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SOIL MICROBIAL ACTIVITY IN A TOPOSEQUENCE UNDER MEDITERRANEAN CLIMATE

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Introduction

The Mediterranean environment is extremely varied in distribution of soil types. Vertisols and soils with vertic features are widespread in Sicily, which is characterised by Mediterranean climate. The large amount of expandable clays in these soils is responsible for argillo-pedoturbation promoting the homogeneity of chemical-physical characteristics along the profile. Few studies have been conducted on their microbiological properties, in terms of energy dynamics and microbial populations, even though the carbon flux from terrestrial to atmospheric systems depends on them (Howards *et al.,* 1998).

The present study investigated a reforested area of *Eucaliptus spp*. in order to characterise soil microbial activity and soil organic matter fractions of a toposequence in a gypsiferous hilly area in central Sicily. It is part of a vaster investigation carried out by Fierotti *et al.,* (1995) in Land Suitability for the *Eucaliptus camaldulensis*, and by Dazzi and Monteleone (this publication) in soil features and soil-landform relationships. The afforestation programme, that was undertaken to sustain paper mill industries, could strongly affect carbon turnover in soil and soil organic matter "quality", depending on the plant species employed.

Many authors have demonstrated an interrelation between vegetation and soil characteristics (Buol *et al.,* 1997; Fyles and Coté, 1994; Howard *et al.,* 1998, Pinzari *et al.,* 1998). As a result, the possible influence exerted by *Eucaliptus spp*. on some chemical and biochemical soil properties was investigated. In fact *Eucaliptus spp*. are not Sicilian native species, and the success of this kind of reforestation is not certain. Besides, Eucalyptus leaves contain high amounts of long aliphatic hydrocarbon chains, with aldehyde, chetons and esthers compounds (Harper *et al.,* 2000). Thus, litter decomposition carried out by soil microbiota could be more difficult, and could influence the carbon turnover into the soil.

Our aim was to investigate microbial metabolism and substrate availability, and compare it with organic carbon *status* in soil. The study was based on microbial activity parameters (qCO_2, C_{mic}/C_{org}), organic carbon fractions (C_{org}, humic and fulvic carbon), humification parameters and kinetics of organic carbon mineralisation.

Materials and Methods

Study Area

The study area is Mustigarufi wood, located in a gypsiferous hilly area in central Sicily near Caltanissetta (Figure 1). An extensive reforestation programme was carried out during the 1960s to prevent soil erosion processes. Quick growing species suitable for warm climate were used, mainly *Eucaliptus camaldulensis,* but also *Eucaliptus occidentalis, Pinus halepensis, Cupressus sempervirens* and *Cupressus orizontalis*.

Figure 1. Geographical location of the study area. Sicily, Mustigarufi wood.
Schematic representation of the toposequence along Salito river.
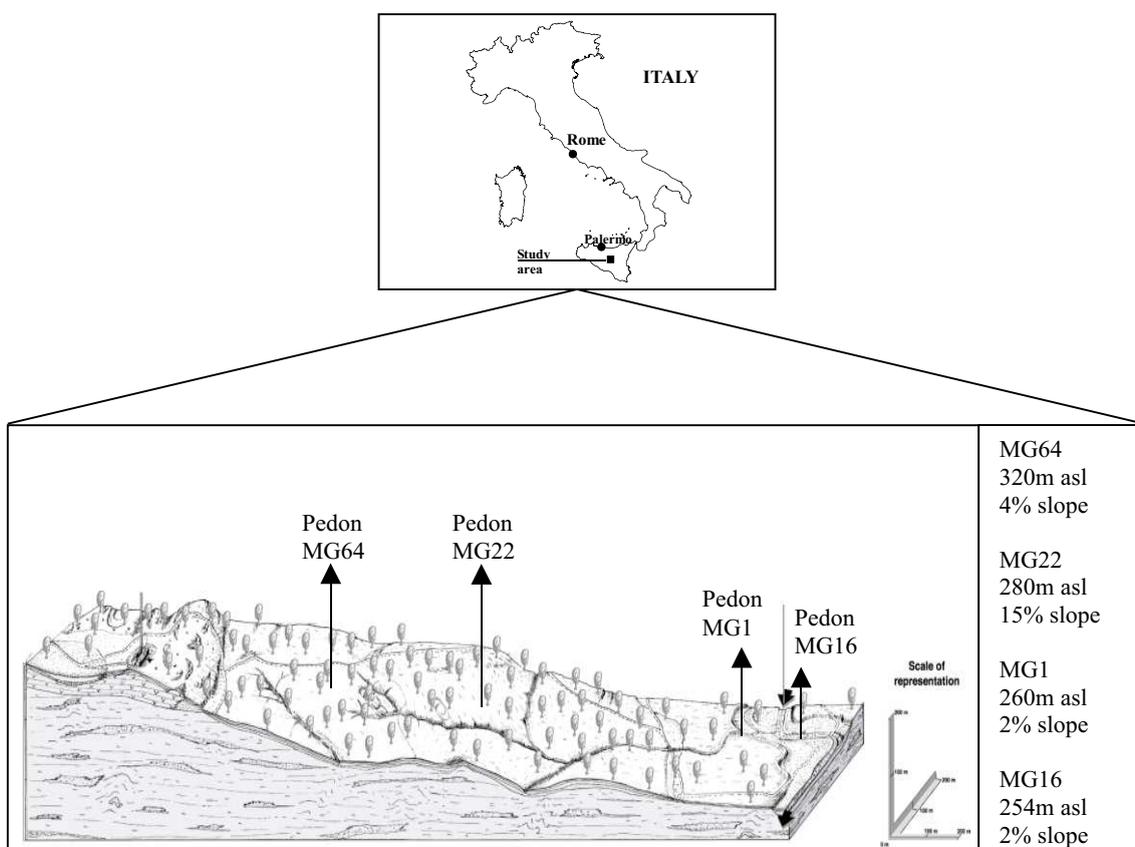
According to pluriannual average data recorded at a thermopluviometric station close to Caltanissetta (Fierotti *et al.,* 1995), average monthly temperature was 16.3°C; maximum in July (25.7°C) and August (25.8°C), and minimum in January (8.2°C) and February (8.7°C).

Average rainfall was 440 mm, with maximum in January and December (78 and 73 mm, respectively) and minimum in June and July (10 and 12 mm, respectively). The ombrothermic graph according to Bagnouls-Gaussen (Figure 2) defined the climate of the area as Mediterranean, with cool to cold, wet winters and warm to hot, dry summers. The Soil Moisture Regime was defined as "Xeric", and the Soil Temperature Regime as "Thermic".

Figure 2. Ombrothermic graph according to Bagnolous-Gaussen. Average monthly temperature T (°C), and average monthly rainfall R, (mm).
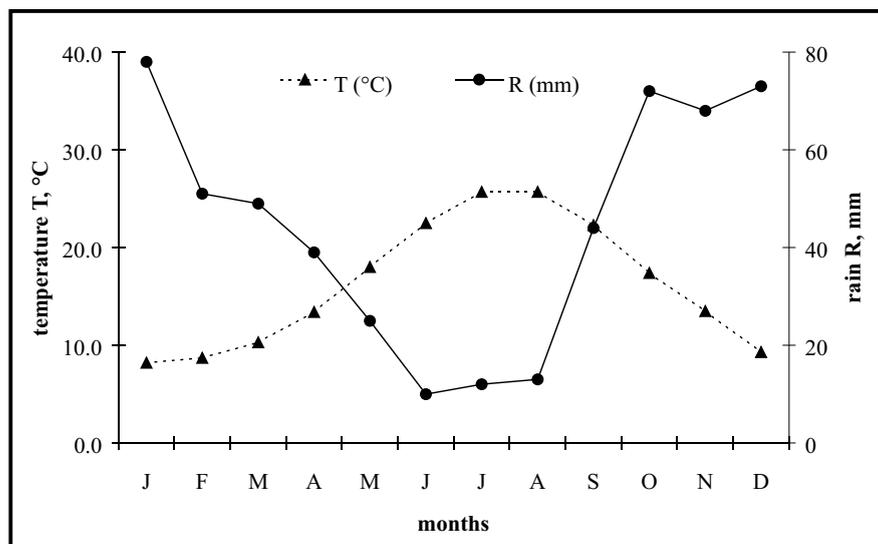
Soils

Four genetically consistent pedons along a toposequence of free-drained soils near the Salito river were studied (Dazzi and Monteleone, this publication). Pedon MG64 formed in the first section of the slope (Figure 1) and its profile is moderately deep and the texture is clayey. Reaction ranges from neutral to subalkaline. The moderate carbonate content increases in the B horizons in concretions forms. This soil shows good mineralogical composition homogeneity: there is a prevalence of clay minerals (59-76%), moderate amounts of quartz (14-20%), and lower amounts of calcite (4-8%) and feldspars (2-5%). The presence of solphatic lenses in the clay substratum seems to influence the profile's mineralogy slightly, except for the deeper horizons where there is a good amount of gypsum (11%).

Pedon MG22 developed in the second part of the hillslope shows a deep and very clayey profile. Reaction is subalkaline with moderate amounts of carbonates. There is a sharp prevalence of clay minerals (up to 83%) of the swelling type confirming the vertic features of this soil. Minor quantities of quartz and feldspars are present (8-12% and 2-3% respectively). The amounts of gypsum (up to 5% between 50 and 80 cm) can be related to low sulphate water circulation inside the soil.

At the end of the slope there is an orographic terrace shaped by the Salito River and set on marly clay. This lithomorphology is at the origin of the MG1 pedon whose very deep profile has vertic characteristics. The texture is always clayey and clay content is over 50%. Reaction is subalkaline that is influenced also by the high amounts of carbonates. The soil posses large levels of clay minerals (58-71%) and moderate amounts of calcite (12-17%), quartz (12-18%) and feldspars (2-12%). There are traces of gypsum as well. Lastly, MG16 pedon that is located on very sandy terraced alluvium shows a very deep profile. The texture is sandy-loam. The soil has a subalkaline reaction, extensive calcium carbonate content and very low amount of gypsum (about 1%).

Mineralogical composition is very homogeneous: there are moderate amounts of clay minerals (34-45%) and of calcite (about 25%). There is a notable presence of quartz (21% in the topsoil and 35% in the subsoil) and lower amounts of feldspars (6-11%). The scarce presence of gypsum testifies circulation of low sulphatic waters along the profile.

Pedons MG64 and MG22 evolve on substrata in which gypsum is absent, but show a gypsic horizon. Pedon MG64 is pedologically characterised by an O-A-Bk-BCy profile and can be classified as a *Gypsic Calcixerept*. Pedon MG22 shows a similar morphological sequence (O-A-Bkss-Byss-C), and can be regarded as a *Gypsic Vertic Haploxerept*.

Finally, in the flat area, pedon MG1 shows an O-A-Bwss-C profile and can be classified as *Vertic Haploxerept*. Pedon MG16 shows an O-A-C profile, having all the features that allow it to be classified as a *Typic Xerofluvent*.

A previous investigation on Land Suitability for the *Eucaliptus camaldulensis*, carried out by Fierotti *et al.,* (1995) revealed that, according to the FAO methodology, pedons MG1 and MG64 were "not suitable" because of soil texture limitations, pedon MG22 was "not suitable" because of texture and salinity limitations, while pedon MG16 was only "marginally suitable" for limitations due to soil salinity. Samples were collected from each horizon for laboratory analysis. Air-dried samples were gently crushed and passed through a 2 mm sieve; fine earth was used for analysis.

Analytical Methods

Determination of total nitrogen, N_{tot}, was obtained on two replicates by Elemental Analysis using Nitrogen Analyzer LECO FP 228 (Bremner and Tabatabai, 1971). Extractable fraction of Nitrogen, N_{ext}, by extraction with KCl 1N (1:10) and Autoanalyzer Technicon II reading was determined (Wall *et al.,* 1975 per NH_4^+ and Kampshake et al.,1967 per NO_3^-).

Total Organic Carbon, C_{org}, was determined according to the Springer and Klee method consisting of an oxidation with 0.33N $K_2Cr_7O_7$ at 160°C in acidic condition (Alef and Nannipieri, 1995).

Extraction of the soil organic matter was carried out by 0.1N NaOH and 0.1N $Na_4P_2O_7$ at 65°C for 48 hrs in N_2 atmosphere. Humic acids (HA) were precipitated by acidification (pH<1.5) of the extract and fulvic acid (FA), which remained in solution, were purified on a polyvynilpyrrolidone column and then recollected to the humic portion (Ciavatta *et al.,* 1990). Total extractable carbon, C_{ext}, and humic plus fulvic acid carbon, $C_{(HA+FA)}$, were determined by dichromate oxidation method, according to Ciavatta *et al.,* (1990). Duplicate determinations were carried out.

Humification parameters, such as the Humification Rate (HR) and the Degree of Humification (DH), were calculated according to Ciavatta *et al.,* (1990), as follows:

$$HR\ (\%) = 100\ \ C_{(HA+FA)} / C_{org}$$
$$DH\ (\%) = 100\ \ C_{(HA+FA)} / C_{ext.}$$

Humin content (the insoluble organic carbon) was calculated as the difference $C_{org} - C_{ext}$.

Water retention of the soil was determined on sieved samples by a pressure cell apparatus (Richard and Fireman, 1943), and physiological performance of the samples was tested under standard laboratory conditions (-33 kPa water retention and T 30°C).

Biomass carbon, C_{mic}, was measured by the fumigation-extraction method of Vance *et al.,* (1987) with some minor modifications. Measurements were performed on air-dried soils, pre-conditioned by a 10 day incubation in open glass jars, at 33 kPa water tension, and 30°C. Incubation was employed for restoring, within limits (Stotzky *et al.,* 1962), the microbial activity of air-dried soils to that of soils in the field. Three replicates of each soil sample were used.

Estimate of CO_2 concentration evolved by microbial biomass in soil sample was performed by Isermayer method (1952), based on the measurements of CO_2 production by soil biomass in a closed system (1,000 ml jar with a rubber ring and pegs), with some slight modification. Two beakers, containing, respectively the soil sample and the NaOH solution, were placed in each jar. At the bottom of each jar, 5 ml of CO_2 free water, to avoid the soil moisture depletion by the NaOH (Stotzky, 1965), were placed. Two replicates of each soil sample (25 g of each, oven dry-weight equivalent) were rewetted to their 33 kPa water tension and incubated at 30°C. CO_2 evolution was measured during 32 days. Average values were expressed as mg C-CO_2 kg^{-1} day^{-1} of soil oven dry-weight equivalent. The 32-day measurements were used as values of basal respiration, R_b.

Carbon turnover dynamics by kinetic of soil organic matter mineralisation (Riffaldi *et al.,* 1996) were studied. Nonlinear least square regression analysis was used to calculate parameters affecting C-mineralisation from daily CO_2 evolution data. The best fit was obtained with the exponential model of CO_2-C accumulation according to the negative exponential decay model: $C_m = C_0 (1-e^{-kt})$, where C_m is the cumulative value of mineralised carbon during t days, k is the rate constant, and C_0 is the potentially mineralisable carbon.

Metabolic quotient, $q(CO_2)$, defined as specific soil respiration of the microbial biomass, was calculated from basal respiration values with the formula: $q(CO_2)$ = (mg C-CO_2) (mg C_{mic} kg^{-1}) hour^{-1} (Anderson and Domsch, 1990). The ratio (C_{mic}:C_{org})% was used as an index of the contribution of microbial biomass to soil organic C (Anderson and Domsch, 1989).

Results and discussion

Initial observation of the four pedons showed that, despite their different classification, chemical parameters (particularly organic carbon and total nitrogen values, Table 1) were rather homogeneous along the profiles.

Soil organic carbon values (Table 1) were not high and decreased with depth, pointing out the limited organic matter storage in the forest floor (data on the amounts of input materials to forest floor are not available).

Table 1. Average values of organic carbon and nitrogen parameters.

	C_{org} (1)	C/N (2)	C_{ext}/C_{org} (3)	C_{ha+fa} (4)	DH (5)	HR (6)	N_{tot} (7)	N_{ext} (8)
MG16								
0-15 cm	13.5	9.8	0.76	12.3	95	73	1.1	0.015
15-30	7.1	5.6	0.66	10.1	100	78	0.7	0.014
30-50	7.3	5.7	0.77	10.4	100	78	0.6	0.017
50-90	5.3	4.3	0.68	10.6	100	81	0.5	0.020
MG1								
0-30	14.3	11.9	0.80	10.2	100	84	1.4	0.020
30-70	8.8	4.2	0.66	8.0	73	48	1.1	0.017
70-125	7.0	2.8	0.56	7.8	71	40	0.9	0.017
MG22								
0-20	17.0	13.0	0.72	11.3	100	77	1.5	0.018
20-50	7.9	5.1	0.67	7.9	97	64	1.0	0.017
50-80	4.8	2.7	0.50	6.7	100	57	0.7	0.017
80-95	4.1	2.0	0.49	8.2	98	48	0.5	0.017
95-110	3.8	2.0	0.39	4.7	100	54	0.8	0.018
MG64								
0-15	19.8	14.5	0.68	13.2	100	73	1.5	0.019
15-60	9.7	6.3	0.59	9.7	100	65	1.0	0.017
60-80	7.9	7.2	0.70	9.9	100	91	0.8	0.016
80-100	5.2	3.2	0.52	8.7	100	61	0.6	0.016

(1) Total Organic Carbon, g C kg^{-1} soil; (2) Carbon to Nitrogen ratio; (3) extractable carbon to total organic carbon ratio; (4) Humic and Fulvic Acid Carbon g C kg^{-1} soil; (5)= Humification Degree, %; (6) Humification Rate, %; (7) Total Nitrogen, g N kg^{-1} soil; (8) Extractable Nitrogen, g N kg^{-1} soil.

In particular, a sharp decline in carbon content from the surface horizon to the subsequent one was observed (about the half), thereafter the carbon decrease was more gradual as the depth in the hillslope land pedons increased, and values were almost constant in the terrace landform pedons. Among the four pedons, the terrace landform (M1 and M16) soils contained slightly lower amounts of organic carbon than the two hillslope ones (M64 and M 22), while chemical solubility of soil organic matter in the extracting solution was slightly lower in the hillslope pedons than in the two terrace landform ones, as shown by the C_{ext}/C_{org} ratios (Table 1) and by the humin carbon contents (Figure 3).

According to a classical approach based on different solubility at acidic conditions of extracted organic macromolecules, calculation of the humification parameters might indicate the extent of humification processes. Humification rate (HR%) provides quantitative information about the humic plus fulvic content normalised with respect to total organic carbon, while humification degree (DH%) provides the percentage of the humified carbon in the extracted organic fraction.

The results reported in Table 1 revealed that high HR values in all the four soils: in pedon MG16 values were increased slightly along the profile, indicating the presence of active humification process in deeper horizons leading to accumulation of humified compounds, while in the other profiles HR values decreased with depth.
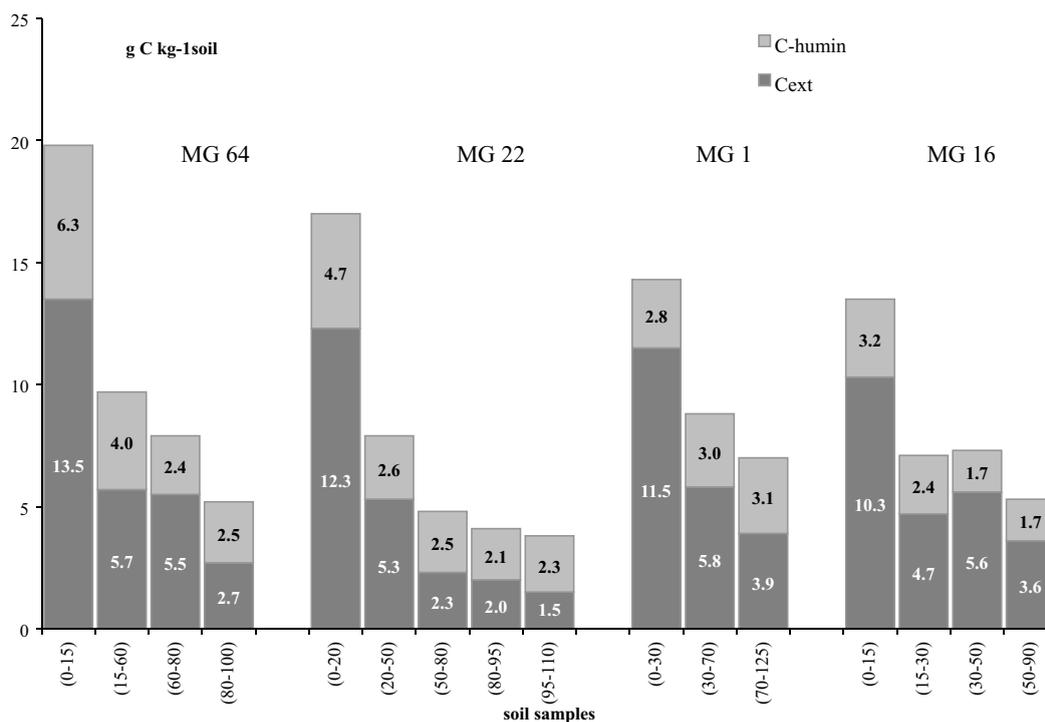
Figure 3. Soil organic C (extractable and humin fractions).

With the exception of the deeper horizons in MG1 pedon, humification degree (DH) values were close to 100%, indicating that the humified fraction represented almost all the extractable carbon (C_{ext}) in the $NaOH/Na_4P_2O_7$ solution. This finding indicated scarce availability of labile organic matter for soil micro-organisms metabolism and seemed to denote a potential condition of degradation, since the more stabilised fractions of soil organic matter (the humified one) can be mineralised and depleted by soil micro-organisms.

Nitrogen data showed a satisfactory content of total nitrogen along all the four profiles, the decrease of the C/N ratio being due essentially to C decrease along the profile. The presence of consistent amounts of both the NH_4^+ and NO_3^- forms in all the horizons seems to indicate that nitrogen content does not limit soil microbial activity, as shown by the low C to N ratio values along the soil profiles, especially in depth.

The soil respiration data and microbial biomass activity parameters reported in Figure 4 and Table 2, seemed to agree with the soil organic matter *status*. In fact, C-CO$_2$ cumulative production (C_m) declined along the four soil profiles, as did basal respiration (R_b) and respiration *"flush"* on the first day (R_{1day}). In particular, cumulative respiration (C_m) declined sharply from the surface horizons to the deeper layers, according to the trend shown by total organic carbon values. The relatively high C_m values in the deeper horizons indicated intensive microbes activity connected with organic matter content. In fact, the amounts of mineralised C ranged from 3.3 to 4.8 % of total organic carbon in soil.

On the basis of microbial biomass measurements (Figure 4), the soil of pedon MG16 seems to sustain greater microbial community along the profile: in fact, the living organic pool

amounted to more than 2% of total organic carbon at 90 cm of depth (Table 2). On the contrary, in the superficial horizons the size of microbial biomass was low compared with total organic carbon content, and the activity of the former was very high. Differently from the other pedons, MG 16 has developed on a sandy terraced alluvium: it contains minor quantities of clays, it is less compacted, permitting a better CO_2 exchange. Lower C_{mic}/C_{org} ratio in the surface horizons can be attributed to higher complexity of organic matter or to the presence of allelopathic compounds (Harper *et al.,* 2000).
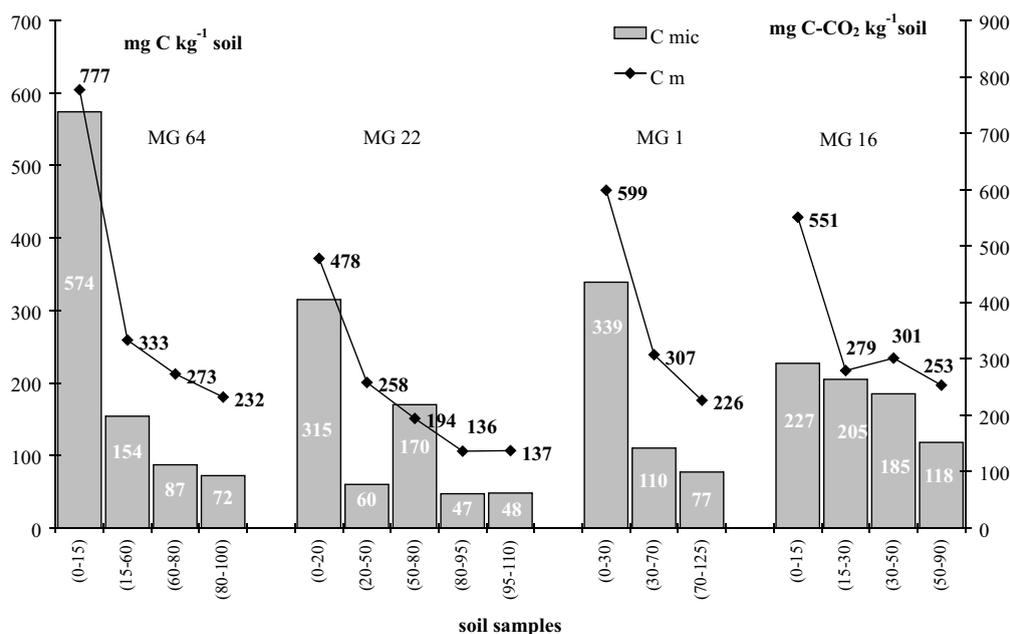
Figure 4. Microbial biomass C and total $C-CO_2$ production.

Carbon mineralisation rates (k) of the superficial horizons obtained by respiration kinetic curves ($C_m=C_0*(1-e^{-kt})$) were lower than observed in the deeper ones, and this finding indicated that C turnover in the superficial horizons takes more time than in the deeper ones. Analysis of both the metabolic quotient (qCO_2) and the metabolic rate (k) is highly informative with regard to the difference in the energy balance. In the surface horizon of the MG16 pedon, the metabolic quotient was high and the kinetic rate low indicating scarce energy efficiency; in fact higher metabolic activity in enzymes production, useful for organic substrates degradation, induces high respiration values, but the reaction rate is low. Diversely, in deeper horizons the opposite occurred, i.e. energy efficiency was higher (low qCO_2 values) and mineralisation process faster (high k).

On the contrary, in the other three pedons both mineralisation rates and metabolic quotients increased along the profile. The higher k values indicated that organic matter turnover was more rapid than in the surface horizons, while the high qCO_2 in connection with the C_{mic} to C_{org} ratio suggested a stress condition for soil micro-organisms (Anderson and Domsh, 1990). In this case the presence of gypsum may have played a role, especially in hillslope soils (Dazzi and Monteleone, this publication).

Table 2. Average values of microbial biomass parameters.

		C_{mic}/C_{org} (1)	C_0 (2)	R_{1day} (3)	R_b (4)	qCO_2 (5)	K (6)
MG16	(0-15)	0.0168	757	60	13	0.0024	0.0355
	(15-30)	0.0298	299	37	5	0.0010	0.0640
	(30-50)	0.0253	353	41	6	0.0014	0.0571
	(50-90)	0.0223	259	41	5	0.0018	0.0690
MG1	(0-30)	0.0237	688	59	10	0.0012	0.0551
	(30-70)	0.0125	327	32	5	0.0019	0.0669
	(70-125)	0.0110	226	32	3	0.0014	0.0876
MG22	(0-20)	0.0185	553	52	10	0.0013	0.0506
	(20-50)	0.0076	259	39	3	0.0019	0.0900
	(50-80)	0.0354	201	26	2	0.0005	0.0786
	(80-95)	0.0115	150	10	2	0.0018	0.0606
	(95-110)	0.0126	153	11	2	0.0017	0.0593
MG64	(0-15)	0.0290	946	100	5	0.0011	0.0461
	(15-60)	0.0159	363	47	8	0.0021	0.0568
	(60-80)	0.0110	277	38	5	0.0025	0.0734
	(80-100)	0.0138	248	29	5	0.0029	0.0611

(1)= Microbial Carbon: Total Organic Carbon ratio; (2)= Potentially mineralizable C, mg $C-CO_2$ kg^{-1} soil; (3)= CO_2 production at 1st day of incubation; (4)= Microbial Biomass Basal Respiration, CO_2 evolution at 14th day, mg $C-CO_2$ kg^{-1} soil; (5)= metabolic quotient, (mg $C-CO_2$) (mg C_{mic} kg^{-1} soil)$^{-1}$ h^{-1}; (6)= rate constant of carbon mineralisation.

Two possible hypotheses can be considered to explain the diverse micro-organisms activity at different soil profile depths: i) different soil organic matter "quality", i.e. different energy required by the same micro-organism consortium to mineralise different organic substrata; or, ii) different micro-organism community composition. Unfortunately, we have no additional data to support either of the hypotheses. On the other hand, it is known that environmental conditions, such as temperature and moisture level, are limiting factors of organic matter mineralisation, especially under Mediterranean climate, where drought is a limiting factor.

In litter bags incubation experiments, Rovira et Vallejo (1997) found higher C and N mineralisation of plant materials (*Eucaliptus globulus, Quercus ilex* and *Pinus halepensis*) at 20 cm of depth than at 5 cm. These authors suggested that under Mediterranean conditions the pedoclimate in deep layers is more favourable to microbial activity than in surface ones. However, temperature and moisture were not limiting factors in the standardised laboratory incubation conditions adopted in our research, thus the microbial activity recorded during our experiments was related to the potential performances of soil micro-organisms. Once again, different availability of organic substrates or different microbial communities may occur.

Conclusions

The results obtained in this study show some interesting features of carbon turnover and substrate utilisation by soil micro-organisms along the soil profiles.

The four pedons considered along the elevational transect showed scarce organic carbon storage in the forest floor, limited to the surface horizons: in fact a sharp decline in total organic carbon was observed through the profile from the surface layers to those below. Fractionation of soil organic carbon revealed low humin values and high amounts of soluble humic and fulvic fractions. Humification parameters, such as humification degree (DH), showed that the humified fraction reperesented almost all the extractable carbon, thus labile organic fractions were not available for micro-organisms metabolisms. This could denote potential soil degradation.

Soil organic carbon content may depend on climatic fluctuations and the amounts of organic input: in a given pedoclimate organic matter turnover is controlled not only by the inputs but also by the outputs represented by organic matter mineralisation. Unfortunately, no data on litter input to soil are available, but the low carbon mineralisation rates in the surface horizons indicated that carbon turnover was slower than in the deeper layers. Moreover, surface horizons seemed to sustain a smaller microbial community than the deeper layers, as shown by the C_{mic} to C_{org} ratio. An exception was the terrace pedon MG 16, where microbial biomass in the surface horizon seemed to undergo stress.

Cumulative respiration (C_m) declined sharply from the surface horizons to the deeper layers, following the same trend as total organic carbon values. The relatively high C_m values in the deeper horizons indicated intensive microbial activity, connected with the organic matter content. With the exception of MG 16 pedon, both mineralisation rates and metabolic quotients increased along the profile: the higher k values indicated more rapid organic matter turnover than observed in the surface horizons, while the high qCO_2 in connection with the C_{mic} to C_{org} ratio seemed indicate a stress condition for soil microorganisms, in which the presence of gypsum may have played a role, especially in hillslope soils (Dazzi and Monteleone, this publication).

Two possible hypotheses were considered to explain the diverse micro-organisms activity at different soil profile depths: different energy required by the same micro-organism consortium to mineralise organic substrata having different availability, or different micro-organism community composition. In depth investigation on the energetic status of soil organic matter in connection with a metabolic screening on soil micro-organisms may furnish data supporting one of these hypotheses.

Previous investigations suggested that thermal analysis methods, such as differential scanning calorimetry and thermogravimetry, could provide information on the energy in the soil organic pool (Grisi *et al.,* 1998), and that the chemical and structural characteristics of humic substances reflect soil organic matter turnover in Vertisols (Dell'Abate *et al.,* 2002). Moreover, community level physiological profile (CLPP) studies may highlight possible differences in the microbial community.

These two tasks are the next objectives to explain the effects of the afforestation programme carried out forty years ago on soil quality. From an economical point of view it was unsuccessful because of the very low wood productivity, while for purposes of soil protection the presence of tree covering limits soil erosion.

On the other hand, a previous investigation on Land Suitability for the *Eucaliptus camaldulensis* carried out by Fierotti *et al.,* (1995) showed that pedons MG1 and MG64 were "not suitable" for soil texture limitations, pedon MG22 was "not suitable" for texture and salinity limitations, while pedon MG16 was only "marginally suitable" for limitations due to soil salinity.

In fact, Eucaliptus is sensitive to high clay content in soil: for this pedon texture is not a limiting factor to Eucaliptus growth, thus it is less compacted permitting a better O_2 exchange. In agreement with this, the results of the present investigation showed more favourable conditions for microbial metabolism in pedon MG 16 than in the other pedons: energy efficiency was better (low qCO2 values) and mineralisation rates faster (high k) in the deeper layers than in the surface horizon and in the other pedons.

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