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Microbial activity and organic matter composition in Mediterranean humus forms

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ABSTRACT

The aim of this study was to characterise humus forms, intended as organic profile morphologies, and the associated soil chemical, biochemical (β-glucosidase, phosphatase and urease activities) and organic matter (SOM) properties in Mediterranean forest soils. Samples were collected from the organic layers (OF + OH) and the mineral topsoil (M01) in 31 *Quercus* spp. sites. To highlight the differences in soil organic matter (SOM) composition among humus forms, pyrolysis–gas chromatography (Py–GC) was used and the ratios of specific pyrolysis products were calculated. The main results achieved were that humus forms can be clearly separated according to enzyme activities and SOM pyrolytic fragments of the first mineral layer (M01). Humus forms showed a progression from mull macro, which was the richest in soil nutrients and with the highest enzyme activities, across amphi macro and amphi meso to an opposite end represented by mull meso and moder. This trend was also observed in the qualitative changes of SOM composition. In particular, the high ratios of pyrrole to phenol suggested that in macrostructured humus forms soil organic matter is more degraded, whereas high values of acetic acid and of the aliphatic to aromatic compounds ratio in moder and mull meso forms, indicate accumulation of fresh and biodegradable material. This study points to the high potential of humus forms as indicators of forest soil processes.

1. Introduction

Mediterranean ecosystems are characterised by stress factors, such as long drought periods (IPCC, 2007; Mooney, 1989), that can compromise their fragile equilibriums (Sardans and Peñuelas, 2007). Such events may strongly affect microbial activity involved in nutrient mineralisation, with potential shifts to soil degradation dynamics (Bastida et al., 2006), with a progressive depletion of nutrients (Terradas, 2001) due to soil erosion and drought (Sardans and Peñuelas, 2005).

The influence of climate on topsoil, with the development of specific humus forms, has been discussed at the scale of the French continental territory (Ponge et al., 2011) and in Alpine soils (Ascher et al., 2012). Seasonal variability has also been investigated as a controlling factor for Mediterranean microbial communities (Andreetta et al., 2012). These results point to a potential key role of humus forms as indicator of ecosystem changes.

Significant correlations between soil microbial community and both humus macro-morphology (Trap et al., 2011a) and micro-morphology (Frouz and Nováková, 2005) were found, respectively, along a beech (*Fagus sylvatica L.*) forest chronosequence and along a spontaneous succession on post-mining sites.

Enzyme activities are a sensitive indicator of microbial functions (Caldwell, 2005), of stresses to the microbial community and of potential soil degradation (Doran and Parkin, 1994; Jordan et al., 1995), and are more sensitive than other soil properties to environmentally driven soil changes (Filip, 2002; Miralles et al., 2007; Nannipieri et al., 1990). Thus, they are valuable indicators of changes in soil qualities (Dick, 1992). The most dynamic part of the soil profile is the topsoil, where morphological and physical characteristics develop at the fastest rates (Turk et al., 2008). Thus, analysing Mediterranean soils along a gradient of sensitivity to ecosystem changes (mineral soil < humus forms < enzyme activities) seems to be a potential way for predicting disagreeable responses of the forest environment. Although humus forms were considered as an indicator of soil nutrient regimes (Wilson et al., 2001) and as an expression of the rate at which nutrients are circulating within terrestrial ecosystems (Ponge, 2003; Ponge and Chavalier, 2006), a deeper understanding of the biochemical mechanisms by which these processes are modulated is still lacking.

Thickness of the forest floor and the structure of organo-mineral soil horizons are mainly influenced by pedofauna, that acts as an ecosystem engineer (Ponge, 2003; Salmon et al., 2005). Different soil fauna populations lead to different modes of organic matter transport and incorporation into the mineral soil, either by earthworms (Bernier, 1998; Wironen and Moore, 2006), *Collembola* (Chauvat and Ponge, 2002; Salmon et al., 2005), enchytraeids (Kounda-Kiki et al., 2006; Sadaka and Ponge, 2003) and other litter decomposer facies (Chauvat et al., 2007; Topoliantz et al., 2006). Thus, variation of humus forms

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reflects changes in the rate of organic matter degradation and decomposition (Duchaufour, 1997). Links between humus forms, humified complex substances and non humified extracted substances were found in Alpine soils (Bonifacio et al., 2011). Differences in aliphatic compound concentration and distribution, considering specific biomarkers for cutin and suberin, were identified in two contrasting soils and corresponding humus forms, suggesting that these last may potentially provide information on soil organic carbon (SOC) dynamics (Andreetta et al., 2013).

The use of pyrolysis–gas chromatography (Py–GC) has been proposed as a useful tool to evaluate, through separation, identification and relative quantification of individual SOM constituents, the fate of specific compounds in soils (e.g. Buurman et al., 2008; Ceccanti et al., 2007; Van Bergen et al., 1997; Vancampenhout et al., 2009, 2010). Although Py–GC provides just a partial information on OM quality, clearly not representing the overall macromolecular structure (Grandy and Neff, 2008), it is a simple, rapid and cheap technique that has been widely used for the interpretation of OM changes in the soil (Ayuso et al., 1996; Ceccanti et al., 2007; Hernandez et al., 2006; Leinweber and Schuten, 1998; Marinari et al., 2007). The chromatogram presents a characteristic SOM fingerprint, which can be used to discriminate between humus forms (Alcaniz et al., 1987, 1988; Bracewell and Robertson, 1975; Hempfling and Schulten, 1990). Moreover, ratios between specific compounds can be utilised as mineralisation and humification indexes and can enrich the interpretation of the results (Ceccanti et al., 1986, 2007; Macci et al., 2012a, 2012b).

In order to highlight the focal position of different Mediterranean humus forms within biogeochemical cycles, a series of specific objectives were set: a) assessing the activity of enzymes linked to the cycles of major elements; b) correlating soil chemical and physical properties with enzyme activities, to explore specific soil biochemical mechanisms lying behind morphology of humus forms; c) evaluating chemico-structural properties of SOM by Py–GC; and d) identifying changes following mineralisation and humification between humus forms.

2. Material and methods

2.1. Study area and sampling design

31 *Quercus* forest sites were selected within the Italian plots included in the European ICP-Forests network, based on a 16 km × 16 km grid (Van Ranst et al., 1998), modified to 15 by 18 km in Italy. Sites are located in central Italy, with *Quercus* species typical of Mediterranean forests, such as *Quercus ilex*, *Quercus suber*, *Quercus cerris* and *Quercus pubescens*. Sampling was carried out according to standard ICP-Forests protocols Expert Panel on Soil and Forest Soil Coordinating Centre, 2006). At each site, composite samples were made from samples collected at five different points (Fig. 1 A). Organic horizons OF and OH were sampled together by a 25 × 25 cm frame, as OFH layer, due to their small and inconsistent thickness. Mineral soil was sampled to wholly represent fixed soil depth intervals. In this work, we examine the organic OFH layer and the first depth interval of the mineral soil, M01 (0–10 cm). A cylinder method was used to determine bulk density (BD) at 0–5 cm and 5–10 cm. A complete soil profile was described according to FAO (2006), sampled by genetic horizons and classified according to World Reference Base (IUSS Working Group WRB, 2006) (Table 1).

2.2. Humus form classification

The humus form classification used in this study is an application of the proposal by the European Humus Research Group (Zanella et al., 2011). The soil structure (IUSS Working Group WRB, 2006) of the first mineral horizon and the presence/absence of the OH horizon are used as the main diagnostic properties, while the thickness of the organic layers was not considered due to their minor and ephemeral importance in the Mediterranean forest soils (Andreetta et al., 2011). Classification of Mediterranean humus forms should take into account of the fact that OL, OF and OH thickness is low, and highly variable in space. Conversely, A horizon characteristics are more stable across any given site. Humus forms were thus grouped into five types:

- Moder: massive E-AE or bio-microstructured A horizon (peds Ø ≤ 1 mm); OL, OF and OH horizons present.
- Mull meso: bio-mesostructured A horizon (1 mm < Ø ≤ 5 mm); OH horizon absent.
- Amphi meso: bio-mesostructured A horizon (1 mm < Ø ≤ 5 mm); OL, OF and OH horizons present.
- Amphi macro: bio-macrostructured A horizon (Ø >5 mm); OL, OF and OH horizons present.
- Mull macro: bio-macrostructured A horizon (Ø >5 mm); OH horizon absent.

2.3. Soil physical and chemical analyses

Soil samples were air dried and sieved at 2 mm. Soil texture was determined after sample pre-treatment with Na-hexametaphosphate to obtain complete particle dispersion. Sand (53 μm–2 mm) was separated from clay and silt by wet sieving. Clay (<2 μm) and silt (2–53 μm) were determined by the pipette method (Gee and Bauder, 1986). The soil pH was potentiometrically measured in the supernatant suspension of a 1:2.5 soil:water mixture. Total organic carbon (TOC) and nitrogen (TN) were determined by dry combustion with a RC-412 multiphase carbon and a FP-528 protein/nitrogen determinator, respectively (LECO Corporation, USA). Total P was extracted by $NaHCO_3$ at pH 8.5 (Olsen et al., 1954)

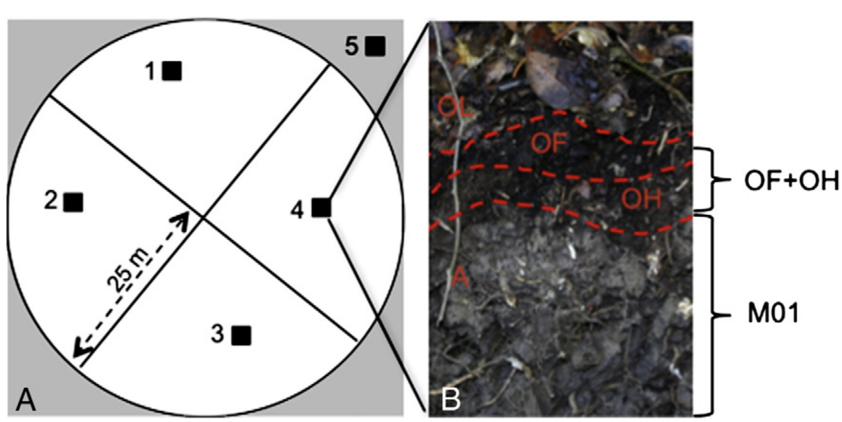

Fig. 1. (A) Representation of the sampling plot. (B) Representative humus profile with indication of the collected layers: organic horizons (OF and OH) which were combined in OFH and the first 10 cm of mineral soil M01.