PRELIMINARY INVESTIGATION ON SOIL MICROBIAL DIVERSITY : EFFECT OF PEDOGENIC SUBSTRATE

ENQUÊTE PRÉLIMINAIRE SUR LA DIVERSITÉ MICROBIENNE DU SOL: EFFET DU SUBSTRAT PEDOGENETIQUE

INDAGINE PRELIMINARE SULLA DIVERSITA' MICROBICA DEL SUOLO: EFFETTO DEL SUBSTRATO PEDOGENETICO

Armando Pignataro [(1,2) *], Maria Cristina Moscatelli [(2)], Stefano Mocali [(1)], Rosario Napoli [(1)], Stefano Grego [(3)], Anna Benedetti [(1)]

[(1)] C.R.A. Agricultural Research Council, Roma.
[(2)] DIBAF, Department for innovation in biological, agro-food and forest systems, Viterbo
[(3)] DAFNE, Depart. of Science and Technology for Agriculture, Forest, Nature and Energy
* Corresponding author: e-mail: armando.pignataro@entecra.it

Abstract

Soil biological functions, in particular linked to the activities of microbial communities, are influenced by the interaction between the species (canopy, quantity and quality of litter, roots and rhizodepositions) and the type of soil. The present study focused on the influence of different pedogenic substrates on the composition and the activities of microbial soil communities. Three systems with the same plant cover (*Quercus cerris* spp.) and same topographic conditions but with different pedogenic material (Andosol, Entisol, Inceptisol) were chosen. The soils were sampled in June 2009 in three Natural Reserves in the Centre of Italy (Selva di Meana/Monte Peglia, Monte Rufeno, Lago di Vico) at 0-20cm in horizon A. Functional diversity was calculated by estimating eight enzyme activities and the Community Level Physiological Profile (CLPP), together with soil chemical characterization.

Key words: *soil, forest ecosystem, microbial diversity, enzymes, CLPP (MicroResp, Biolog)*

Résumé

Les fonctions biologiques d'un sol, principalement garanties par l'activité des communautés microbiennes, sont influencées par l'interaction entre le type de couverture végétale (morphologie de la canopée, quantité et qualité de la litière, appareil racinaire et rhizodepositions) et le type de sol. Cette étude vise à attirer l'attention sur l'influence de sols différents sur la composition et les activités des communautés microbiennes du sol. Nous avons comparé trois systèmes avec couverture (*Quercus cerris* spp.) et autres conditions topographiques (altitude, pluviométrie, exposition) semblables, mais avec un substrat pédogénétique différent (Andosol, Entisol, Inceptisol). L'échantillonnage a été effectué en été 2009 dans trois réserves naturelles de l'Italie centrale (Selva di Meana / Monte

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Peglia, Mont Rufeno, Lac de Vico) à une profondeur de 0-20cm, horizon A. La diversité fonctionnelle a été déterminée par l'estimation de huit activités enzymatiques et par la détermination de l'empreinte physiologique des communautés microbiennes (PPLA).
Mots clés: *sols, écosystèmes forestiers, diversité microbienne, enzymes, PPLA (MicroResp, Biolog)*

Riassunto

Le funzioni biologiche di un suolo, garantite sopratutto dall'attività delle comunità microbiche, sono influenzate dall'interazione tra la copertura vegetale (morfologia della chioma, quantità e qualità della lettiera, apparato radicale e rizodeposizioni) e il tipo di suolo. Il presente studio intende focalizzare l'attenzione sull'influenza del diverso substrato pedologico nei confronti della composizione e delle attività della comunità microbica del suolo. Sono stati confrontati tre sistemi caratterizzati da una medesima copertura (*Quercus cerris* spp.) e condizioni topografiche simili (altitudine, piovosità, esposizione) ma con un diverso substrato pedogenetico (Andosuolo, Entisuolo, Inceptisuolo). Il campionamento è stato effettuato nell'estate del 2009 in tre riserve naturali del Centro Italia (Selva di Meana/Monte Peglia, Monte Rufeno, Lago di Vico) ad una profondità di 0-20cm nell'orizzonte A. La diversità funzionale microbica è stata determinata attraverso la stima di otto attività enzimatiche e tramite la determinazione dell'impronta fisiologica delle comunità microbiche (CLPP), unitamente alla caratterizzazione chimica dei suoli.
Parole chiave: suolo, ecosistema forestale, diversità microbica, enzimi, CLPP (MicroResp, Biolog)

Introduction

Italian soils are extremely various as genesis, features, properties and distribution. Their lithological variety and different morphological processes occurred in the centuries and clustered the national territory in morphological areas with different characteristics. They can be employed both for anthropical and natural use: natural forest ecosystems represent the place where biodiversity, both aboveground and belowground, is preserved. Soils host a big variety of microbial species, most of them still unknown. The main role carried out by soil microbial component for ecosystem functioning is guaranteed by its diversification (Giller et al., 1997).
Soil microorganisms, in fact, have a main role in biotic natural systems where they are key players in nutrient turnover.
Microorganisms respond differently to prevailing environmental conditions and forest stand characteristics (i.e. soil and vegetation properties) influence the composition of the soil microbial community in a specific way (Hackl et al., 2005).
The main aim of this paper is to evaluate the differences in soil microbial communities induced by specific pedogenic processes of soils and functional characteristics (soil temperature and moisture) under the same tree species (*Quercus cerris* spp.) but different parental material. The information on the

microbial community composition in undisturbed forest soils could then be used to facilitate interpretation of microbial community data derived from measurements in managed or even damaged or degraded soils.

Materials and methods

Site description and soil sampling. Soils have been sampled at 0-20cm in three different natural reserves: The Natural Reserve "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 550m a.s.l., annual rainfall 900mm. The Natural Reserve of "Monte Rufeno" is located in Lazio region (Italy), Viterbo district, 32T 0736390 UTM 4742149, exposition North-West, mean altitude 600m a.s.l., annual rainfall 800mm. The Natural Reserve "Lago di Vico" is located in Lazio region (Italy), Viterbo district, N42°19'47'' E12°08'08'', exposition North, mean altitude 550m a.s.l. , Six soil samples were collected in each area. Soil cores were collected, in June 2009, at least 10m from the nearest tree and 25m distant from each other. All soil samples were dried and sieved at 2mm. 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 prior to biochemical and microbiological analyses (Pinzari et al., 1999). Turkey Oak *(Quercus cerris* spp.*)* is the main tree species. The parent material has been classified, with the International Classification World Reference Base (2006), as follows: Monte Peglia is on a Haplic Litosol (Entisol very thin), Monte Rufeno is on a Dystric Cambisol (acid soil with a cambic horizon) and Lago di Vico is on a Melanic Andosol (Andosol with Melanic epipedon).

Physical data. Soil physical data regarding textural classes of the three selected soils are coming from previous studies: for the Haplic Litosol of Monte Peglia from the CRA National Soil Database (Napoli R., personal communication), for the Dystric Cambisol of Monte Rufeno from the CRA-RPS soil data of the forestry monitoring CONECOFOR Project (Alianiello et al., 2002) and for the Melanic Andosol of Lago di Vico from previous pedological studies of the same site (Lorenzoni and Quantin, 1990).

Chemical and biochemical analyses. Soil pH was determined in three replicates in water (pH_{H2O}) and KCl 1N with a soil:solution ratio of 1:2.5, while the moisture content was determined by oven drying samples at 105°C. The analysis of total organic Carbon (C_{org}) and total N (N_{tot}) was based on dry combustion using an elemental analyser (Thermo Soil NC – Flash EA1112). Microbial biomass carbon (MBC) was estimated following the Fumigation Extraction (FE) method (Vance et al., 1987), with some modifications, on air-dried soils conditioned by an incubation for 10 days in open glass jars at -33kPa water tension and 30°C.

Enzymatic activities. By the employment of fluorogenic methylumbelliferil (MUF)-substrates, according to Marx et al. (2001) and Vepsalainen et al. (2001),

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eight enzyme activities were measured. The enzymes studied are involved in the main biogeochemical cycles: Carbon (β-glucosidase, β-xylosidase, β-cellobiopyranoside), Nitrogen (leucine aminopeptidase, N-acetyl-β-glucosaminidase) (Snajdr et al., 2008), Sulphur (arylsulfatase) and Phosphorus (acid phosphatase), moreover acetate esterase was included as a proxy of endocellular activity.

Community level physiological profile. The community level physiological profile (CLPP) was performed using the MicroResp™ soil respiration system (MicroResp, Macaulay Scientific Consulting Ltd, Aberdeen, UK) according to Campbell (2003) and the Biolog™ (Biolog Inc., Haywood, California, USA) according to Garland and Mills (1996). The carbon substrates employed in MicroResp were selected on their ecological relevance and for the importance of roots inputs for microbial metabolism. The fifteen substrates consisted of five carbohydrates (D-glucose (GLU), N-acetyl-Glucosamine (NAG), D-Galactose (Ga), D-fructose (FR), L-arabinose (ARA)), five aminoacids (L-leucine (LEU), L-arginine (ARG), Glycine (GLY), L-aspartic acid (ASP) and g-amino-butyric acid (BUT)), three carboxylic acids (citric acid (CIT), oxalic acid (OX) and L-ascorbic acid (ASC)), and two phenolic acids (vanillic (VAN) and syringic acid (SIR)). Furthermore the carbohydrates to aminoacids ratio (CH/AA) was calculated using Biolog data.

Statistical analysis. The analysis of variance (ANOVA) was performed, on six replicates for each management, to evaluate the main differences among the tested treatments. Duncan's Post hoc test has been applied. Statistical analyses were performed using SPSS 16.0 Linux edition. Statistical significance was determined at $p \leq 0.05$.

Results

The main soil physical and chemical properties are reported in table 1.

Table 1 - *Physical, chemical and biochemical properties.*

	Monte Peglia	**Monte Rufeno**	**Lago di Vico**
U%	15	25	30
% Clay	19	21	9
% Silt	45	49	42
% Sand	36	30	49
pH	$6,18^{b}$ ±0,08	$5,85^{a}$ ±0,05	$6,22^{b}$ ±0,05
% C_{org}	$6,30^{a}$ ±0,67	$2,85^{b}$ ±0,15	$14,27^{c}$ ±0,40
% N_{tot}	$0,47^{a}$ ±0,03	$0,18^{b}$ ±0,01	$1,15^{c}$ ±0,03
C/N ratio	$14,69^{a}$ ±0,90	$15,53^{a}$ ±0,35	$12,38^{b}$ ±0,01
P (µg P g-1)	$6,04^{b}$ ±0,73	$8,18^{a}$ ±0,46	$6,72^{b}$ ±0,12
CEC $cmol_{(+)kg}^{-1}$	10^{a} ±2,6	$7,5^{a}$ ±2,07	$23,5^{b}$ ±2,70
qmic (µg C bio $TOC^{-1)}$	$0,66^{b}$ ±0,057	$1,80^{a}$ ±0,052	$0,65^{b}$ ±0,055

Significant differences among sites were observed in the C_{org} and of N_{tot} content. Since the three areas are characterised by significant differences in organic carbon content all biochemical data were normalized for their relative amount of organic C to reduce other sources of variation. Enzyme activities (Fig.1) and CLPP-MicroResp (Fig.2) ranked in the three sites as follows: Monte Rufeno > Monte Peglia > Lago di Vico. In particular in the last site microbial activity decreased by one order of magnitude than in the other ones. The community level physiological profile performed with MicroResp (Fig.2) and Biolog (Fig. 3 and table 2) showed a high consumption of substrates in Monte Rufeno. In particular glucose substrate, used in both techniques, is one of the most employed by soil microbial communities. The parameters derived from Biolog data are reported in table 3, in particular it is possible to observe that the microbial growth (slope, hours and area) for the microorganisms in Monte Peglia is slower than in the other sites.

Figure 1 – *Enzyme activities*

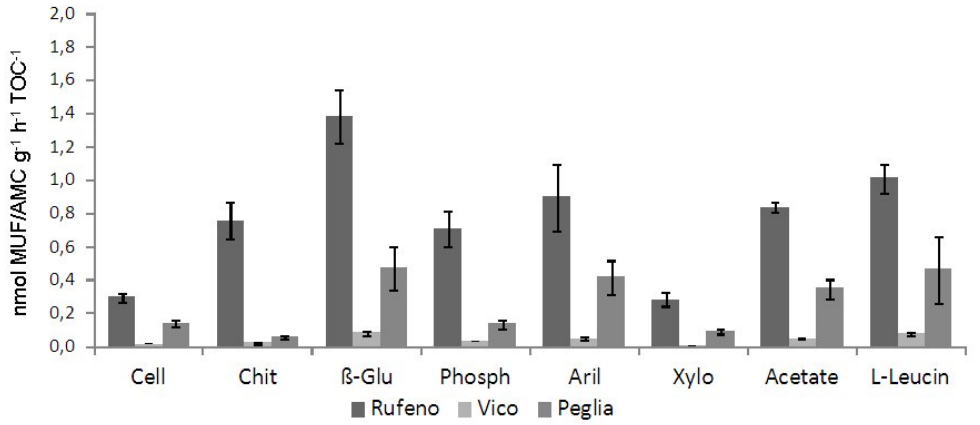

Figure 2 - *CLPP-MicroResp activities*

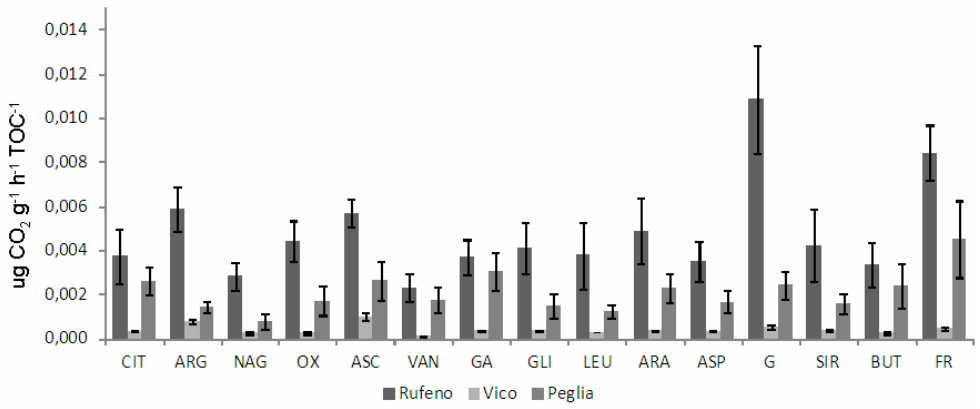

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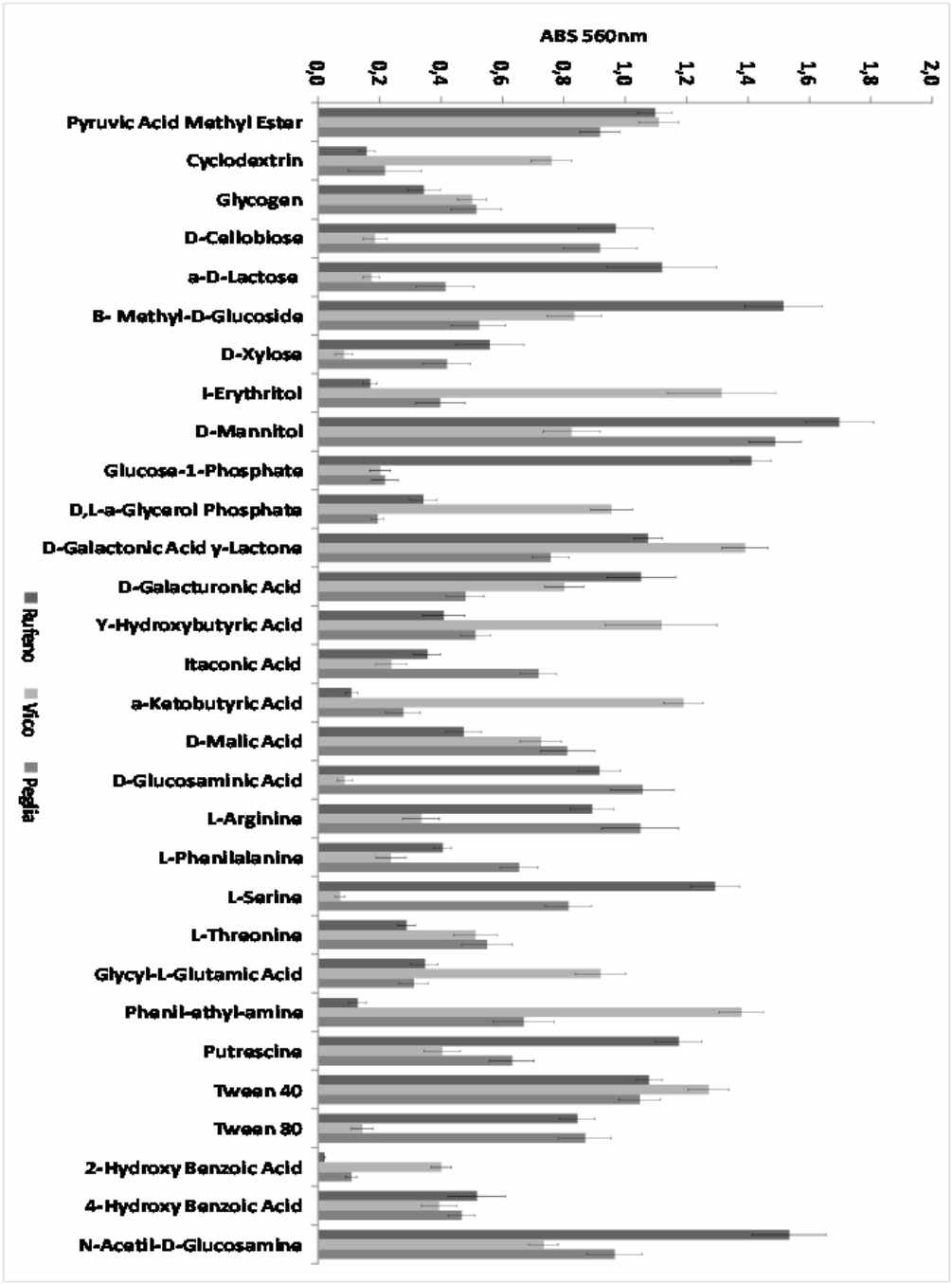
Figure 3 - *CLPP-Biolog activities*



Among the different substrates, used with the BIOLOG technique, polymers (such as Tween 40 and 2-hydroxy benzoic acid) used were better employed by the microbial biomass in the Andosol (Lago di Vico) than in the Inceptisol and Entisol. The CH/AA ratio was significantly higher in Monte Rufeno (Table 3).

Table 3 - *Biolog parameters: AWCD measured at curve plateau (K) and flex slope (r), number of hours to reach the flex (s), area under the curve.*

	Monte Peglia	**Monte Rufeno**	**Lago di Vico**
K	$1{,}183^{a}$ ±0,200	$1{,}436^{a}$ ±0,13	$1{,}335^{a}$ ±0,06
r	$0{,}0271^{a}$ ±0,003	$0{,}042^{b}$ ±0,003	$0{,}052^{b}$ ±0,002
s	$114{,}57^{a}$ ±46,85	$82{,}46^{b}$ ±16,62	$80{,}74^{b}$ ±5,79
Area	$93{,}11^{a}$ ±13,90	$163{,}32^{b}$ ±14,00	$153{,}03^{b}$ ±6,25
CH-to-AA ratio	$1{,}119^{b}$ ±0,61	$4{,}282^{a}$ ±1,84	$1{,}60^{b}$ ±0,46

Discussion and conclusions

The studies assessing the effect of parent material on soil microbial properties are several, in particular those regarding the natural forest ecosystems (Vittori Antisari et al., 2001; Stursova, 2011; Chodak et al., 2010).

The differences in physical and chemical properties of the studied soils were reflected in their microbial activity, in fact Monte Rufeno exhibited higher values for enzyme activities and MicroResp. Lago di Vico soil potentially showed the best conditions for the growth and metabolism of microorganisms (higher CEC, sub-acidic pH and higher C_{org} and N content, lower C:N ratio); conversely this site was characterized by a "static system" showing the lowest microbial activities. These results could suggest different hypotheses that will be tested in the future: i) a likely stress condition for soil microorganisms caused by the low quality of dissolved organic matter (DOC) of soil (i.e. presence of recalcitrant chemical material) or ii) the site typical microclimate which maintains a high level of soil moisture for a long period of the year which, combined to a high content of humic material, could (Lorenzoni et al., 1986) retard all biological processes.

The marked low microbial activity determined in andosols (Lago di Vico) may also be related to the presence of organo-metallic (Al based) compounds of high molecular weight, typically associated with the melanic epipedon on pyroclastic deposits rich in amorphous materials (Piccolo, 1990). Soil moisture, very high trough the whole year, associated with quite low average temperature could represent a cofactor in damping the microbial activity in this particular soil located in Vico Caldera's inner slopes.

Monte Peglia (Entisol) showed intermediate values of microbial activity, generally quite low if compared to Monte Rufeno. This site is characterized by a very shallow depth caused by strong surface water erosion. The topographic factor could

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be one of the main reason in controlling the low availability of nutrients in weathered pedogenic materials and soil moisture.
The highest values of microbial activity found at Monte Rufeno could be explained according to the relatively young profile horizons structure and the consequent presence of pedogenic weathering and alteration processes both in A and Bw horizons. Furthermore, at Monte Rufeno, the less dense canopy structure of oak stands allows the sunlight to reach the soil surface for a long period, this can promote a higher microbial decomposition and turnover of organic matter because of the increased topsoil temperature. (Singh et al. 1999). BIOLOG data showed more heterogeneous results and did not allow to evidentiate a higher use of all substrates in a specific site, probably BIOLOG technique was sensitive to the differences in pH and in clay content among the three sites. At Lago di Vico site a higher consumption of complex polymers (Tween 40 and 2-Hydroxy Benzoic Acid) was recorded, suggesting a specific ability of microorganisms to metabolize recalcitrant compounds.
The relative use of amino acids, carboxylic acids and polymers was affected by the soil type. Sharma et al. (1998) suggested that higher CH-to-AA ratios indicate higher content of easily degradable carbon compounds in the soils. The higher values of CH/AA ratio found at Monte Rufeno confirm thus the occurrence of specific organic substrates quality that promoted the elevated levels of microbial activity determined in this site with all the techniques used.
The preliminary results suggest that even if plant cover is the same in each site and the topographic conditions are similar, parent material and the specific pedogenic processes could influence in a strong way the functional characteristics of microbial communities. In order to obtain a more comprehensive picture of parent material influence on soil biodiversity our research will be completed with the study of microbial genetic diversity.

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