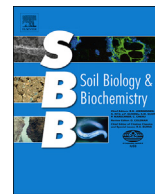




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Effect of land use change on contents and distribution of monosaccharides within density fractions of calcareous soil

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ABSTRACT

In the last century, many calcareous soils in Castilla León (northwestern Spain) have been transformed from natural *Quercus ilex* forest to cropped land. During the past 40 years, cropping was abandoned and some of these soils has been reforested with *Pinus halepensis*. We studied how these land use changes affected the soil organic matter amount (C stocks) and characteristics. Density fractionation combined with ultrasonic dispersion enables separation and study of soil OM fractions, considered on the basis of the mechanisms of physical protection: non-physically protected OM (FF), OM occluded into aggregates (OF), and OM stabilized in organo-mineral complexes (DF). In the present study, whole soils and density fractions were analyzed for organic C amount (OC) and for carbohydrate content. The study aimed to describe the carbohydrate content in whole soils and its SOM density fractions to find out whether the carbohydrates can be used to explain the origin of SOM fractions and if they can depict its response to land management. We found a loss of 67% of the topsoil OC as a result of converting the natural *Quercus ilex* forest to cropped land, ~100 years ago. After crop abandonment and soil reforestation with *Pinus halepensis*, the stock of topsoil OC increased. After ~40 years of pine plantation, an average recovery of 71% of the previously lost OC had been recovered (43% loss of reference topsoil). The changes in OC stocks affected mainly the free fraction (FF) but also the organo-mineral (DF) one. Our results point to the role of physical protection in the C stocks but also that organo-mineral associations could be disrupted under a land use change. Monosaccharide content was significantly different ($p < 0.01$) among land uses. Gas chromatography analysis revealed significant differences in monosaccharide composition between land uses and also among density fractions. Whatever the fraction and land use considered, glucose was the dominant sugar monomer, followed by mannose and xylose.

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1. Introduction

Soil organic matter (SOM) is recognised as an important factor in C driven climate change (Sanderman et al., 2003). Because of this, the capacity of soils to accumulate organic C has received great attention in recent years. Land use and management influence the amount and the dynamics of SOM (Zhang et al., 1999). Numerous studies have shown that conversion of native ecosystems to agriculture disturbs the ecological balance, disrupting the C cycle and generally results in net loss of soil C (e.g., Paustian et al., 1997). However, when arable land is converted to permanent vegetation

the soil organic carbon (OC) increases gradually (Haynes and Beare, 1996). That increase of soil C stock is probably due to a greater incorporation of OM and also to the lack of tillage. Adsorption to soil minerals and occlusion within soil aggregates have been universally demonstrated to shield SOC from decomposition. However, the role of minerals in SOM adsorption and stability depends on their mineralogy and soil characteristics. Studies of the effects of land use change on soil OC in calcareous soils are scarce. However, calcareous soils are of particular interest because they represent approximately 12% of the world soil resources (FAO, 1996).

Changes in management not only influence SOM quantity but may also affect its quality. Of the various components of the SOM, carbohydrates have an important role in nutrient cycling (Hu et al., 1997). Carbohydrates are one of the main components of SOM and are considered readily degradable materials that can provide

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information about SOM dynamics (Sposito, 2008). Carbohydrates comprise about 50–70% of the dry weight of most plants, which makes them the most introduced compounds to soil (Stevenson, 1994). In soil systems, carbohydrates exist in various forms, from simple sugars or oligosaccharides to humic-polysaccharide complexes (Cheshire et al., 1992). The relative composition of carbohydrates can provide information on the origin of carbohydrates (Hu et al., 1997) and the relative content of carbohydrates in organic matter fractions can depict the nature of these fractions and their cycling pattern and response to land management (Abdelrahman et al., 2016). Monosaccharides (MS) in soil are subject to rapid turnover and are involved in biochemical and organo-mineral reactions. They represent a primary source of nutrients and energy for soil micro-organisms and contribute to microbial activity and, therefore, may be a sensitive indicator of several biological properties (Larré-Larrouy et al., 2003). Carbohydrates also play a role in enhancing soil structural stability because they are of particular importance in promoting aggregate stability (Feller and Beare, 1997). However, the effects of land use changes on carbohydrates are largely unknown. Information on the nature and distribution of the soil MS in the soil density fractions is thus important for the understanding of the role of carbohydrates in organic matter stability. Based on the observation that a predominance of hexoses represents microbial derived materials while a predominance of pentose represents plant-derived materials (Guggenberger et al., 1994), the ratios C6/C5 ((mannose + galactose)/(xylose + arabinose)) and Desoxy/C5 ((rhannose + fructose)/(xylose + arabinose)) is frequently used as a proxy to determine the share of microbial OM (Oades, 1984; Six et al., 2006 and Ludwig et al., 2015).

Soil molecular structure of SOC does not necessarily predetermine the persistence of OC in soils and adsorption to soil minerals and occlusion within soil aggregates have been universally demonstrated to shield SOC from decomposition (Han et al., 2016). Soil organic matter density fractionation following ultrasonic dispersion enables the separation and study of three different fractions according to the different mechanisms of physical protection (Sohi et al., 2001). Based on these stabilization mechanisms, it is possible to distinguish three SOM fractions: (1) “free” OM (free fraction, FF), isolated before ultrasonic break-down of stable aggregates; (2) OM occluded within aggregates (occluded fraction, OF), isolated after ultrasonic dispersion to break the aggregates; and (3) organo-mineral fraction (dense fraction, DF) recovered as the residual (heavy) material. Many studies (e.g. Golchin et al., 1994a; Six et al., 2002; John et al., 2005) have shown that the un-protected FF represents a labile SOM pool with a rapid turnover rate. In contrast OF and DF are more stable pools, with turnover times ranging from decades to centuries. Therefore, density fractions may be affected differently by changes in land use.

The aims of the present study were (1) to assess and compare land use effects on soil C content and its distribution among density fractions; (2) to determine the content and origin (microbial or plant-derived) of monosaccharides in whole soils and soil density fractions.

2. Material and methods

2.1. Site description

The study was carried out in calcareous soils in the region of Castilla y León (northwestern Spain), UTM: 30T 384465 E 4639001 N. The mean annual rainfall in the region is around 400 mm, under a xeric moisture regime, and the mean annual temperature 12.3 °C. The altitude of the moor is between 800 and 900 m above sea level, with low slopes (<7%). The soils

(Calcixerept) are quite homogeneous in spite of differences in their land use history. The native vegetation in the studied calcareous soils is Holm-oak wood (*Quercus ilex* subsp *ballota*). In the 19th century, most of the natural forest was converted into agricultural land (cereal crops), but since the 1950s, reforestation with *Pinus halepensis* has been carried out on abandoned agricultural land. Agricultural land is currently cropped with cereals (usually barley), managed by conventional tillage system. The average basal area of the *Quercus ilex* forest is 13 m² ha⁻¹ with an average density of 3200 trees ha⁻¹. The average basal area of the pine plantation is 50 m² ha⁻¹, with an average density of 1184 trees ha⁻¹. For the characterization of the selected plots we support the previous information using historical aerial photos, historical maps and also through discussions with local residents.

The main characteristics of the soil samples are shown in Table 1. We can observe a systematic higher % of coarse material in soils under oak forest related to a stone removal management applied to the cropped lands.

2.2. Sampling procedures

A land use map of the calcareous moor of Castilla y León was elaborated with a GIS (ArcGIS 10.3 for Windows) following the process shown in Fig. 1.

The map was used to select the sampling plots on the basis of the following criteria: a) *Quercus ilex* forest, cropped land, and *Pinus halepensis* plantations in adjacent areas; b) minimum area of each land use of 1 ha. For this study, plots of the above-mentioned land uses were selected in three different regions, and four representative plots were sampled (0–10 cm depth) in each region. A total of 36 topsoils (0–10 cm depth) (3 regions x 3 land uses x 4 plots) were thus sampled. A composite sample (~2 kg) by joining 3 soil sub-samples, was obtained for each plot. Soils under forest uses were sampled in points under canopy trees.

Visible plant residues and roots were removed; soil was air-dried, sieved (<2 mm) and stored in plastic bags until analysis. For bulk density determinations, all of the soil extracted from the soil pit was weighed. The volume of the soil was calculated from the volume of water required to fill the hole (after impermeabilization of the soil pit with plastic sheeting) following USDA (1999).

2.3. Density fractionation of soil

A density fractionation procedure was applied. The method follows the concept of Golchin et al. (1994b), who differentiated three degrees of physical protection of OM: FF, non-protected; OF, occluded within aggregates - extractable by sonication; and DF, retained in the dense residual material after sonication.

Briefly, 5 g of soil sample were placed in small centrifuge bottles (50 ml capacity), and 35 ml of NaI at 1.8 g ml⁻¹ density were added. The bottles were shaken gently and the floating material, considered as the FF, was then recovered by centrifugation at 800g for 30 min at 18 °C, and filtered over a vacuum filter, using a glass fiber filter (Whatman GF/F), by washing with deionized water. The residue remaining was placed in an ice bath and sonicated at 300 J ml⁻¹ with a probe-type ultrasonic disintegrator (Branson 450 W).

The floating material, considered as the OF, was recovered by centrifugation and washed in the same way as the FF. The remaining material, considered the DF, was washed with deionized water. All fractions were dried at 40 °C, weighed, ground in a mortar and pestle, and analyzed for C and N.

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