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# Labile, recalcitrant, and inert organic matter in Mediterranean forest soils

Pere Rovira<sup>a,\*</sup>, V. Ramón Vallejo<sup>b</sup>

a Department of de Biologia Vegetal, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain <sup>b</sup>CEAM, Parc Tecnològic, Charles Darwin 14, 46980 Paterna, València, Spain

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#### Abstract

The biochemical quality of soil organic matter (SOM) was studied in various profiles under *Quercus rotundifolia* Lam. stands on calcareous parent material. Special attention was paid to the question of how biochemical quality is affected by position within the soil profile (upper versus lower horizons). The following global SOM characteristics were investigated: (a) overall recalcitrance, using hydrolysis with either hydrochloric or sulphuric acid; (b) hydrolyzable carbohydrates and polyphenolics; (c) extractability by hot water and quality of the extract; and (d) abundance of inert forms of SOM: charcoal and soot-graphite. The recalcitrance of soil organic carbon (OC) decreases with depth, following the order: H horizons > A horizons > B horizons. In contrast, the recalcitrance of nitrogen is roughly maintained with depth. The ratio carbohydrate C to total OC increases from H to B horizons, due to the increasing importance of cellulosic polysaccharides in B horizons, whereas other carbohydrates are maintained throughout the soil profile at a relatively constant level, 12–15% of the total OC in the horizon. Whereas the quality of the hydrolyzable carbon (measured by the carbohydrate to polyphenolic C ratio) decreases with depth from H to B horizons, the quality of the hot-water extractable organic matter is much higher in B horizons than in A or H horizons. The relative importance of both charcoal and soot-graphitic C and N tends to increase with depth. The ratio black/total is usually higher for N than for C, a result that suggests that inert SOM may represent a relevant compartment in the nitrogen cycle. Overall, our data suggest that in Mediterranean forest soils the organic matter in B horizons could be less stable than often thought.

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Keywords: Mediterranean forest; Black carbon; Soil organic matter; Recalcitrant organic matter; Acid hydrolysis; Available organic matter; Carbohydrates; Polyphenolics; Nitrogen

### 1. Introduction

Soil organic matter (SOM) consists of a variety of compounds with different chemical characteristics and physical availability. These features combine with the prevailing climatic constraints to determine the rate of SOM decomposition, i.e., on the one hand, the repartition of SOM between easily biodegradable and refractory compounds and, on the other hand, the repartition of SOM between physically available and physically protected compounds.

To quantify free and protected SOM pools, the use of physical fractionation procedures is widespread [\(Christen-](#page--1-0)

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[sen, 1992\)](#page--1-0). In contrast, there is less agreement about how to quantify SOM biochemical quality. Ideally, this 'quality' must reflect biodegradability in the absence of physical protection and hence be based on chemical composition independent of physical position within the mineral matrix. Standard humus fractionation methods ([Stevenson, 1982](#page--1-0)) are not completely satisfactory because they are timeexpensive and the fractions obtained (fulvic acids, humic acids, humin) do not clearly correspond to functionally different fractions. Advanced methods like pyrolysis (Py-GC, Py-MS) or <sup>13</sup>C-CPMAS-NMR can provide important information on SOM structural features [\(Schnitzer, 1990\)](#page--1-0), but translating this information to a quantitative measure of SOM quality is complicated.

A useful alternative is acid hydrolysis, proposed by [Stout](#page--1-0) [et al. \(1981\)](#page--1-0) as a simple method to evaluate SOM quality;

<sup>\*</sup>Corresponding author. Tel.: +34934021462; fax: +34934112842. E-mail address: [provira21@yahoo.es \(P. Rovira\).](mailto:provira21@yahoo.es)

this procedure is easy to perform and can be applied to the large series of samples generally employed in ecological research. The non-hydrolyzable residue may include young SOM (e.g., [Bottner and Peyronel, 1977\)](#page--1-0), but most radiocarbon studies have shown that the residue from acid hydrolysis is consistently older than the hydrolyzable fraction, whether the hydrolysis is applied to the whole SOM or to a given chemical fraction such as humic acids or humin ([Goh and Pullar, 1977;](#page--1-0) [Goh and Molloy, 1978;](#page--1-0) [Leavitt et al., 1997;](#page--1-0) [Paul et al., 1997\)](#page--1-0). Refractory plant compounds include lignin, suberin, and fatty acids ([Mind](#page--1-0)[erman, 1968](#page--1-0)), all of which are resistant to acid hydrolysis. [Pelz et al. \(2005\)](#page--1-0) studied the isotopic composition of several SOM fractions, in a context of crop change (from C3 to C4 plants), and found that the non-hydrolyzable residue has clearly the lowest penetration of recent C.

The biochemical quality of SOM, as measured by its hydrolyzability, decreases with decomposition ([Rovira and](#page--1-0) [Vallejo, 2002\)](#page--1-0). Since SOM is older in deep horizons [\(Guillet,](#page--1-0) [1979](#page--1-0)), biochemical quality should decrease with depth. Surprisingly, when acid hydrolysis was applied to mineral soil at several depths the opposite result was obtained, i.e., biochemical quality increased with depth [\(Goh et al., 1984;](#page--1-0) [Joergensen and Meyer, 1990](#page--1-0); [Tan et al., 2004\)](#page--1-0). The scarce available data suggest that the relationship between SOM biochemical quality and position within the soil profile can be complex and thus deserves further attention.

For a more complete picture of SOM biochemical quality, other features must be taken into account. The biochemical quality of the whole SOM could not translate to the immediately available fractions, and hence the latter had to be included in the study. Also, in addition to recalcitrant compounds, the presence of an inert pool has often been mentioned, either from theoretical approaches (RothC model: [Jenkinson, 1990\)](#page--1-0), or from analytical studies ([Poirier et al., 2000](#page--1-0)). Owing to wildfires, black carbon (BC) is expected to be a main component of such an inert fraction in Mediterranean ecosystems [\(Poirier et al., 2000\)](#page--1-0).

The aim of this paper is to characterize SOM in Mediterranean forest soils from the point of view of its biochemical quality. We applied various chemical treatments (acid hydrolysis, BC analysis, hot water extraction) to a set of soil profiles in the Mediterranean area to obtain insight on: (i) the abundance of labile, recalcitrant, and inert pools of SOM; (ii) how SOM biochemical quality depends on the position in the soil profile; (iii) how the overall SOM quality affects the quality of the most available SOM pools.

#### 2. Materials and methods

## 2.1. Soils

The soil profiles studied [\(Table 1](#page--1-0)) were all located in the area of Lleida (Catalonia, NE Spain), under Quercus rotundifolia stands. All parent materials were calcareous (limestone, calcareous sandstone, and marl). Since position within the soil profile was one of the main issues in this study, soil horizons were classified in three large groups: (i) organic L and F horizons were not included in the study; (ii) the first horizon under the F horizon, usually dark, highly organic and poor in recognizable plant debris, was considered the 'H' horizon if its organic carbon (OC) content was  $>20\%$ . Otherwise, it was considered the 'A' horizon; (iii) the first horizon below the H horizon was considered the 'A' horizon; and (iv) any horizon below the A horizon was considered a 'B' horizon. A soil profile can have several B horizons, but only a single H and a single A horizon.

The profiles were usually poorly differentiated; the A horizon was always present, whereas the B and/or H horizons may be absent. The sampled horizons were airdried and crushed to pass through a 2 mm mesh. The texture of the fine earth  $(< 2 \text{ mm})$  was obtained by the pipette method; total OC by dichromate oxidation, using an aluminum digestion block ([Nelson and Sommers, 1996\)](#page--1-0); total N by using a CARLO ERBA analyzer; carbonates by the calcimeter method; pH in water, in a suspension 1:2.5 w/v.

#### 2.2. Acid hydrolysis

To obtain the most complete picture possible, we applied two different protocols:

- (a) Single-step hydrolysis with HCl. 100 mg (in H horizons) or 400–500 mg (in A and B horizons) were hydrolyzed with 20 ml of 6 M HCl in sealed Pyrex tubes, at  $105^{\circ}$ C for 18 h. The hydrolysate was discarded. The unhydrolyzed residue was washed in deionized water with repeated centrifugations and decantations, and then transferred to pre-weighed vials, dried at  $60^{\circ}$ C to constant weight, and analysed for C and N using a CARLO ERBA analyzer.
- (b) Two-step hydrolysis with  $H_2SO_4$  ([Oades et al., 1970;](#page--1-0) [Rovira and Vallejo, 2000\)](#page--1-0). 100 mg (in H horizons) or 400–500 mg (in A and B horizons) were hydrolyzed with 20 ml of  $2.5 M H<sub>2</sub>SO<sub>4</sub>$  in sealed Pyrex tubes, at  $105^{\circ}$ C for 30 min. The hydrolysate (labile pool I) was recovered by centrifugation. The residue was washed with water and dried. Then, 2 ml of  $13 M H_2SO_4$  was added, and the tubes were placed in an end-over-end shaker overnight. After diluting the acid with water to 1 M, the residue was hydrolyzed 3 h at  $105^{\circ}$ C. The hydrolysate (labile pool II) was also recovered by centrifugation. The residue (recalcitrant pool) was washed again, dried at  $60^{\circ}$ C, weighed, and analysed for C and N with a CARLO ERBA analyzer.

In both methods, we define the 'recalcitrance index' (RI) as follows:

- $RI_C =$  (unhydrolyzed C/total OC)  $\times$  100,
- $RI_N =$  (unhydrolyzed N/total N)  $\times$  100.

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