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Graphical statistical approach to soil organic matter resilience using analytical pyrolysis data

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ABSTRACT

Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) of humic acids (HAs) from 30 agricultural soils from a volcanic island (Tenerife, Spain) was used to discern the molecular characteristics of soil organic matter (SOM) associated to resilience. For faster perceptual identification of the results, the yields of the pyrolysis products in the form of surface density plots were compared in an update of the Van Krevelen graphical statistical method. This approach, with respect to data reduction and visualization, was also used to collectively represent statistical indices that were obtained after simple and partial least squares (PLS) regression. The resulting plots illustrate different SOM structural domains (for example, carbohydrate- and lignin-derived and condensed lipid). The content of SOM and total mineralization coefficient (TMC) values can be well estimated from the relative abundance of 57 major pyrolysis compounds: SOM content and composition parallels the accumulation of lignin- and carbohydrate-derived structures (lignocellulosic material) and the depletion of condensed polyalkyl structures. In other words, in the volcanic ash soils that were studied, we found that the higher the amount of SOM, the lower its quality in terms of resilience. Although no cause-and-effect is inferred from this fact, it is evident that the resistance to biodegradation of the SOM is related to its molecular composition.

1. Introduction

There is a controversy about whether the resilience of soil organic matter (SOM) is intrinsic to its molecular composition or whether it depends on physical protection by soil minerals [1]. Several classical studies have suggested that SOM resistance to biodegradation may be explained by intrinsic factors, such as a chaotic macromolecular structure consisting of a disordered arrangement of "building blocks" [2–4]. However, it is clear that some extrinsic factors may also play a role in the retention of organic C in soil [5]. Among these extrinsic factors there are the physical occlusion of SOM into micro-aggregates [6,7] and the formation of organo-mineral complexes with fine clays or amorphous oxides [3,4,8,9] with a substantial bearing on soil agro-ecological quality [10–12]. Notwithstanding, it is apparent that additional research is required in order to establish the extent to which soil carbon sequestration may depend on multiple interrelated factors [13–17].

Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) involves a thermolytic degradation of macromolecules into small fragments that may be separated and identified by GC-MS [18–19]. This degradation technique is able to release meaningful structural fragments even from C—C bonded chemically recalcitrant materials [20]. In addition, SOM pyrolysis generates a wide range of products that can be related to its origin (e.g., methoxyphenols from lignin, furans from polysaccharides, N-molecules from proteins, etc. [21–22]). The technique has been used successfully to assess the SOM quality or maturity i.e., the extent to which plant biomacromolecules such as lignin are transformed into amorphous colloidal material of chaotic structure known as humic acids (HAs). In this material, structural domains consisting of plant or microbial macromolecules may remain to an extent that depends on its progressive stages of maturity [23–25]. In particular, progressive changes in the signature of lignin constituents during humification reflect the transformation of plant biomacromolecules into stabilized SOM [26].

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In the present paper, a new method is described for using Py-GC-MS data that are generated from soils. A chemometric study of the chromatographic data from volcanic soils under agricultural management (Tenerife Island, Spain), including several predictive data mining analyses, was carried out in order to identify molecular proxies with potential use as soil carbon storage indexes. With this aim, total abundances of the major pyrolysis products were compared using the classical Van Krevelen graphical statistical method [27], updated as surface density values in the space defined by the compound-specific H/C and O/C atomic ratios [28]. These 3D Van Krevelen plots, which may be represented as surfaces or contour diagrams, were used in our approach to represent not only the sizes of the chromatographic peaks, but also statistical indices that were calculated by uni- or multivariate data analyses, or subtraction values between compound yields from different groups of samples for comparison purposes. In all cases, the purpose was to identify, within the pyrolysis chromatographic data, molecular descriptors for SOM resilience.

2. Materials and methods

2.1. Studied sites and soil samples

Tenerife (Spain) is a volcanic island that is one of seven in the Canary islands, located at 28.280 N and 16.290 W, with a maximum altitude of 3,718 m (Teide volcano). The geology of Tenerife island includes basaltic lava flows (predominant) and pyroclastic materials [29].

The present work analyzes 30 soils corresponding to vineyards subjected to different management practices (Table 1). The sampling points were selected with the intention to include the maximum C range and variability in quantitative environmental factors with a presumptive role in soil C storage. The main characteristics and agricultural system for each plot are summarized in Table 1. Further details have been reported elsewhere in the body of literature [30].

2.2. General analysis of soil

Soil samples were collected in triplicate from 100 m^2 plots from the upper 20–30 cm of the profile (Ap horizon), and immediately transported in a fridge to the laboratory to avoid microbial degradations. Before analysis, the samples were air-dried and homogenized to < 2 mm (fine earth); gravel (> 2 mm) was weighed and discarded.

For pH measurements, a 1:2.5 soil-to-water mixture was used; N content was measured by micro-Kjeldahl digestion. Soil organic carbon (SOC) was determined by using the wet oxidation method [31]. Particle-size was measured using the hydrometer method [32].

2.3. Isolation and purification of humic acids

The isolation of SOM fractions was undertaken using alkaline reagents [33]. In brief, total extractable humic substances were isolated with 0.1 mol L^{-1} $Na_4P_2O_7$ and 0.1 mol L^{-1} NaOH. The first step is needed to remove Al associated with SOM and release the humic substances that are complexed with hydrous amorphous oxides, which are frequent in volcanic soils. Then, the total humic extract was divided into (i) HAs, the acid-insoluble fraction, (ii) fulvic acids (fraction soluble at all pH conditions), and (iii) humin (non-extractable insoluble fraction). In order to remove the accompanying mineral content that was coextracted or incorporated in the course of the extraction, the HAs were dissolved in 0.5 mol L^{-1} NaOH and centrifuged at 43,500 g; the centrifugation pellets were discarded and the sodium humates were re-precipitated with acid (6 mol L^{-1} HCl). The HAs were finally dialyzed into cellophane bags (Visking® dialysis tubing, molecular weight cut-off 18,000 D) and desiccated at 40 °C.

2.4. Incubation experiment

The CO_2 released by the soils in the course of its biological transformation was determined under standard laboratory conditions. Soil samples (20 g) at 66% of water holding capacity (at atmospheric pressure) were incubated in Erlenmeyer flasks for 30 days at 27 °C. The CO_2 accumulated in the flasks was determined periodically (every 1–5 days, depending on the stage of the experiment) using a gas analyzer (Carmhograph-12; Whöstoff, Germany). During each measurement, the atmosphere of the flasks was replaced with CO_2-free air passed through a soda lime filter. Data were expressed as total mineralization coefficients (TMC) in mg C kg C $soil^{-1}$ day^{-1} [34].

2.5. Pyrolysis-gas chromatography-mass spectrometry

Analytical pyrolysis was performed in the HA fractions samples using a Pyrojector® (SGE Analytical Science, Melbourne, Australia). Approximately one mg HAs in a quartz capillary tube was introduced into a preheated furnace at 500 °C. The pyrolysis products were separated and analyzed in a GC-MS system (Finnigan Trace GC Ultra) with a Trace dual stage quadrupole (DSQ) mass spectrometer (Thermo Finnigan LLC., Austin TX, USA) fitted with a fused silica capillary non-polar general-purpose column HP-1 wall-coated, silphenylene polysiloxane, 30 m × 0.25 mm i.d. × 0.25 μm film thickness (Quadrex Corp., Woodbridge, CT, USA). Carrier gas was He and flow was adjusted to 1 mL min^{-1}. The total chromatographic time was 60 min. The initial temperature was kept to 50 °C for one minute, and then increased up to 100 °C at 30 °C min^{-1}, from 100–300 °C at 10 °C min^{-1} and was isothermal at 320 °C.

Tentative assignment and relative quantitation of pyrolysis compounds were undertaken by using: i) selected ion traces in reconstructed ion chromatograms for the major expected compounds (i.e., the well-known, frequent SOM pyrolysis products), ii) comparison with own laboratory databases of chromatographic data (i.e., MS, retention time (RT), response factors, literature references, etc.) of pyrolysis compounds, based on previous injection either of commercial standards or purified preparations of plant and microbial biomacromolecules (e.g., lignin, suberins, polysaccharides, etc.) which were used to confirm the RTs of the peaks for the main series of homologues and iii) from their electron impact mass spectra (70 eV) using the NIST and Wiley spectral libraries for automatic library searching, in addition to "ad hoc" self-developed computer programmes for manual heuristic searching (mainly using reverse fitting) in the aforementioned specific databases of SOM pyrolysis compounds. The peak areas (as total area counts) of the different chromatographic peaks were integrated and calculated as total abundances.

In the case of most diagnostic pyrolysis compounds, no attempt for peak deconvolution was carried out, since the major problems of overlapped peaks corresponded to coelution with well-defined alkyl series of alkanes, alkenes and fatty acids. The well-defined series were previously extracted as single ion traces using the ions at *m/z* 57, 55 and 73, respectively. Integration values of the peaks in the traces for these single ions were transformed into total ion counts via multiplying by suitable response factors that were calculated from independent injections of commercial standards. A similar semiquantitative routine, in the case of any other typical pyrolytic series (i.e., methoxyphenols, alkylbenzenes, catechols, etc.), was carried out by integrating the selected peaks in ion chromatograms at known RT. The total abundances as total ion chromatographic signal were calculated using conversion

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