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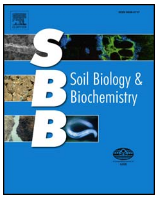

# Rock-Eval pyrolysis discriminates soil macro-aggregates formed by plants and earthworms

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## ABSTRACT

Plants and earthworms, as soil ecosystem engineers, play a crucial role during stabilisation of organic matter in soil through its incorporation into soil aggregates. It is therefore essential to better understand the mechanisms and interactions of soil engineering organisms regarding soil organic matter stabilisation. Several methods have already been successfully applied to differentiate soil aggregates by their origin, but they cannot specify the degree of organic matter stability within soil aggregates. Rock-Eval pyrolysis has already been proved to be pertinent for analyses of soil organic matter bulk chemistry and thermal stability, but it has not yet been directly applied to identify biogenic organic matter signatures within soil aggregates. In this study, Rock-Eval pyrolysis was used for the identification of the soil aggregate origin as well as for the determination of the soil organic matter bulk chemistry and thermal stability in a controlled experiment. Mesocosms were set up, containing treatments with a plant, an earthworm species, or both. Water stable soil macro-aggregates > 250 μm were sampled and tested with Rock-Eval pyrolysis after a two-month incubation period. Rock-Eval pyrolysis was able to differentiate soil macro-aggregates by their origin, and to identify a specific signature for each treatment. Macro-aggregates from the plant and earthworm treatment were characterized by a mixed signature incoming from the two soil engineers, indicating that both engineers contribute concomitantly to soil aggregate formation. Organic matter thermal stability was not positively affected by earthworms and even tends to decrease for the plant treatment, emphasising that organic matter was mainly physically protected during the incubation period, but not stabilised. However, future research is required to test if signatures for the tested organisms are species-specific or generally assignable to other plant and earthworm species.

## 1. Introduction

Earthworms and plants are essential soil ecosystem engineers in temperate systems due to their ability to structure soils through the formation of water-stable soil macro-aggregates. Plants form soil macro-aggregates either through mechanical enmeshment of soil particles by roots or through the secretion of root exudates cementing soil particles together (Degens et al., 1994; Angers and Caron, 1998). Earthworms fractionate soil organic matter (SOM) and build up a stable soil structure through SOM incorporation into soil macro-aggregates (Lavelle et al., 1997; Brown et al., 2000; Tanner, 2001). They combine mineral and organic matter by feeding on soil and glue selected soil particles together with saliva and mucus as they pass through the digestive tract (Blanchart et al., 1997; Brown et al., 2000; Lavelle and Spain, 2001). These biological processes combined with SOM biogeochemical stabilisation mechanisms determine the residence time of SOM in the pedosphere (Schmidt et al., 2011). A changing paradigm was presented by Schmidt et al. (2011), indicating that SOM stability is driven through biological and physicochemical influences from the surrounding environment rather than by its molecular structure. Labile organic matter (OM) can thus persist for decades if it is protected against microbial decay through adsorption to mineral surfaces or occlusion into soil aggregates (Christensen, 1996; Sollins et al., 1996; von Lützow et al., 2006; Jastrow et al., 2007). According to this, processes of SOM turnover rates have to be investigated not only at the molecular scale, but must be extended to the scale of the ecosystem functioning

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(Schmidt et al., 2011). This primarily concerns the mechanisms of how ecosystem engineers incorporate SOM into soil aggregates, e.g. using plant root exudates or mucus from earthworms' digestive tracts. However, the identification of the soil aggregates' origin according to the biological fingerprints from their respective engineers remains challenging. Aggregates from field samples are not only characterized by fresh biogenic OM through its modification of plants and earthworms, but also contain previously incorporated OM from the bulk soil, either found as particulate organic matter or as organic matter associated to the fine soil fraction. Using controlled laboratory experiments (i.e. equal initial organic matter content and bulk chemistry, no external litter input, introduction of specific plants or earthworms), differences in SOM signatures in aggregates can be discriminated and thus assigned to the activity of each specific soil engineer. Near infrared spectroscopy (NIRS) has been successfully applied to the differentiation of the soil aggregate origin in several microcosm experiments under controlled conditions (Hedde et al., 2005; Velasquez et al., 2007; Zhang et al., 2009; Huerta et al., 2013; Zangerlé et al., 2011, 2014). This method allows the quantitative determination of biogenic structures of soil aggregates based on the identification of functional OM groups at specific infrared wavelengths. This analysis draws a specific OM fingerprint that can be assigned to one specific ecosystem engineer. However, previous studies indicate that certain requirements for the soil substrate have to be fulfilled in order to obtain optimum data accuracy (Chodak, 2008). Assessment of total C and N values were imprecise in soils with low TOC contents (i.e. 0.3%) (Dalal and Henry, 1986), when calibrating NIRS data. Furthermore, C-O bounds of carbonates were shown to affect NIRS results in soils containing extraordinary high carbonate contents (Cozzolino and Moron, 2004; Chodak, 2008). An alternative method is thus required in order to distinguish soil aggregates even under unfavourable soil conditions for measurements.

Rock-Eval pyrolysis has been described as a low-cost and a technically less demanding method for the characterization and quantification of soil carbon, as it does not require any previous treatment of the sample (Lafargue et al., 1998; Disnar et al., 2003). Compounds of organic and inorganic matter are identified through a stepwise pyrolysis of a sample in an inert/oxygen atmosphere by which SOM and carbon-bearing minerals are broken down according to its thermal stability (Lafargue et al., 1998; Behar et al., 2001). Initially developed for the exploration of oil and gas reservoirs (Espitalié et al., 1985), this method has already proved pertinent for the evaluation of several biogeochemical problems, such as for the exploration of contaminated sites (Lafargue et al., 1998) or for the estimation of OM decay and transformation rates in soil and sediments (Sebag et al., 2006; Marchand et al., 2008; Carrie et al., 2009; Hare et al., 2014; Albrecht et al., 2015). Recently, the method was applied to the identification of SOM thermal stability in soil horizons from soils around the world based on more than 1000 samples (Sebag et al., 2016). This approach has not yet been performed on OM identification in soil aggregates formed by plants and earthworms.

We thus aim to test the applicability of Rock-Eval pyrolysis for the first time on water stable soil macro-aggregates created by ecosystem engineers, coupled with controlled sediment and OM inputs. In doing so, we attempt to distinguish these aggregates according to their origin from either plants, earthworms or both. Based on the technical features offered by Rock-Eval pyrolysis, our study was conducted using a three-step analysis including (i) a quantitative OM analysis through the determination of organic and mineral carbon contents, (ii) a qualitative OM analysis through the calculation of standard Rock-Eval parameters and, (iii) a thermal stability analysis of the OM using new indices, as proposed in Sebag et al. (2016). Considering these analyses, we developed the following hypotheses: (i) the composition of organic matter in soil macro-aggregates can be discriminated and thus assigned to engineering effects of plants and earthworms, respectively, and, (ii) the ecosystem engineers affect the OM bulk chemistry during the aggregation process. We furthermore expect (iii) that thermal stability of OM in macro-aggregates is improved if soil engineers contribute to aggregate formation.

## 2. Material and methods

### 2.1. Incubation experiment

A mesocosm experiment was set up and incubated in pots over 8 weeks in a climatic chamber under controlled conditions. Twenty pots (10 cm in height, 7 cm in diameter at the bottom increasing to 11 cm at the top) were prepared and allocated to four different treatments with five replicates each, containing plants (P), earthworms (EW), and both plants and earthworms (P + EW). The remaining five pots were kept as a control (CT) but treated under the same conditions. Pots were wrapped in an aluminium foil and covered with a net of 1 mm mesh size at the bottom to allow drainage and prevent anoxic conditions. A transparent plastic cylinder was installed on top to prevent earthworms from escaping. The pots were filled with a silty alluvial sediment composed of 3% clay, 67% silt, and 30% sand content, a $pH_{water}$ value of 7.95, and 30% total carbonates. This sediment was collected at the restored section of the Thur River floodplain at Niederneunforn (8°77′12″ E, 47°59′10″ N), Thurgau canton, Switzerland. It is a recent deposit, overlying a Calcaric Fluvisol (Siltic) (IUSS Working Group WRB, 2015). The sediment fraction was oven-dried at 40 °C for 72 h in order to preserve the SOM fraction, and sieved by hand at 2 mm. In the field, seedlings of the pioneer plant species *Phalaris arundinacea*, weighing between 5 and 7 g were sampled, and adult earthworms of the endogeic species *Allolobophora chlorotica* were collected using the “hot” mustard extraction method (Lawrence and Bowers, 2002). Dead leaves from the willow tree *Salix viminalis* were air-dried and crushed by hand to provide food for earthworms during the incubation experiment. Pots were filled with 600 g of sediment mixed with 1 g of *Salix viminalis* leaves, rewetting the sediment after each 2 cm of filling. One seedling of *Phalaris arundinacea* was planted in the pots for P and P + EW treatments. A group of three adult earthworms of similar total biomass was added to each pot for EW and P + EW treatments.

Pots were incubated for 8 weeks at 18 ± 3 °C, 65% humidity, and a 16/8 h day-night time rhythm simulated in a climate chamber. Humidity in the pots was controlled once a week over the total weight of the pots and rewetted, if necessary, to keep the soil moisture content at field capacity. Irrigation was performed using a fog irrigation nozzle in order to preserve new macro-aggregates built at the soil surface. Pots were randomly arranged under the artificial lights after each humidity control to avoid a potential position effect inside the climate chamber.

### 2.2. Aggregate sampling

Macro-aggregates of 0.250–2 mm size were sampled after 8 weeks of incubation using two sieves arranged on top of each other. Aggregates larger than 2 mm and smaller than 0.250 mm were neglected, whereas those remaining on the 0.250 mm sieve were carefully plunged into demineralized water at 25 °C for 5 min (Murer et al., 1993). This treatment preserves macro-aggregates that have an increased stability compared to aggregates formed by desiccation and remoistening (Tisdall and Oades, 1982; Jastrow and Miller, 1991; Six et al., 2000). These water-stable macro-aggregates are formed by a combination of biogeochemical processes, to which plants and earthworms contribute to a large extent (Shipitalo and Le Bayon, 2004; Milleret et al., 2009b; Fonte et al., 2012). Macro-aggregates were then air-dried over an entire week and finely crushed for further analyses. Soil material from the CT pots was only sampled and air-dried before being crushed.