05$)

Soils	WSC (mg kg^{-1})	Carbohydrates soluble (mg glucose kg^{-1})	Water soluble polyphenols (mg phenol kg^{-1})
L1	785 (118)	92 (15)	143 (12)
L2	611 (93)	77 (14)	80 (21)
L3	486 (85)	95 (11)	64 (14)
L4	259 (24)	55 (9)	51 (17)
L5	252 (18)	36 (6)	49 (12)
L6	248 (16)	23 (8)	39 (13)
G1	862 (123)	145 (15)	101 (15)
G2	464 (26)	133 (11)	87 (21)
G3	341 (39)	55 (9)	62 (12)
G4	223 (23)	43 (8)	81 (14)
G5	293 (20)	55 (15)	80 (16)
LSD	± 128	± 21	± 24

3.2. Microbiological parameters

MBC content of limestone soils was lower when the plant cover was less developed (sites L4–L6, low shrub see Table 5). In the same way, in the granitic soils, the highest values of MBC were found in G1 and G2, where there was a lower degree of plant cover degradation. In general, smaller differences in MBC values existed among the acid soils than among the limestone soils (Table 5).

Soil microbial respiration is an useful index for measuring soil microbial activity (Nannipieri et al., 1990). As in the case of MBC, the limestone soils with the greatest degree of plant cover degradation (low bushes of broom, esparto grass and thyme) showed the lowest basal respiration values (Table 5). In the acid soils studied, differences in basal respiration were only observed between those with a cover of holm oak (G1) and the rest (Table 5).

Microbial activity can be assessed by a certain number of parameters, some of which depend on the activity of the overall community whereas others are related to specific members of that community. Most of these parameters are not related to microbial population size but to the activity or function of these populations. qCO_2 provides a method to relate both the size and activity of soil microbial populations (Anderson and Domsch, 1993). Table 5 lists the values obtained for qCO_2, all of which were between 1.68 and 7.49. The highest differences in this parameter were seen in acid soils: qCO_2 decreased with the plant cover decline.

3.3. Enzyme activities

Although limestone soils did not show great differences in dehydrogenase activity, the lower values were found in

Table 5
Microbiological parameters and dehydrogenase activity of soils developed on limestone (L) or granite (G) supporting different levels of plant cover (for description of sampling sites and soil type, see Table 1. In parenthesis, standard deviation for $n = 6$, INTF: Iodo nitrotetrazolium Formazan; unit qCO_2, ng C–CO_2 mg C mic g^{-1} h^{-1}, for LSD values, $p < 0.05$)

Soils	MBC (μgC g^{-1} Soil)	Dehydrogenase activity (ng INTF g^{-1} Soil)	Bio C × 100/TOC	Basal respiration (μg C–CO_2 g^{-1} d^{-1})	qCO_2
L1	1426 (153)	152 (11)	3.42	126 (18)	3.68
L2	1015 (80)	150 (9)	2.66	118 (14)	4.84
L3	777 (133)	153 (7)	2.06	98 (12)	5.25
L4	486 (83)	77 (14)	5.65	32 (9)	2.74
L5	589 (106)	103 (12)	3.90	54 (12)	3.82
L6	498 (60)	87 (14)	3.95	34 (9)	2.84
G1	428 (47)	62 (79)	3.01	77 (15)	7.49
G2	438 (52)	47 (5)	2.96	33 (6)	3.10
G3	341 (28)	48 (7)	3.80	14 (5)	1.71
G4	247 (20)	38 (6)	6.02	10 (3)	1.68
G5	265 (21)	36 (10)	4.00	18 (5)	2.83
LSD	± 95	± 10		± 11	

zones with reduced vegetation (L4–L6, low shrub, Table 5), indicating that the metabolic activity of the microorganisms is greater when the plant cover is not declined. The maximum value for dehydrogenase activity in limestone and acid soils was found in G1 and L1, tree forest. This parameter does not differ in soils covered by matorral when they are acidic. Urease activity is agronomically important because it hydrolyses urea. In the limestone soils, urease and protease which hydrolyses *N*-α-benzoyl-L-argininamide activities (both enzymes of the N cycle) showed high values when forest was the predominant cover and were minimum at site L6 where the vegetation was sparse low bush (Table 6). The enzymes of the N cycle showed a different behavior in the granitic soils, higher values being observed in soils covered by matorral than in soils with holm oak (Table 6).

Phosphatase catalyses the release of inorganic phosphorus from organic-bound phosphorus returned to soil as litter and other organic debris, and this activity plays a important role in the phosphorus cycling. The lowest phosphatase activities were observed in the soil with reduced plant cover (Table 6). β-glucosidase catalyses the hydrolysis of the non-reducing terminal ends of β-glucosides, with the release of glucose. The soils with the most degraded plant cover showed the lowest β-glucosidase activity (Table 6).

4. Discussion

4.1. Chemical parameters

The effect of the original parent material on some chemical parameters was greater than that of the vegetation. Therefore, soils in similar conditions and with a similar vegetation (L1 and G1, tree forest) had different pH and EC values. According to Garcia and Hernandez (1996), salinity has a strong negative effect on the microbial activity in these soils, especially when the salinity is due to chlorides as opposed to sulphates. However, the salinity of these soils did not decrease the microbial activity probably because the salinity levels registered are far from those which can inhibit microbial activity (380 dS m^{-1}, Garcia and Hernandez, 1996).

Changes in soil use lead to changes in vegetation and gradual degradation, which in turn affects both quality and quantity of soil organic matter. Spanish soils in a Mediterranean climate lose quality due to a gradual decrease of their organic matter content and microbial activity (Garcia et al., 1994). Percentage of plant cover could also be responsible for this behavior: this percentage in L1 (limestone soil) was 85% while in G1 (acid soil) the vegetation cover was 58% (Table 1). Acidic soil conditions can inhibit plant growth, leading to a lower input of plant residues in the soil, which means lower organic C inputs to soil and lower soil organic matter content (TOC).

According to Van Veen et al. (1985), soils with a lower plant cover (and in our case, with lower labile organic matter fractions) do not have a higher potential microbial activity because the labile organic matter fractions express the ecosystem's potential to support an energy load sufficient for microbial activity. Low biodegradability of dissolved (WSC) organic C in an oak-forest floor has been observed by Qualls and Haines (1992). However we have found a good correlation between MBC and WSC (0.667, $p < 0.001$), indicating that the labile carbon fractions can sustain the level of microbial biomass in soil. According to De Luca and Keeny (1993), who defined the water-soluble carbohydrate content as a reflection of the microbial activity of soils, plant cover degradation induces a decrease in microbial activity. The original parent material had a strong influence on the labile organic fractions: acid soils which showed lower input of residues to soil (lower percentage of

Table 6
Hydrolase activities of soils developed on limestone (L) or granite (G) supporting different level of plant cover (for description sampling sites and soil type, see Table 1. In parenthesis, standard deviation for $n = 6$, PNP: para nitrophenol, for LSD values, $p < 0.05$)

Soils	Uease activity (μmol NH_3 g^{-1} h^{-1})	Protease BAA activity (μmol NH_3 g^{-1} h^{-1})	Phosphatase activity (μmol PNP g^{-1} h^{-1})	β-glucosidase activity (μmol PNP g^{-1} h^{-1})
L1	1.61 (0.12)	1.65 (0.08)	0.29 (0.02)	0.28 (0.01)
L2	0.77 (0.07)	0.92 (0.07)	0.28 (0.01)	0.17 (0.01)
L3	0.61 (0.03)	0.44 (0.04)	0.21 (0.01)	0.15 (0.01)
L4	0.11 (0.01)	0.16 (0.01)	0.10 (0.01)	0.09 (0.01)
L5	0.23 (0.06)	0.39 (0.01)	0.14 (0.01)	0.13 (0.01)
L6	0.10 (0.06)	0.14 (0.01)	0.12 (0.01)	0.13 (0.01)
G1	0.34 (0.01)	0.35 (0.03)	0.36 (0.03)	0.05 (0.01)
G2	1.73 (0.50)	0.51 (0.03)	0.27 (0.03)	0.05 (0.01)
G3	1.35 (0.03)	0.58 (0.02)	0.16 (0.02)	0.05 (0.00)
G4	0.66 (0.07)	0.13 (0.04)	0.11 (0.02)	0.03 (0.01)
G5	0.59 (0.01)	0.56 (0.04)	0.13 (0.02)	0.04 (0.01)
LSD	± 0.16	± 0.10	± 0.06	± 0.02

plant cover, Table 1) also showed lower values of these organic fractions.

4.2. Microbiological parameters

According to Powlson et al. (1987), microbial biomass is a sensitive index of changes in the organic C content of soils. Plant cover decline affected the MBC. A degraded plant cover means a lower soil organic matter content (Table 2) and so the microorganisms have less organic matter to decompose, resulting in lower basal respiration values. In agreement with Polwson et al. (1987), we have found in this study a significant correlation between TOC and MBC (0.87, $p < 0.0001$).

The MBC/TOC ratio is a useful measure for monitoring the soil organic matter and provides a more sensitive index that organic carbon measured alone. In our study, this ratio showed values of 2–6% (Table 5). MBC usually constitutes 1–5% of TOC and absolute values for this ratio differ markedly between soils and are greatly influenced by the organic matter content (Sparling, 1992; Brookes, 1995). In the soils studied here, a higher ratio is seemed to be associated with a more declined plant cover (Table 5). This could seem to be in disagreement with the values of soil respiration observed (Table 5) which were higher in the soils with lower plant cover decline. In fact, the latter soils also showed higher MBC contents but the higher input of C in these soils from plant remains leads to a lower MBC/TOC. One factor that affects the MBC/TOC is that MBC can show marked seasonal changes. In our case this was not important since all the samples were collected in spring, which, as mentioned above, is the season with the highest microbial activity under Mediterranean conditions (Garcia et al., 1997a). However, other factors arising from the method which is used to estimate MBC (preincubation of soil samples, wet, sieving) might cause some alterations in this ratio (Tate et al., 1991).

In general, qCO_2 tended to be lower as degradation of plant cover was more pronounced, perhaps because when the plant cover is degraded there is a reduction in specific respiration due to reduction of the water soluble substrates directly available to the microorganisms. According to Santruskova and Straskava (1991) a high value is associated with immature ecosystems. A positive correlation existed between qCO_2 and the labile carbon fractions (WSC: 0.693, $p < 0.001$; water soluble carbohydrates: 0.792, $p < 0.001$), indicating that the specific respiration is strongly influenced by the content in the soil of compounds which can be used as an energy resource by microorganisms. qCO_2 values may also be influenced by soil clay content. So, in cultivated soils Chausod et al. (1986) observed an inverse relationship between specific respiration (qCO_2) and clay content in cultivated soils. However, we did not observe any influence of soil clay content on qCO_2 values when a variety of different soils under Mediterranean climate was studied (Garcia et al., 1994). Ross and Tate (1993) also observed similar values for qCO_2 in soils with different clay contents.

4.3. Enzyme activities

Soil enzymes are biological catalysts of specific reactions and these reactions, in turn, depend on a variety of factors. Numerous studies on enzymes exist and comparisons have been made in various soils subjected to different climatic conditions and management practices (Burns, 1978; Ladd, 1985; Garcia et al., 1997b).

Dehydrogenase activity has been used to assess microbial activity, although this use has been criticized by different authors; Benefield et al. (1977) indicated that dehydrogenase activity is not an accurate parameter for determining the electron flow rate to O_2 because the electron acceptors used in the dehydrogenase assays are less efficient than O_2. However, Garcia et al. (1997c) found that dehydrogenase

activity is a good index of the status of soil microbial activity in semiarid Mediterranean areas. Enzymes involved in the dehydrogenase assay are mainly intracellular, so that a correlation between dehydrogenase and oxygen uptake or CO_2 release by bacterial population is expected. In fact, dehydrogenase has been widely used to measure the catabolic activities in soil, which are correlated with microbial activity (Skujins, 1976) and to compare soils under different crops, as well as natural and cultivated soils (Masciandaro et al., 1998).

The type of substrate affected the dehydrogenase activity since the values in acid soils were a third of those recorded in limestone soils. The higher percentage of plant cover in limestone substrate soils (and the plant residues returned to soil) can be responsible for this fact. The highest dehydrogenase activity was recorded in the less degraded forest soil, which varied significantly from the more degraded ones. Dehydrogenase activity showed a significant correlation with the content of TOC (0.896, $p < 0.0001$), and MBC (0.951, $p < 0.0001$), although other authors as Sparling (1992) found no correlation between dehydrogenase and microbial biomass.

Plant cover decline is linked with changes in the N cycle, because the values of urease and protease activities fell in soils with plant cover decreased. The synthesis of urease in soil has been studied by McCarthy et al. (1992); they found that the metabolite derived by NH_4^+ immobilization inhibited urease synthesis. In our case, decrease of urease activity was not related to the content of NH_4^+ (Table 3). The type of plant residues returned to soil as well as root exudates influence these values. Nitrogenated substrates that induce the synthesis of these enzymes in soils from granite may be produced in greater quantity when the plant cover is a matorral.

The variations in phosphatase activity of forest system may be due to variations in plant species composition (Beck, 1974). The low values of phosphatase activity in limestone soils with degraded plant cover (Table 6) could be related to the quantity of available inorganic P, which may inhibit the synthesis of this enzyme (Nannipieri et al., 1990), although in our case, the inorganic P content in the soils was not high (Table 3). The higher phosphatase activity found in non-disturbed soils covered by holm oak may be attributable to the depletion of available P by soil microorganisms and/or the adsorption of Pi, which lead to the synthesis of phosphatase by the soil microbial population as they attempt to obtain P from less readily available organic sources.

Alkaline soils have shown markedly lower phosphatase activities when assayed at pH < 8 compared to acid soils. In our study, the phosphatase activity observed in alkaline soils was higher than that of the acid soils; hence, the type of vegetation can greatly affect the activity of that enzyme. There are many studies that indicate no correlation between enzymes and vegetal cropping in agrosystems (Dick, 1997), but in natural systems, these enzymes are able to inform about ecology of plant succession or plant productivity.

The scarcity of plant remains under degraded vegetal cover is probably responsible for the absence of β-glucosidase activity when plant cover decline is produced. This is in agreement with Hayano and Tubaki (1985), who indicated that a lower quantity of plant residues contributed to a lesser degree of enzyme synthesis.

It is clear that the decline of plant cover markedly affect soil microbial parameters. The high concentration of various soil enzymes such as dehydrogenase, urease or phosphatase in the sites with a more mature plant cover than in the more degraded sites would be due to higher organic carbon levels and microbial population numbers in the former soils. The reduced microbial activity in the soils with degraded plant cover may lead to lower mineralization and hence to a slower successional process. Parent materials have a great influence on organic carbon fractions and soil microbial activity: values for these parameters in acid soils are lower than in limestone soils, probably due to the lowest input of C in acid soils.

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