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Water-extractable organic matter and enzyme activity in three forest soils of the Mediterranean area

Felicia Grosso [a], Fabio Temussi [b], Flavia De Nicola [a, *]

[a] Dipartimento di Scienze e Tecnologie, Università del Sannio, via Port'Arsa 11, I-82100 Benevento, Italy
[b] UdR Napoli 4 INCA, Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Complesso Universitario Monte S. Angelo, Via Cintia, I-80126 Napoli, Italy

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ABSTRACT

Soil enzyme activities mediate key ecosystem functions of degradation, transformation and carbon mineralization. The study of microbial activity and its relations with water-extractable organic matter (WEOM) can be crucial to understand the dynamics of soil organic carbon pool. We investigated FDA-hydrolytic, β-glucosidase, cellulase, *o*-diphenol oxidase activities in soils under *Fagus sylvatica* (beech), *Quercus ilex* (holm-oak) and *Quercus cerris* (turkey-oak) stands. We investigated WEOM by liquid-state nuclear magnetic resonance (NMR) spectroscopy, useful to highlight the major functionalities in this fraction of soil organic matter. The highest enzyme activities, on mass basis, were recorded in soil under beech, with the highest organic carbon content. Reporting enzyme activities on organic carbon basis, it was possible to reveal enzyme enrichment for β-glucosidase and diphenol oxidase activities in soil under turkey-oak, with low organic matter. The ^{1}H NMR spectra of WEOM highlighted a great richness of soluble organic compounds in soils with high organic carbon content, such as beech and holm-oak soils. All spectra are dominated by carbohydrate resonances. Spectra of WEOM from each stand exhibited specific signals. In WEOM from holm-oak, signals from substituted aliphatics account for up 28% of the total spectrum; in this sample signals from acetic and formic acids predominate, likely relating to the lower microbial utilization, according to the low heterotrophic (FDA-hydrolytic) activity. Only in WEOM from beech, signals from aromatics were detected probably related to the lower lignin degradation in soil, as expressed by the low phenol oxidase activity. However, the relationships among WEOM quality, tree species and microbial activity need further investigations.

1. Introduction

In terrestrial ecosystems, soil microbial communities represent a mechanistic link between tree species and ecosystem functions, mediating key ecological processes. Plant debris contributes to soil organic matter that represents the main regulating factor of the microbial communities, being a nutrient source and acting as a substrate for microorganisms [1]. Soil heterotrophic microorganisms play a key role in the organic matter decomposition, releasing several enzymes that catalyse the nutrient recycling in forms available for plants and other organisms. The measure of the microbial enzymatic activities is useful to evaluate the soil fertility and to quantify the turnover of organic compounds, particularly high in forest and grassland systems [2]. Microbial enzymatic activities, direct expression of the functioning of microbial communities, depend on the amount and type of litter inputs and root exudates that vary with tree species [2,3]. Plant species may also influence soil microbial processes through their effects on soil physico-chemical characteristics, such as pH and water content [4].

Microorganisms mainly use low molecular weight compounds, obtained either directly from solution or following breakdown of the polymers in soil through hydrolytic enzymes. The dominant low molecular weight compounds of soil organic carbon pool are sugars, organic acids, amino acids, amino sugars and nucleotides, which are used by organisms in most metabolic processes. These substrates move through the soluble phase to reach and pass through microbial membranes [5].

Generally, the soil organic matter present in soil solutions in macro-pores is defined as dissolved organic matter (DOM). Water-extractable organic matter (WEOM) is widely recognized as a surrogate for in situ DOM and indicates the soluble fraction of organic matter extracted from the soil under various laboratory conditions [6]. Although the DOM is only a small part of soil organic matter, it

* Corresponding author.
E-mail address: fdenicol@unisannio.it (F. De Nicola).

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Table 1
Characteristics of sites and beech (B), holm-oak (H) and turkey-oak (T) stands, expressed as mean (s.e.) of five replicate plots.

Site	Latitude	Longitude	Altitude (m a.s.l.)	Slope (%)	Soil type	Forest association	Litter (cm)	Stand age (yr)	Stand density (stem/plot)	Stand basal area (m^2/ha)	Stand height (m)
B	41°25′ N	14°28′ E	1336	6.30	Molli-eutrisilic andosol, Molli-vitric Andosols	*Campanulo trichocalycinae–Fagetum sylvaticae*	4	100	25	448.02 (86.54)	18.8 (3.88)
H	41°21′ N	14°24′ E	680	64.7	Molli-vitric Andosol, Molli-eutrisilic andosols	*Fraxino orni–Quercetum ilicis*	4	80	27	151.74 (17.59)	7.48 (1.01)
T	41°16′ N	14°24′ E	184	16.7	Luvi-vitric andosols	*Lathyro digitati–Quercetum cerris*	1.5	30	10	62.00 (11.12)	14.98 (3.27)

has a strong influence on several ecologically relevant processes in soil. For example, it is a potential available source of carbon for microorganisms, modulating the structure of the soil microbial communities based on the bioavailability of carbon. Besides it can enhance the mobility of heavy metals and organic pollutants, thus affecting soil contaminant transport and micronutrient availability, and contributes to mineral weathering (see Ref. [7] and references therein). The dissolved fraction generally reflects in molecular composition the soil total organic matter, because the soluble phase tends to be in equilibrium with the solid phase of organic matter [6]. The tree species strongly affect quantity and chemical composition of soil organic matter, both as total and dissolved forms [7].

Nuclear magnetic resonance (NMR) techniques are useful tools to study the structural components present at molecular level and their interactions and the application in environmental chemistry is growing. Liquid-state NMR can provide high-resolution spectra in order to define the basic structural components in a complex environmental mixture, such as the soil WEOM [8]. Thus the application of NMR spectroscopy becomes an important tool to highlight the major moieties present in soil organic matter. Up to now there are little spectroscopy data available about the WEOM across forest ecosystems in the Mediterranean area [9].

The study of WEOM and its relation with soil microbial activity in forest systems can be of paramount importance to understand the dynamics of soil organic carbon pool and its implication in carbon fluxes and climate change. On this basis, goal of this study was to compare soil microbial activities and WEOM in different forest types in the Mediterranean area. This with the aim to investigate the relationships between soil enzyme activities and chemical composition of soil WEOM associated with different dominant tree species. At this purpose, we measured several soil microbial enzyme activities related to matter cycle, and characterized WEOM, by ^{1}H NMR liquid-state, in stands of *Fagus sylvatica* L., *Quercus ilex* L. and *Quercus cerris* L.

2. Material and methods

2.1. Study sites and soil sampling

The study sites are located in the Matese area (Apennines district, southern Italy) covered for 69% by forests. *F. sylvatica* forests predominate at altitudes above 1000 m a.s.l., whereas forests of Mediterranean evergreen oaks, with *Q. ilex* and *Quercus rotundifolia*, and thermophilous oak woods, with *Q. cerris* and *Quercus frainetto*, predominate below 1000 m a.s.l., even if in many areas the native oak forests have been replanted with *Castanea sativa*. Due to the outstanding natural value, the Matese area is included in the European Union special conservation zones for natural habitats and wild flora and fauna (Council Directive 92/43/EEC). The climate is characterized by a mean annual rainfall of 848 mm and a mean annual air temperature of 16 °C. In the hottest months (June–September, mean temperature 25 °C) meanly 80 mm precipitation were recorded (data from www.sito.regione.campania.it/agricoltura/meteo/agrometeo.htm).

The study was carried out in three different forest stands (Table 1) located on a calcareous substrate covered with pyroclastic materials:

- B, a mono-species *F. sylvatica* L. (beech) stand;
- H, a stand dominated by *Q. ilex* L. (holm-oak);
- T, a stand dominated by *Q. cerris* L. (turkey-oak).

There were some differences in the ground vegetation cover of the stands; shrubs and herbs were more abundant in the oak stands and scantly represented in the beech stand. Each stand was characterized for soil water holding capacity, detected on undisturbed soil cores via gravimetric method after soil water saturation: the soil water holding capacity results equal to 195% d.w. in B, 131% d.w. in H and 37% d.w. in T, respectively.

In order to evaluate the differences in the soil chemical, physical and biological properties among the three studied stands, a completely randomized design consisting of 5 field replicates for each stand, represented by adjacent plots (5 × 5 m each), was set up. In autumn 2012, in each plot 8 soil samples (core diameter: 10 cm) were collected from the surface layer (0–5 cm depth) after litter removing, and pooled to obtain a homogeneous and representative sample. We chose this depth for our sampling, because most of the microbial biomass and activity occur in the surface layer and the effects of tree species (through litter chemistry) should be stronger and more distinct within the soil superficial layer.

2.2. Soil physico-chemical analysis

Soil samples were sieved (<2 mm) and, successively, analysed for the water content, via the gravimetric method after oven-drying (75 °C), the pH in water suspension (1:50 = w/v = soil:water) via the potentiometric method, and the organic carbon by dichromate oxidation and titration using the Walkley–Black method [10]. All analyses were carried out on three laboratory replicates per plot.

2.3. Enzyme activities

Enzyme activities were measured on soil samples, sieved and stored at 4 °C, within five days from the sampling. The fluorescein diacetate hydrolytic activity was determined by incubating soil samples at 25 °C for 30 min with phosphate buffer (pH 7.6), adding 3, 6-diacetyl fluorescein (FDA, at a final concentration of 2 mg ml^{-1} in acetone) as substrate, and measuring the absorbance of the released fluorescein at 490 nm [11]. The β-glucosidase activity was assessed by incubating soil samples at 37 °C for 45 min with MUB buffer (pH 6.0), adding *p*-nitrophenyl β-D-glucopyranoside (PNGP, 0.05 M) as substrate, and measuring the absorbance of the released *p*-nitrophenol (PNP) at 398 nm [12]. The cellulase activity was assessed by incubating soil samples at 50 °C for 24 h with acetate buffer (pH 5.5), adding carboxymethyl-cellulose (CMC) sodium salt (0.7% w/v) as substrate, and measuring the absorbance of the released sugars at 690 nm [13]. The *o*-diphenol oxidases activity was determined by incubating soil samples at 30 °C for 10 min with