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Assessment of soil microbial functional diversity in a coppiced forest system

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ABSTRACT

Among the bioindicators used to determine soil quality a vast interest is devoted to those related to soil biodiversity; in fact, the ability of a system to withstand stress and abiotic and biotic disturbances depends on its level of biodiversity which is the basis of the functionality of ecosystems. Functional diversity is widely used to gain insight into microbial performances, particularly in presence of a factor of disturbance. In this study we present the changes of microbial functional diversity and other soil chemical and biochemical properties following forest coppicing. The study was conducted in central Italy, in a natural reservation under *Quercus cerris* spp. plant cover; soils were sampled after three years from coppicing and in an aged coppice taken as control. Trees cutting provoked a decrease of soil total organic carbon (TOC) and a pH increase suggesting a priming effect on native organic matter and qualitative changes in soil solution composition. Microbial indicators (microbial biomass carbon, MBC), basal and cumulative respiration, and indexes (microbial and metabolic quotients) were significantly affected by forest management. Enzyme activities and microbial catabolic activity measured by means of community level physiological profile (CLPP) techniques (MicroResp and Biolog) increased in coppiced plots indicating higher decomposition processes promoted by plant debris and rhizodepositions released after cutting.

The calculation of the diversity indexes using both techniques (enzyme activities and CLPP) suggested interesting speculations and perspectives on possible interpretations of these results.

1. Introduction

In the last two decades the interest on soil biodiversity and ecosystem functioning has become more and more important in ecological sciences. Soil hosts several microbial species, even if most of them are still unknown (Miller and Lodge, 1997); belowground biodiversity, in fact, represents the 95% of total diversity on Earth but only 5% of it has been classified (Gentile, 2006; Menta et al., 2008). The key role of soil microbial communities for the functioning of terrestrial ecosystems is guaranteed by their diversification in terms of structure and/or activity. Soil microbial processes include degradation of organic residues, transformations of soil organic matter, mineralization and immobilization of nutrients, formation and stabilization of soil aggregates (Nannipieri et al., 2003; Gregorich et al., 1997; Haynes and Beare, 1996).

In forest ecosystems, most of the studies on soil microbial community regard the vertical distribution along soil profile (Ascher et al., 2012; Noe et al., 2012) and/or the response to anthropic disturbances such as prescribed burning, erosion, harvesting, forest management, changes in plant diversity and soil pollution (Epelde et al., 2010; Giai and Boerner, 2007; Lalor et al., 2007; Leckie, 2005). The clearing of trees, in particular, causing the restructuring of vegetation, modification in litter quality and quantity, alteration of root exudates, leaching of plant nutrients and changes in the microclimate, can create conditions capable of affecting soil biota and influencing microbial communities diversity (Marshall, 2000).

To have a better understanding of the role of the soil microbial communities it is essential to know their functional and genetic diversity. According to Insam et al. (1989) the microbial functional diversity represents the sum of the ecological processes developed by the organisms of a community and it can be expressed through species or important groups to maintain several functions in the soil, while the genetic one represents gene and genotype variations.

The functional diversity of soil microbial communities results from genetic variability within a taxon, environmental effects on gene expression and ecological interactions among taxa. Distinct from the genetic diversity of the soil microbial biomass (Wellington et al., 2003; Emmerling et al., 2002; Zak et al., 1994) which assesses potential diversity, functional diversity is related to the actual activities resulting from that potential, so that "functional rather than taxonomic diversity may provide greater insight into microbial roles in ecosystems" (Zak et al., 1994).

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To assess the functional diversity of soil microbial communities both enzyme activities and the metabolic activity of soil samples can be measured. In particular, the metabolic activity of microbial communities can be determined by using two community level physiological profile techniques (CLPP), such as Biolog™ (Garland and Mills, 1991) and MicroResp™ (Campbell et al., 2003).

To date few studies focused on soil microbial diversity changes associated with cutting and burning of vegetation, afforestation or reforestation (Zhu et al., 2012) in Mediterranean soils, especially in Italy (Rutigliano et al., 2004). Furthermore, few of these studies used both enzyme activities and CLPPs approaches to study soil biodiversity. Coherent results, using both techniques, were recorded in two restored mixed-oak forests (Giai and Boerner, 2007), in a study on litter distribution along soil profile (Alarcon-Gutierrez et al., 2009), in a N fertilization experiment in a Mediterranean forest (Dalmonech et al., 2010), and in a managed forest (Gómez-Luna et al., 2012).

The present work was carried out in a forest ecosystem (*Quercus cerris* spp) in Central Italy managed as a coppice with standards. The aim of this work was to evaluate the effect of coppice on the size, metabolic activities and functional diversity of soil microbial communities. Microbial functional diversity was determined by means of enzyme activities and CLPP techniques (Biolog and MicroResp).

2. Material and methods

2.1. Site description and soil sampling

The Natural Reservation "Selva di Meana Monte Peglia" is located in Umbria region (Italy), Terni and Perugia district (N42°43′36″, E12°11′78″), exposition North–West, mean altitude 550 m a.s.l., annual rainfall 900 mm. The total area is 4.535 ha. Turkey oak (*Quercus cerris* spp) is the main species. The forest has been managed as a 'coppice with standards' with residues left on soil surface.

In June 2009 six plots were selected for soil sampling: the first three, for a total of 17.5 ha, were coppiced in 2006 (coppice with standards, CS), while the last three, for a total of 24.0 ha, were coppiced 50 years before and can thus be considered as an aged coppice (AC). For soil sampling particular attention was paid to the uniformity of the lithological substrate which was classified as *Haplic litosol* (FAO classification). Two soil samples were collected in each plot in the A horizon after removal of litter layer and O horizon. Soil samples were collected, within a homogeneous area, at least 10 m from the nearest tree, in order to be uniformly influenced by the root system and 25 m distant from each other. All soil samples (for a total of 12 cores) were air-dried and sieved at 2 mm before being analysed. The moisture content was adjusted to 60% of their water holding capacity (WHC), and soil samples where then left to equilibrate at room temperature in the dark for 10 days before biochemical and microbiological analyses.

2.2. Chemical and biochemical analyses

Soil pH was determined in three replicates in water pH_{H_2O} and KCl 1 N (pH_{KCl}) with a soil:solution ratio of 1:2.5, while the moisture content was determined by oven drying samples at 105 °C.

For TOC and total N (N_{tot}) soils were dried at 70 °C, then 20 mg were weighed into Ag-foil capsules. The analysis was based on dry combustion using an elemental analyser (Thermo Soil NC – Flash EA1112).

MBC was estimated following the fumigation extraction (FE) method (Vance et al., 1987). Two portions of moist soil (20 g oven-dry soil) were weighed, the first one (not fumigated) was immediately extracted with 80 ml of 0.5 M K_2SO_4 for 30 min by oscillating shaking at 200 rpm and filtered (Whatman no. 42); the second one was fumigated for 24 h at 25 °C with ethanol-free $CHCl_3$ and then extracted as described above. Organic C in the extracts was determined after oxidation with 0.4 N $K_2Cr_2O_7$ at 100 °C for 30 min.

Soil respiration was measured in a closed system (1000 ml jar with a rubber ring and pegs) following Badalucco et al. (1992). Three replicates of each soil sample were used. CO_2 evolution was measured after 1, 2, 4, 7, 10, 14, 17, 21, 28, 35, 42 days. Average values are given in mg CO_2-C/kg of soil oven dry-weight equivalent. The mean values of the hourly CO_2 evolved after the 10 days of incubation were used as the basal respiration (BR), because after that period the soil CO_2 production rate reached a constant value.

The organic C mineralization has been calculated by cumulative values of respiration through an exponential model of organic matter decomposition $C_m = C_0\,(1 - e^{-kt})$ (Riffaldi et al., 1996). In this model C_m corresponds to the cumulative C mineralized over time t of observation (days), while C_0 is the potentially mineralizable C, k is the rate constant and C_0k is the initial potential rate of C mineralization. Moreover, we calculated two different microbial indexes: the metabolic quotient (qCO_2) which represents the ratio of basal respiration to biomass C, and the microbial quotient (q_{mic}), which is the ratio of microbial C to organic C.

2.3. Enzyme activities

According to Marx et al. (2001) and Vepsalainen et al. (2001) enzyme activities were measured using fluorogenic methylumbelliferil (MUF)-substrates. The eight enzymes studied are involved in the main biogeochemical cycles: carbon (β-glucosidase, β-xylosidase, β-cellobiopyranoside), nitrogen (leucine aminopeptidase, N-acetyl-β-glucosaminidase), sulphur (arylsulphatase) and phosphorus (acid phosphatase). Furthermore, acetate esterase was included as a proxy of endocellular activity (Wittmann et al., 2004).

1 g of soil was weighed into a sterile jar and 50 ml of water. Soil suspension was obtained by homogenising with Ultra Turrax at 9600 rpm for three minutes, then 100 μl were withdrawn and dispensed into a 96 well microplate. Substrates were prepared with acetate buffer 0.5 M pH5.5 and an aliquot of 100 μl (1 mM) was added to each well reaching a final concentration of 500 μM. Fluorescence (excitation 360 nm, emission 450 nm) was measured with an automatic fluorimetric plate-reader (Fluoroskan Ascent) and readings were performed after 0, 30, 60, 120 and 180 min of incubation at 30 °C.

Shannon's diversity index ($H' = \sum p_i \log_2 p_i$) was computed from enzymatic rates as a measure of functional diversity (Bending et al., 2004), where p_i is the ratio of the activity of a particular enzyme to the sum of all enzymatic activities.

The Simpson–Yule index (Magurran, 1988) calculates evenness using the formula: $CE = 1/\sum p_i^2$, where p_i is the ratio of the activity of a particular enzyme to the sum of all enzymatic activities.

Catabolic versatility (CV) (Sharma et al., 1998) is defined by the following equation: CV = M/SD, where M is the mean absorbance value of all wells and SD the standard deviation of absorbance values.

2.4. CLPPs (MicroResp™ and Biolog™)

The community level physiological profile (CLPP) was determined using the MicroResp™ soil respiration system (MicroResp, Macaulay Scientific Consulting Ltd, Aberdeen, UK) according to Campbell et al. (2003) and the Biolog™ system (Biolog Inc., Haywood, California, USA) according to Garland (1996).

MicroResp™ is a technique based on the employment of bulk soil and it combines the advantages of Biolog™ and SIR (Campbell et al., 2003). The C substrates employed were selected on their

ecological relevance as representative of roots inputs for microbial metabolism.

The fifteen substrates consisted of five carbohydrates (D-glucose, N-acetyl-glucosamine, D-galactose, D-fructose, L-arabinose), five aminoacids (L-leucine, L-arginine, glycine, L-aspartic acid and γ-amino-butyric acid), three carboxylic acids (citric acid, oxalic acid and L-ascorbic acid), and two phenolic acids (vanillic and syringic acid). Phenolic acids were selected because they are representative of more recalcitrant organic compounds. Substrates concentration were chosen from values utilised in previous CLPP studies using MicroResp™ (Dalmonech et al., 2010; Lalor et al., 2007). Soil was added to substrates dissolved in deionized water; a water control was present in each microplate. For each soil three field replicates were employed. The evolution of CO_2 was estimated by the employment of a colorimetric method immediately before and after 6 h of incubation at 28 °C. The absorbance was read at 590 nm. At the end the absorbance was normalised for any differences recorded at time zero and then converted to % CO_2 using the calibration curve $y = A + B/(1 + D \times A_i)$, where $A = -0.34$, $B = -1.46$, $D = -7.88$. The % CO_2 was converted to μg CO_2 g^{-1} h^{-1} production rate using gas constant, T °C, headspace volume, soil dry weight (d.w.) and incubation time.

Biolog™ is nowadays much in favour of measuring microbial functional diversity in soil because the utilisation of available C is the key factor governing microbial growth in soil (Insam and Rangger, 1997; Garland and Mills, 1991). This technique is based on tetrazolium dye reduction as an indicator of sole-carbon-source utilization. ECOplates™ have been used to apply this technique to ecological studies to estimate metabolic potential of microbial communities (Insam and Rangger, 1997). Three grams of rewetted soil were suspended in 30 ml of 0.85% NaCl solution and bead-beated for 30 min at 5000 rpm. Sample suspensions were then centrifuged for 2 min at 3000 rpm. Each well of ECOplates was inoculated with 150 μl of sample supernatant. Two analytical replicates were performed for each sample. All samples were incubated at 30 °C and the optical density (OD) read at 590 nm for each plate every three hours over a period of 150 h. The average well colour development (AWCD) of the different replicates was calculated according to Garland and Mills (1991), where AWCD equals the sum of the difference between the OD of control (no substrate) and substrate wells divided by 31 (no. of substrates). Further parameters derived from the growth curves were r and s: where r is the flex slope and s the number of hours to reach the flex. They were obtained by fitting the curve of OD_{592} vs time to a density-dependent logistic growth equation (Lindstrom et al., 1998):

$$Y = \mathrm{OD}_{592} = \frac{K}{1 + e^{-r(t-s)}}$$

where K denotes the asymptote or maximum degree of colour development (OD_{592}), r the exponential rate of OD_{592} change (h^{-1}), t the time following inoculation of the microplates (h), and s the time at the midpoint of the exponential portion of the curve (i.e., when $y = K/2$) (h).

The diversity indexes for both CLPPs were computed similarly to those for enzymatic activities, except substituting the term p_i with the respiration rate of each single C-substrate as SIR (Degens et al., 2000).

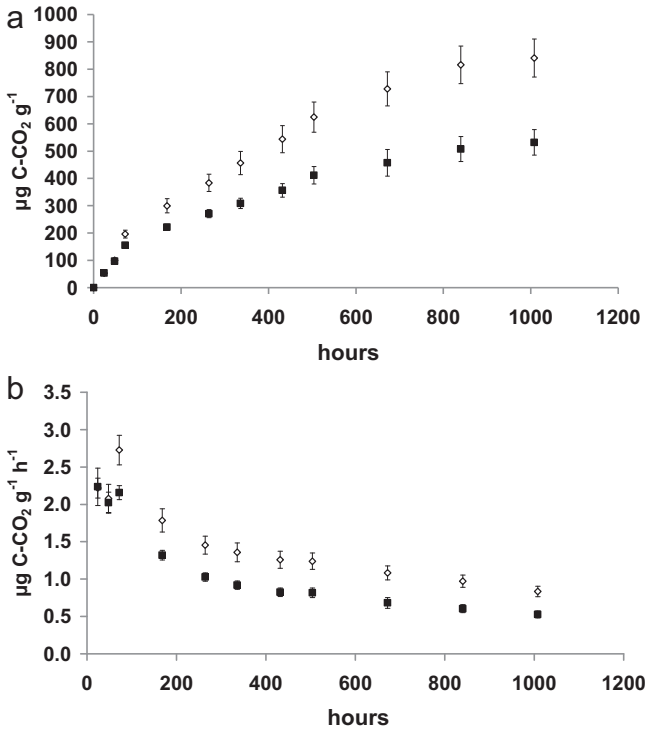

Fig. 1. Cumulative (a) and basal respiration (b) measured in CS (white diamond) and AC (black square) soils. Standard error bars are reported, $n = 6$.

2.5. Statistical analysis

Two tailed Student's t-test was performed (six replicates for each management) to evaluate the significance of differences between the two treatments. All statistical analyses were performed using SPSS 16 Linux edition. Statistical significance was determined at $P \leq 0.05$.

3. Results

3.1. Chemical and biochemical analyses

Soil pH (active and exchangeable acidity) was significantly increased after coppice (CS) while TOC content was reduced by 13% ($P < 0.05$) (Table 1). No significant variations were observed neither for soil N_{tot} nor for C/N ratio (Table 1). Soil MBC (+46%, $P < 0.05$), microbial quotient (q_{mic}) (+36%, $P < 0.05$), metabolic quotient (qCO_2) (+42%, $P < 0.05$), basal (+52%, $P < 0.01$) and cumulative respiration (+58%, $P < 0.01$) and the potentially mineralizable C (C_0) (+63%, $P < 0.05$) were all significantly increased in the managed forest ecosystem while the rate constant (k) decreased by 29% ($P < 0.01$) (Table 2 and Fig. 1).

Table 1
Main chemical parameters: pH, total organic carbon (TOC), total nitrogen (TN), C/N ratio determined in coppiced (CS) and aged coppice (AC) plots.

	pH (H_2O)		pH (KCl)		TOC (%)		TN (%)		C/N ratio	
	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
CS	6.72	*0.12*	6,51	*0.14*	5.49	*0.73*	0.526	*0.15*	12.74	*0.9*
AC	5.84	*0.35*	5.74	*0.08*	6.30	*0.68*	0.472	*0.05*	11.91	*0.8*
Student's t-test Management	*		*		*		ns		ns	

Standard errors are reported in Italics, $n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns: not significant.

Table 2
Microbial Biomass Carbon (MBC), microbial quotient (q_{mic}), metabolic quotient (qCO_2), potentially mineralizable C (C_0), kinetic constant (K), initial potential rate of C mineralization (C_0K), microbial cumulative respiration and basal respiration determined in coppiced (CS) and aged coppice (AC) plots.

	MBC (μg C biomass g^{-1})		q_{mic} (μg C biomass μg C org^{-1})		qCO_2 (μg C-CO_2 h^{-1} μg C $biomass^{-1}$)		Cumulative respiration (μg C-CO_2 g^{-1})		Basal respiration (μg C-CO_2 h^{-1} g^{-1})		C_0		K		C_0K	
	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
CS	589.7	*77*	0.90	*0.12*	0.27	*0.018*	840.97	*69.6*	1.171	*0.10*	993.9	*86.6*	0.048	*0.004*	47.23	*4.41*
AC	403.5	*23*	0.66	*0.05*	0.19	*0.016*	531.81	*46.7*	0.771	*0.06*	608.5	*98.7*	0.068	*0.009*	37.93	*0.76*
Student's t-test Management	*		*		*		**		**		*		**		ns	

Standard errors are reported in Italics, $n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns: not significant.

3.2. Enzyme activities

CS plots showed a generally higher activity (+47.8%, averaged across all the enzymes) than the AC (Table 3). Fig. 2 reports the percentage variation of each enzyme activity due to coppice with respect to AC plots. The highest effect was recorded for β-glucosidase which increased by almost 90% ($P < 0.05$). All three diversity indexes calculated with the enzymatic activities showed significant higher diversity for the managed ecosystem, in particular both Simpson-Yule and Catabolic Versatility showed the highest values in the managed ecosystem (7.11 vs 5.48 and 8.61 vs 3.15, $P < 0.05$, respectively).

3.3. MicroResp™ and Biolog™

The community level physiological profile (CLPP), measured by means of MicroResp™, showed a general higher consumption of the substrates in the coppiced plots (Table 4). The highest rates were observed in the following ranking for the different groups of substrates: aminoacids > phenolic acids > carboxylic acids > carbohydrates > amide (Fig. 3). MicroResp data were highly correlated with enzyme activities ($r = 0.709$, $P < 0.05$, Fig. 4) both averaged across managements. Although a general increase of all diversity indexes in the AC plots was observed, only Simpson-Yule (CE) significantly discriminated between the treatments (10.64 vs 12.50, $P < 0.05$).

Fig. 5 shows Biolog-AWCD values for soil microbial communities both in CS and AC plots, calculated during a 190.5 h incubation experiment. Microbial activity, as measured by AWCD, continued to increase with time and was higher for coppiced plots. The parameters derived from the growth curve (K, r and area) were not statistically different between the two treatments, except for s (number of hours to reach the flex) which was significantly higher in CS plots (124.7 vs 101.5, $P < 0.05$).

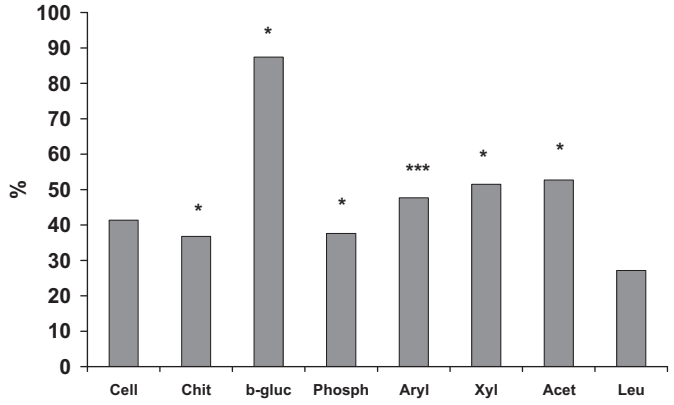

Fig. 2. Effect of coppice on enzyme activities expressed as percentage variation. Cellulase (Cell), chitinase (Chit), β-glucosidase (β-Gluc), acid phosphatase (Phosph), arylsulfatase (Aryl), xylosidase (Xyl), acetate esterase (Acet), L-leucine-aminopeptidase (Leu). $n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns not significant.

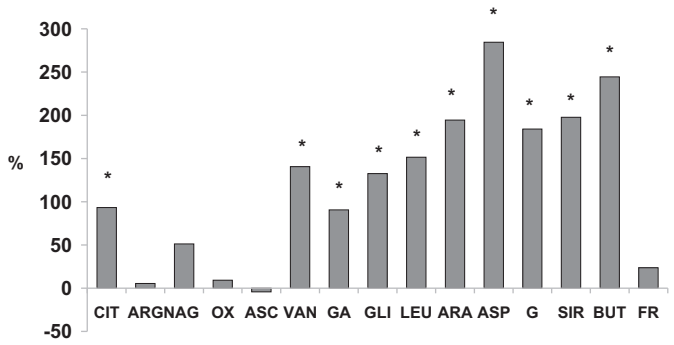

Fig. 3. Effect of coppice on CLPP-MicroResp expressed as percentage variation. Citric acid (Cit), oxalic acid (Ox), ascorbic acid (Asc), arginine (Arg), glycine (Gly), leucine (Leu), aspartic acid (Asp), butyric acid (But), galactose (Ga), arabinose (Ara), glucose (G), fructose (Fr), vanillic acid (Van), syringic acid (Sir), N-acetyl-glucosamine (Nag). $n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns not significant.

Despite the MicroResp™ results, Biolog™ C sources utilization was extremely heterogeneous. Some chemical groups as aminoacids and carboxylic acids had a higher activity in the AC than in the coppiced plots (data not shown).

The diversity indexes calculated with Biolog™ data gave higher values for the AC plots and were significant only for CE and CV (21.64 vs 23.22 and 1.53 vs 2.06, $P < 0.05$, respectively).

4. Discussion

4.1. Chemical and biochemical properties

In our study, significant effects were observed for soil pH and TOC which were modified after coppice. Grady and Hart (2006) did not find any difference in chemical parameters between a clear cut

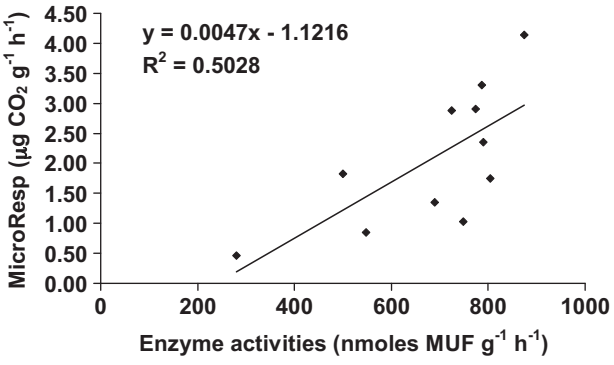

Fig. 4. Correlation between MicroResp and enzyme activities averaged across managements ($r = 0.709$, $P < 0.05$, $n = 11$).

Table 3
Enzyme activities, expressed in nmoles MUF $g^{-1}h^{-1}$, determined in coppiced (CS) and aged coppice (AC) plots.

	Cellulase		Chitinase		β-Glucosidase		Phosphatase		Arilsulphatase		Xylosidase		Acetate esterase		L-Leucine aminopeptidase	
	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
CS	107.0	*8.3*	4.03	*0.24*	445.7	*23.2*	830.4	*51.4*	346.8	*12.5*	93.25	*6.0*	3125.0	264.0	945.9	*46.8*
AC	75.7	*12.5*	2.95	*0.35*	237.8	*47.8*	603.4	*107.9*	234.8	*10.6*	61.57	*2.7*	2047.0	257.3	744.0	*74.7*
Student's t-test Management	ns		*		*		*		***		*		*		ns	

Standard errors are reported in Italics, $n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns: not significant.

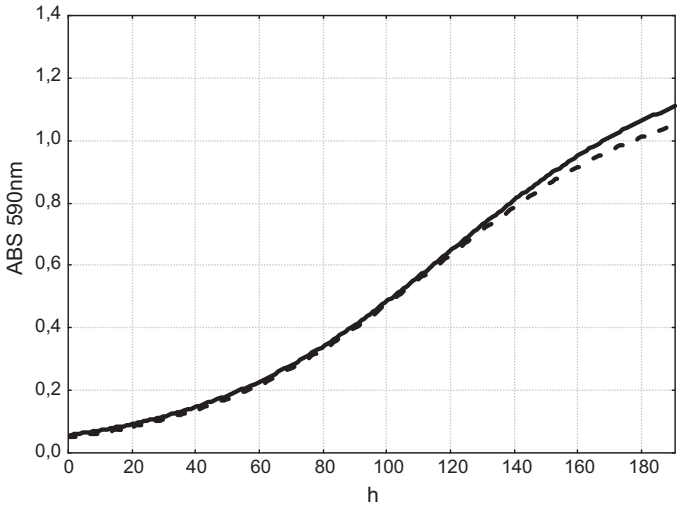

Fig. 5. Biolog average well colour development (AWCD) curves at the end of the incubation experiment measured in CS (straight line) and AC (dotted line).

system and a 30 years old unmanaged plot. Soil pH is known as one of the most sensible parameters to ecosystem variations (Berger et al., 2004; Pizzeghello et al., 2001). In this work the increase of pH_{H_2O} and pH_{KCl} suggested qualitative changes, arisen during the 3 years after coppice, in the soil solution and in the cationic composition of the organic component of soil colloidal fraction (Oorts et al., 2003). A consequence of trees cutting is the cessation of annual litter fall and root exudation, as fine roots of cut trees become subject to degradation. In particular, the intense extrusion of H^+ occurring in a living root when acquiring nutrients, was interrupted after coppice. Consequently, the reduced H^+ concentration in the soil solution combined to an increase of other cations released from litter decomposition could have qualitatively changed the cationic composition of ions adsorbed on soil solid phase. The increase of soil pH was also reported after similar forest management practices such as clear cut (Brais et al., 2003; Siira-Pietikäinen et al., 2001) and after thinning (Chauvat et al., 2003).

In this study, trees coppice significantly decreased soil TOC content as a likely consequence of either the intense microbial decomposition activity observed (enhanced enzyme activities and respiration) or a strongly reduced input of plant litter in the years following trees cutting. In soils of natural forests a general condition of homeostasis leads to a long-term substrate constraint which controls microbial functioning. Conversely, new inputs of fresh C due to coppicing as root exudates, plant residues and low molecular weight organic substances, can activate microbial groups that were dormant or inactive, with synthesis of a broad variety of enzymes and possible SOM decomposition, leading to a "priming effect" (Kuzyakov, 2010). Giai and Boerner (2007) found no significant differences in soil organic C content among different forest management strategies (prescribed fire, thinning, the combination of fire and thinning, and an untreated control), though they observed a significant increase in C/N ratio in the thin-only treatment. They supposed this increase in C/N ratio to be related to accumulations on the forest floor of woody remains from the trees cut during the thinning treatment. Several authors (Brais et al., 2003; Pietikainen et al., 1995; Lundgren, 1982) observed that the greater part of changes in nutrient cycling occurred during the first years after management, and included an increase in forest floor organic C, N_{tot}, base cations availability, and a decrease in microbial C/N ratio. These changes may have occurred in response to reduced vegetation uptake and woody debris abundance.

It is well known that microbial biomass plays a key role in the processes of soil organic matter dynamics and nutrient availability

Table 4
CLPP-MicroResp determined in coppiced (CS) and aged coppice (AC) plots. Citric acid (Cit), oxalic acid (Ox), ascorbic acid (Asc), arginine (Arg), glycine (Gly), leucine (Leu), aspartic acid (Asp), butyric acid (But), galactose (Ga), arabinose (Ara), glucose (G), fructose (Fr), vanillic acid (Van), syringic acid (Sir), N-acetyl-glucosamine (Nag). CA is carboxylic acids, AA is amino acids, CH is carbohydrates and A is amide.

Chemical groups	Substrates	CS		AC		Student's *t-test*
		$\mu g\,CO_2\,g^{-1}\,h^{-1}$	s.e.	$\mu g\,CO_2\,g^{-1}\,h^{-1}$	s.e.	Management
CA	Cit	2.910	0.255	1.507	0.200	*
CA	Ox	1.027	0.066	0.942	0.057	ns
CA	Asc	1.403	0.098	1.467	0.108	ns
AA	Arg	0.919	0.213	0.872	0.052	ns
AA	Gly	2.746	0.093	1.180	0.066	*
AA	Leu	2.618	0.112	1.041	0.048	*
AA	Asp	3.553	0.206	1.414	0.065	*
AA	But	2.262	0.383	1.076	0.099	*
CH	Ga	4.505	0.227	2.364	0.191	*
CH	Ara	3.447	0.113	1.916	0.087	*
CH	Glu	3.467	0.173	2.107	0.030	*
CH	Fr	3.018	0.093	2.441	0.173	ns
PA	Van	2.306	0.227	0.959	0.078	*
PA	Sir	2.457	0.103	1.225	0.081	*
A	Nag	0.655	0.217	0.433	0.026	ns

Standard errors are reported in Italics, $n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns: not significant.

in soil ecosystem. Soil management practices strongly affect microbial biomass, particularly the inputs of C substrates (Brookes et al., 1990); therefore clear cut or coppice may have either positive or negative effects on soil microbes.

In this study the microbial biomass increase in coppiced plots was probably due to enhanced availability of C substrates, deriving from litter and rhizodepositions (Merila et al., 2002; Saetre and Baath, 2000). Chauvat et al. (2003), in a chronosequence of four spruce forest stands, found no differences in soil microbial biomass content. In boreal forest soils Bååth et al. (1995), using PLFA, demonstrated that, few years after a strong management (prescribed burning, clear-cut, harvesting), all managed sites were structurally similar among them, but that they were significantly different from unharvested sites. In our study, also the new herbaceous and shrubs plant cover, colonizing the coppiced plot, may have enhanced MBC through changes in root exudates and litter. In fact, Hannam et al. (2005) found that the chemical composition of forest floor was different and that pre-harvest stand type, rather than disturbance by harvesting, had the strongest influence on microbial community.

In our study microbial respiration increased significantly both as basal and cumulative respiration after coppicing. Lytle and Cronan (1998) found that basal respiration in coniferous forest floors increases in the months following clearcutting, and they ascribed this effect to a flush of fine root death and decay in the immediate aftermath of harvesting. However, this initial flush is ephemeral and subsequent years should show a marked reduction in available C for heterotrophic metabolism, due to the loss of rhizodeposition, throughfall, and litterfall (Bradley et al., 2001).

In this work we can formulate two hypotheses: (a) fine root mortality and decomposition of the leaf litter detritus contributed to enhance soil respiration. In fact, in a study performed in a turkey oak forest, same geographical area as this study, the fine root inventory showed that 59% of roots, in terms of biomass, were recognized as dead in the recently coppiced plot (De Parri, 2001); (b) the improved solar radiation availability on the forest floor after coppicing promoted the development of a grass/herb layer, a rapid root turnover and a diverse kind of rhizodeposition could have contributed to fuel soil microbes and thus their metabolic activity (Sylver and Miya, 2001; Raich and Tufekcioglu, 2000). Higher C_0, C_0k and lower k, derived from the kinetic model used, confirm qualitative changes of C substrates and increase of microbial mineralizing activity in CS plots (Riffaldi et al., 1996).The metabolic quotient is considered as an index of microbial efficiency in utilizing the available resources; when high values of qCO_2 are recorded, soil microbes are operating inefficiently and are diverting a high proportion of C to maintenance requirements than biosynthesis (Anderson and Domsch, 1993). This may occur under stresses ranging from climate changes, fertilization, pollution, human impact and management practices (Dilly and Nannipieri, 1998). Furthermore, in Odum's theory of ecosystem succession (Odum, 1969), when ecosystems approach a steady state or "climax" stage of development, the ratio of respiration to biomass declines as biomass becomes more energy efficient. In our study the metabolic quotient increased significantly in CS plots, indicating a disturbance of microbial metabolism probably induced by changes in quantity and quality of available substrates.

4.2. Enzymatic activities

The actual rate of enzyme production and the fate of produced enzymes are modified by environmental effects as well as by ecological interactions (Moscatelli et al., 2005; Kandeler et al., 1996). Management can significantly impact soil enzyme activities, soil physico-chemical properties and substrate quality (Mungai et al., 2005; Frankenberger and Dick, 1983). Giai and Boerner (2007) found that tree canopy thinning resulted in increased acid phosphatase activity with respect to the untreated control, while no significant differences among restoration treatments in chitinase activity were observed. For Hassett and Zak (2005) all harvest treatments tended to reduce the extracellular enzyme activity from 10 to 30%, confirming the observations of Waldrop et al. (2003) who found that post-harvest treatments reduced the activities of extracellular enzymes in the forest floor of mixed forest in California. In this study an opposite trend was found, with a clear increase of almost all enzyme activities probably because the study was performed after three years from coppice while, in the cited works, the elapsed time was 8–10 years. Busse et al. (2006) report, in fact, a resilience response of microbial communities in the surface soil (size, activity, and composition) in a 5–10 years period after harvest. In particular C-cycle enzymes as cellulose, β-glucosidase and xylosidase were particularly enhanced (+41, 87 and 51%, respectively) in CS plots due to the accumulation of lignocellulosic material released after coppicing. Furthermore, the colonization of the coppiced site by fast-growing pioneer plants stimulates the microbial biomass to increase the activity of nutrients acquisition which also depends on the quality of new substrates available (e.g. rhizodeposition). In particular, Kuzyakov (2010) reports that addition of easily available organic substrates to the soil enhances microbial activity and acceleration of SOM mineralization by means of co-metabolism (i.e. enzyme production). Root exudates, in fact, can stimulate the mineralization of existing C pools (Subke et al., 2004; Mary et al., 1993; Dalenberg and Jager, 1989), resulting in the "priming" effect, already mentioned for microbial respiration and TOC.

4.3. Biolog and MicroResp

The use of ECOplates™ to study the community level physiological profile (CLPP-Biolog) is widely performed and more common than MicroResp™ (Chapman et al., 2007). The Biolog™ method essentially targets the fraction of microbial community that can grow within the microtitre plate wells; moreover, it is subjected to changes in the microbial community during the incubation and the contribution of fungi is not measured for their slow growth (Chapman et al., 2007; Nannipieri et al., 2003). Some authors showed that CLPP-Biolog do not necessarily reflects the functional potential of the dominant community members (Smalla et al., 1998) and that it is a culture-based assay which reflects the functional abilities of a very limited fraction of the entire soil microbial community (Ros et al., 2008). Nevertheless it has been also found that even non-culturable cells may respond to substrate supply (Garland and Lehman, 1999). Furthermore, it has been successfully used as a fast and highly reproducible tool to study changes in soil microbial functional diversity in natural environments (Ros et al., 2008; Gomez et al., 2004; Zak et al., 1994).

MicroResp™ was designed to be a 'whole soil' technique while still maintaining the convenience of the 96 well microtitre plate format as Biolog™. Moreover, substrate concentrations can be optimized in order to reflect the concentrations which likely occur in soils (Lalor et al., 2007). It has been recently used to assess soil quality restoration in forest soils (Jiang et al., 2012).

The utilisation of the different C substrates in Biolog™ was significantly increased in coppiced soils only for a few carbohydrates (data not shown), moreover, the growth curves were almost overlapping in CS and AC soils. Among the derived parameters the s value (the time to reach the flex) differed significantly between managements suggesting a longer period of adaptation to a higher variety of different organic substrates. The lack of other significant differences could be probably ascribable to some limitations of Biolog™ approach (Chodak and Niklinska, 2010; Chapman et al., 2007; Nannipieri et al., 2003).

MicroResp™ was more effective than Biolog™ to point the effects of forest management in terms of changes of microbial metabolism as also shown by the significant correlation with enzyme activities. MicroResp showed significant increases in the utilization of the different C sources, in CS soils, with highest rates for aminoacids and phenolic acids. Trees coppice provided a greater availability of C compounds whose assumption by microbes requires additional acquisition of N compounds; furthermore, increased wood debris on forest floor may have selected those microorganisms more efficient in the use of phenolic compounds. The substrates selection process, allowed by MicroResp technique, can improve its discriminatory power, providing greater confidence and ecological relevance in its use as an indicator of microbial functional diversity (Banning et al., 2012).

The two CLPP approaches, here investigated, differed substantially in their ability to distinguish between soil treatments; we can hypothesize that, beyond the methodological differences previously described (i.e. the employment of soil for MicroResp and a cell suspension for Biolog), additional variation can be due to the different C concentration of the substrates used. In fact, the availability of organic substrates in soil has been shown to be a major regulator of the dynamics and composition of heterotrophic microbial communities; varying substrate concentrations can alter microbial community composition as a result of the affinity of different microorganisms for particular substrate concentrations (Griffiths et al., 1999; Wardle, 1992).

4.4. Diversity indexes

Shannon index is the most widely employed in studies of microbial functional diversity (Saul-Tcherkas and Steinberger, 2009; Epelde et al., 2008) while few studies employ more than one index at the same time (Shen et al., 2008; Staddon et al., 1997).

Variations in the functional diversity can be evidenced by variations in C source utilization profiles and the "evenness" of catabolic responses to different C sources in soil microbial population-based diversity studies (Eaton and Farrell, 2004). At this purpose we calculated three different indices: the Shannon as the most employed (Magurran, 2004), the Simpson Yule (Staddon et al., 1997; Kennedy and Smith, 1995) and the Catabolic Versatility (Sharma et al., 1998). Within each analysis (enzyme activities, Biolog and MicroResp) all indexes were coherent and Simpson Yule proved to be the most sensitive to discriminate between managements.

However, significant differences were obtained between CS and AC plots as different trends were observed when using enzyme activities or CLPPs. In fact, microbial functional diversity increased after coppice if measured with enzymes while it decreased when measured with CLPPs. Similar contrasting results were also obtained by Epelde et al. (2008) who found significantly lower values of *H'* obtained from enzyme activities and higher values of *H'* obtained from ECOplates™ in polluted soils. Under natural conditions, the largest source of labile C inputs to the soil is root exudates (Kuzyakov, 2010; Bertin et al., 2003; Hütsch et al., 2002); the quantity and character of root exudates released by trees show a broad variety of low-molecular weight organic compounds, including sugars, amino acids, organic acids and phenolics (Grayston et al., 1997). These compounds are used in CLPPs techniques for measuring microbial catabolic performances. On the other hand enzyme activities generally target the hydrolysis of complex polymers into simple ones. This kind of substrates may prevail on the forest soil after trees cutting as wood debris and litter which mainly consist in cellulose, lignin, tannins etc.

It is well known that microbial functional diversity represents the capacity to perform different ecological processes and to use a wide array of substrates (Kandeler et al., 1996). This is achieved through different biological processes ranging from oxidative processes (e.g. respiration) to hydrolytic processes (e.g. extracellular enzymes). Consequently, calculating microbial diversity indexes using enzyme activities or CLPP methods could likely provide information on different components of microbial functional diversity. We here hypothesize that, measuring diversity using both methodological approaches, we obtained information on diverse ecological processes occurring in soil: on the one hand the exemplification of complex organic substrates obtained after enzymatic hydrolysis and on the other the direct utilization of simple substrates by microorganisms (CLPPs). CLPP-MicroResp™ is in fact considered a direct measurement of microbial communities' catabolic profile providing thus an instant photograph of microbial physiology (Lagomarsino et al., 2007).

This hypothesis, if confirmed by further experimental evidences, may supply additional information on the relative contribution that enzyme activities and CLPP techniques provide for the evaluation of microbial functional diversity. It may also open an interesting research issue to the pursuit of a synthetic index that takes into account two different, but complementary, components of functional diversity.

5. Conclusions

The oak forest coppicing significantly increased soil microbial pool and metabolism probably due to quali-quantitative changes of root products and litter that provided enhanced availability of C substrates.

Microbial functional diversity, assessed by means of CLPP (MicroResp™ and Biolog™) and enzyme activities was affected by forest management; however the different results obtained suggest that the two analytical approaches used may probably target diverse ecological processes depending on the quality of the available substrates.

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