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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 (Espitalité 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.

2.3. Rock-Eval pyrolysis

The organic matter associated with the macro-aggregates was investigated with a sample of about 50–60 mg of fine crushed aggregates using a Rock-Eval 6 pyrolyser (Vinci Technologies) described in Lafargue et al. (1998) and Behar et al. (2001). The method is based on a stepwise pyrolysis and combustion of OM, releasing CO and CO_2 gases monitored by a flame ionisation detector (FID) for pyrolysis and an infrared detector (IR) for combustion under an artificial air supply (N_2O_2 20/80). Released hydrocarbons monitored by FID are graphed by two curves (S1 and S2). The S1 curve is ignored due to its occurrence as a pseudo-peak because soil samples usually do not contain thermo-vaporised free hydrocarbons, whereas the S2 curve represents the sum of all hydrocarbons. The TpS2 reflects the temperature at which the S2 peak reaches its maximum. The S3 curves represent the amount of CO and CO_2 released during pyrolysis in an inert atmosphere. The S4 and S5 curves are formed during the combustion of residual carbon under artificial air supply and detected by the IR detector (Behar et al., 2001; Disnar et al., 2003; Hetényi et al., 2005). Quantitative OM analysis was performed through the determination of the mineral carbon content (MINC), consisting of $CaCO_3$ and refractory organic carbon (CorgR) to a small extent. Furthermore, total organic carbon (TOC) is calculated using the sum of all released carbon components representing pyrolysable carbon (PC) and residual carbon (RC), which is only released during the combustion (Behar et al., 2001; Disnar et al., 2003). Further elemental analysis of SOM was not performed in this study due to the high conformity of TOC values in soil obtained from the Rock-Eval 6 pyrolyser and the Leco CNS-2000 ($R^2 = 0.998$, Disnar et al., 2003) and a Flash 2000 NC Analyzer ($R^2 = 0.987$, Saenger et al., 2013). Information on the OM bulk chemistry was provided by the Hydrogen index (HI), which summarizes the total amount of hydrocarbons appearing as the S2 peak normalized to TOC content, and the Oxygen index (OI), which includes the total amount of oxygen released during the CO and CO_2 production normalized to TOC content. Larger amounts of hydrocarbons are synonymous with easy decayable OM, whereas the amount of more mature OM is increased if more oxygen is released during CO and CO_2 production (Hetényi et al., 2005; 2006; Disnar et al., 2003; Carrie et al., 2012). SOM thermal stability was analysed through the calculation of the refractory OM index (R-index) and immature OM index (I-index) proposed by Sebag et al. (2016). These indices are calculated using relationships between five subdivided areas under the S2 curve according to predefined temperature thresholds ranging from the A1 area (for the lowest temperatures) to the A5 (for the highest ones) (Sebag et al., 2016). Higher temperature areas representing more thermally refractory OM compounds are used for the R-index calculation according to the following equation:

$$\text{R-index} = (A3 + A4 + A5) / 100 \tag{1}$$

whereas, the I-index is calculated using lower temperature areas representing thermally more labile OM compounds (Albrecht et al., 2015) following:

$$\text{I-index} = \log_{10}((A1 + A2)/A3) \tag{2}$$

Referring only to the signal of the S2 curve, R-Index and I-Index are independent from the $CaCO_3$ content. Furthermore, the HI-index was additionally used as an indicator for SOM thermal stability analysis (Gregorich et al., 2015; Barré et al., 2016).

2.4. Carbonate content analysis

Subsequent carbonate analyses were performed on macro-aggregates in order to better interpret the values obtained from the Rock-Eval pyrolysis. Total carbonate content ($CaCO_3$ total) was determined using a Bernard Calcimeter with a readout scale precision of 0.1 ml (Vatan, 1967). The proportion of bioavailable carbonate ($CaCO_3$ active) was measured according to the method of Drouineau-Galet (Drouineau, 1942) using a Metrohm 702 SM Titrino titration apparatus.

2.5. Statistics

As the incubation experiment was set up as a 2 × 2 factorial design, two-way ANOVAs were conducted testing three omnibus effects: the main effect of each engineer P and EW and the interaction effect between these two engineers. All three effects were tested on Rock-Eval standard parameters (TOC, MINC, PC and RC), values from carbonate analyses, and calculated HI, OI, R- and I-indices. In case where an interaction effect could be found, the specific differences in treatments were subsequently identified with Tukey's HSD post-hoc test. If necessary, data were square-rooted in advance to meet the requirements for normal distribution and variance homogeneity. All statistics and data visualizations were performed with R version 3.3.2 (R Development Core Team, 2016) using “ggplot2” and “Cowplot” packages.

3. Results

3.1. Quantitative SOM and carbonate analysis

Results for quantitative SOM analysis indicated low values for all treatments ranging between 1.07 and 1.36% for TOC, between 0.86 and 1.08% for RC and between 0.2 and 0.29% for PC. Mean MINC values were less than 5.2% for all treatments (Table 1). Standard deviations of TOC, PC and RC values were, except for EW, weak for all treatments due to their range below the sampling and analytical error of the Rock-Eval machine (Behar et al., 2001). Values for TOC, PC, RC and MINC tended to be higher for two EW replicates than for the three others. A main effect of plants was found for most of Rock-Eval standard values, increasing significantly the values of TOC, RC, PC ($p\text{-value} < 0.001$) including the ratios of PC/TOC ($p\text{-value} < 0.01$) and PC/RC ($p\text{-value} < 0.05$), and decreasing the ratio of RC/TOC ($p\text{-value} < 0.01$). The main effects of earthworms indicated a significant decrease for PC values ($p\text{-value} < 0.001$), the PC/TOC ratio ($p\text{-value} < 0.001$) and the PC/RC ratio ($p\text{-value} < 0.001$). However, this main effect of earthworms also led to a significant increase of the RC/TOC ratio ($p\text{-value} < 0.01$). Interaction effects were observed in RC values ($P < 0.01$) when testing relations from P to EW treatment and EW to P + EW treatment. Further interaction effects were found in PC/TOC and PC/RC ratios ($p\text{-value} < 0.01$) with earthworms as the strongest parameter. No main or interaction effects were found for MINC, the total carbonate content and the active carbonate content (Table 1). However, mean values of active $CaCO_3$ increased somewhat in treatments where earthworms were present.

3.2. Analysis of SOM bulk chemistry

The mean HI value of CT treatment remained around 160 ± 2.38 mg HC g^{-1} TOC^{-1} (Fig. 1a). A significant main effect of plants was to increase HI values ($p\text{-value} < 0.001$) and of earthworms was to decrease HI values ($p\text{-value} < 0.001$). No interaction effect was found for HI values (Table 1). According to Fig. 1a, P + EW treatment values ranged in between the values for P and EW treatment.

The mean OI value for CT treatment was 408 ± 3.72 mg OC g^{-1} TOC (Fig. 1b). No main or interaction effects for OI values were statistically significant. Standard deviations were for both HI and OI indices, except for EW treatment, below the analytical error of the Rock-Eval machine (Behar et al., 2001).

3.3. OM thermal stability analysis

HI values already presented in section 3.2 are directly transferable to OM thermal stability and are not described here again. The mean R-index value of all macro-aggregates produced (resulting from all the

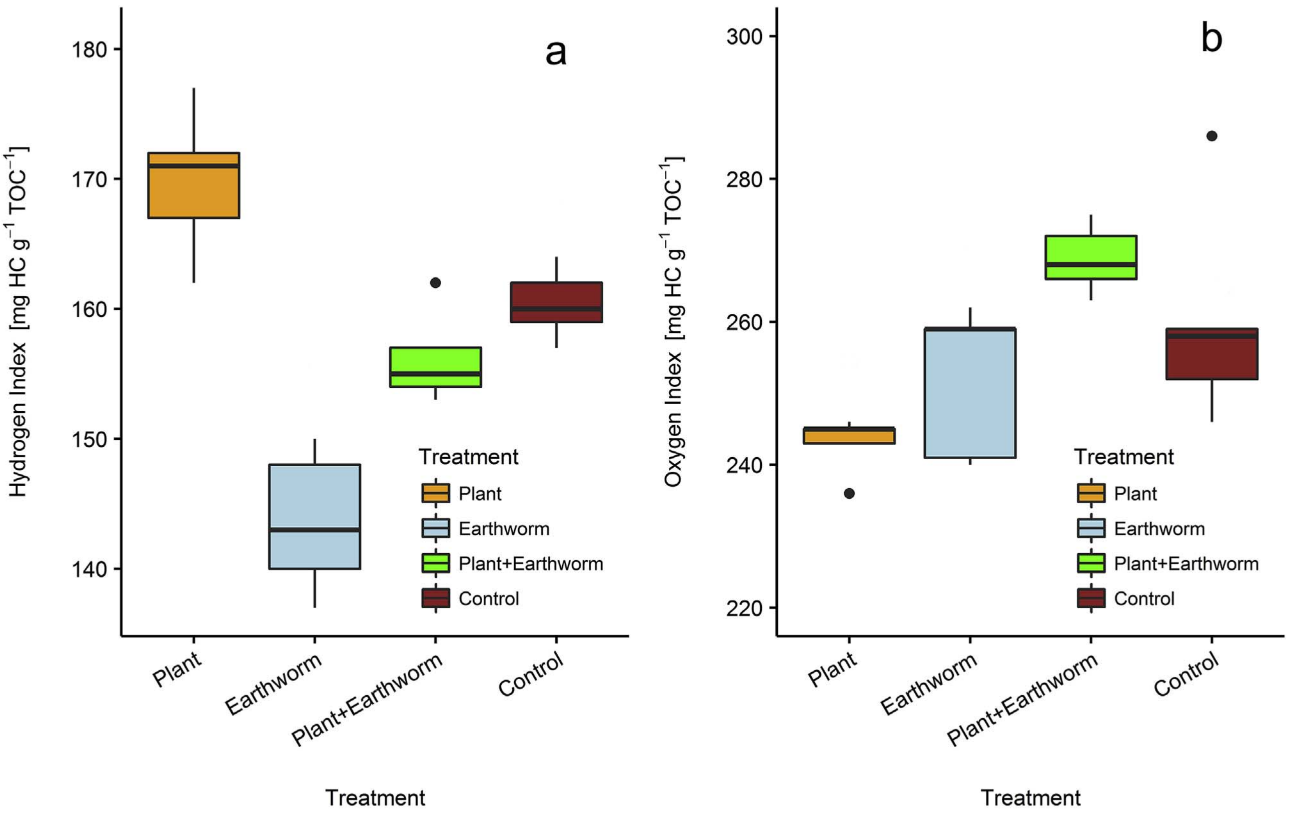

Fig. 1. Two indices used for SOM bulk chemistry analysis: a) Hydrogen Index according to the mesocosm treatments, b) Oxygen Index according to the mesocosm treatments, with n = 5 for all treatments.

Table 1
Average values with standard deviation from the Rock-Eval pyrolysis device according to the mesocosm treatments. “P” stays for plants, “EW” for Earthworms, “P + EW” for plants and earthworms, “CT” for control, “PC” for pyrolysable carbon, “RC” for residual carbon, “TOC” for total organic carbon, “MINC” for mineral carbon, “HI” for hydrogen index, “OI” for oxygen index, “R-index” for refractory index, “I-index” for immature index, "$CaCO_3$ tot" for total carbonates and "$CaCO_3$ act" for active carbonates. p-values represent results from Tukey's HSD tests for the two-way ANOVAs indicating main effects of “Plants” and “Earthworms” and interaction effects of “Plants:Earthworms”.

Parameters	Treatments				Omnibus effects		
	P	EW	P + EW	CT	Plant	Earthworm	Plant:Earthworm
PC (%)	0.28 ± 0.01	0.21 ± 0.01	0.26 ± 0.01	0.23 ± 0.00	$p < 0.001$	$p < 0.01$	n.s.
RC (%)	1.06 ± 0.02	0.93 ± 0.05	1.02 ± 0.03	0.88 ± 0.02	$p < 0.001$	n.s.	$p < 0.01$
TOC (%)	1.34 ± 0.02	1.14 ± 0.06	1.28 ± 0.04	1.10 ± 0.03	$p < 0.001$	n.s.	n.s.
PC/TOC ratio	0.21 ± 0.00	0.18 ± 0.01	0.20 ± 0.00	0.21 ± 0.01	$p < 0.01$	$p < 0.001$	$p < 0.01$
RC/TOC ratio	0.79 ± 0.01	0.81 ± 0.01	0.80 ± 0.01	0.80 ± 0.01	$p < 0.01$	$p < 0.01$	n.s.
PC/RC ratio	0.26 ± 0.01	0.23 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	$p < 0.05$	$p < 0.001$	$p < 0.01$
MINC (%)	0.16 ± 0.01	5.18 ± 0.06	5.19 ± 0.03	5.19 ± 0.03	n.s.	n.s.	n.s.
HI (mg HC)	169 ± 5.05	143 ± 4.84	156 ± 3.20	160 ± 2.38	$p < 0.001$	$p < 0.001$	n.s.
OI (mg)	243 ± 3.61	252 ± 9.73	269 ± 4.37	260 ± 13.5	n.s.	n.s.	n.s.
R-Index	0.58 ± 0.01	0.61 ± 0.01	0.60 ± 0.00	0.60 ± 0.01	$p < 0.01$	$p < 0.05$	n.s.
I-Index	0.20 ± 0.01	0.17 ± 0.02	0.18 ± 0.00	0.16 ± 0.02	$p < 0.01$	n.s.	n.s.
$CaCO_3$ tot (%)	29.03 ± 0.21	28.78 ± 0.65	29.30 ± 0.33	29.58 ± 0.25	n.s.	n.s.	n.s.
$CaCO_3$ act (%)	22.25 ± 1.40	23.00 ± 2.48	24.16 ± 2.49	22.00 ± 2.07	n.s.	n.s.	n.s.

treatments) in this study was 0.60 ± 0.01, and the mean I-index value is 0.18 ± 0.02. Plants affected both R-index and I-index (p-value < 0.01), showing decreasing values for R-index and increasing values for I-index. Earthworms only influenced R-index with increased values (p-value < 0.05) (Table 1). No interaction effects were detectable neither for R-index nor for I-index. Fig. 2 indicates that EW treatment data were in the same range of CT treatment, whereas data of P treatment was shifted towards more fresh OM. Values for P + EW treatment ranged in between the data of P treatment and the EW and CT treatment.

4. Discussion

4.1. Applicability of Rock-Eval pyrolysis to soil aggregates

In our experimental study, HI, OI, R- and I-indices of the sediment ranged within values from A and B horizons that are based on a dataset comprising more than 1000 samples from soil horizons worldwide (Sebag et al., 2016). Deposited floodplain sediments usually consist of topsoil material eroded from the catchment area upstream of a river system and deposited in floodplain ecosystems in lowland areas during flooding events (Nanson & Croke, 1992; Marriot, 1998). We observed that sediment was combined with OM and integrated into soil macro-

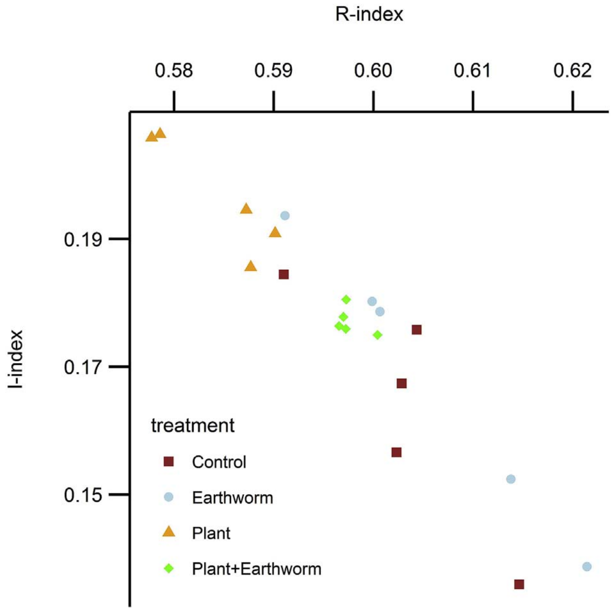

Fig. 2. R-Index plotted against I-index as an indicator for the SOM stability in aggregates, with n = 5 for all treatments.

aggregates by ecosystem engineers during the incubation experiment. Moreover, as the sediment was sieved at 2 mm, smaller pre-existing aggregates were re-arranged by soil engineers at the same time. Results showed modifications of Rock-Eval standard parameters and calculated indices in treatments containing soil engineers. As the presence of an engineer was the only modification during the incubation experiment, it can be assumed that these modifications can be assigned to the contributions of plants and earthworms to soil aggregation. Therefore, it seems possible to determine biological impacts on soil structuration processes based on the technical possibilities of Rock-Eval pyrolysis. The results for standard Rock-Eval parameters and calculated indices were furthermore grouped according to the mesocosm treatments and differed significantly from each other. Although these modifications were low in terms of their absolute number, Rock-Eval pyrolysis was shown to be able to precisely measure the distinctive features of biogenic structures, i.e. plant root exudates and mucus or rather saliva from earthworms' digestive tract, to distinguish them according to their origin. Moreover, analyses of SOM quantity, bulk chemistry, and thermal stability in each treatment emphasize specific ranges of values for each analysis that differ statistically. Therefore, Rock-Eval pyrolysis is able to identify a characterising signature for each treatment through the combination of results obtained with the three-step analysis. This procedure has never been performed on soil macro-aggregates so far and delivers promising results for the identification of soil macro-aggregate origin, and additionally for the quantity, bulk chemistry, and thermal stability of SOM in aggregates, whatever the soil type.

4.2. Plant signature

The OM signature in aggregates stemming from plants was characterized by increased TOC contents, higher HI-index and I-Index values, whereas R-Index values were lower compared to CT treatments. This indicates that fresh and easy decomposable OM compounds were incorporated into the macro-aggregates. Plants provide inputs of labile OM compounds such as root exudates or dead roots (Czarnes et al., 2000; Sebag et al., 2016), which can act as bonding agents for soil particles or may stimulate microbial activity in the surrounding bulk soil (Angers and Caron, 1998; Fonte et al., 2012). This plant signature on macro-aggregates thus reflects the direct influence of the plant itself, as well as the potential effect of biogenic signatures of rhizospheric microorganisms that are stimulated indirectly through the presence of plant roots. Some compounds released by roots, such as carbohydrates and phenols, promote interaction with heterotrophic bacteria and are suspected to be responsible for the colonisation of roots by arbuscular mycorrhizal fungi (AMF) (Becard et al., 1995; Narula et al., 2009). Fine roots that remain in the soil aggregates and are colonised with mycorrhizal fungi may also contribute to the plant's characteristic signature. Root exudates can also affect soil aggregates through the modification of chemical components in the soil. The presence of organic acids in the rhizosphere can lead to the dissolution of $CaCO_3$, which might be a possible explanation for the slight decrease of MINC values observed in the P treatment. However, this effect did not statistically characterise the plant signature. To sum up, plant signature can be understood as a combination of fresh OM residues directly incorporated into soil macro-aggregates and OM products released from interacting organisms in the rhizosphere.

4.3. Earthworm signature

Low HI values make the earthworm signature distinct, as well as its relative enrichment of RC and depletion of PC in relationship to TOC, compared to control treatments. Earthworms were shown to have a preferential feeding behaviour (Curry and Schmidt, 2007), whereby lighter OM fraction containing easier decayable compounds (reflected by PC) are selectively ingested. During the gut transit, OM compounds and mineral matter are mixed and agglutinated with mucus and saliva in the digestive tract (Brown et al., 2000). It can be assumed that these substances, acting as bonding agents, are predominant factors explaining the specific earthworm signature. The ingested OM from bulk soil was recently shown to be occluded into the soil matrix rather than being chemically modified while passing the digestive tract (Angst et al., 2017). Contrary tendencies can be observed regarding the values for MINC. Three MINC values were lower, the remaining two even higher than the MINC values in the CT treatments. It seems likely that two different mechanisms proceed in EW treatments although the setup was equal for all replicates. Groups of earthworms in three of the replicates might alter the CorgR included into the MINC pool, thus leading to its reduction. MINC is increased in the two other treatments that might be explained by the increase of $CaCO_3$ into the MINC pool. These assumptions are strengthened through constant values of TOC, which are usually reduced parallel to the PC preferentially consumed by earthworms. The existing imbalance between a shift of a partial pool in proportion to the overall pool has thus to be offset otherwise. Moreover, it seems unlikely that the C_{org} pool is a source for MINC pool increase in the earthworm treatments because TOC contents in earthworm macro-aggregates do not differ from the TOC values found in CT treatments. Therefore, the increase in the MINC pool seems to be controlled by an external source. Many earthworm species, including *A. chlorotica*, are known to produce calcium carbonate granules by their calciferous glands ranging from 0.125 mm to single $CaCO_3$ crystals (Becze-Deák et al., 1997; Canti, 1998; Canti and Piearce, 2003). The slight increase in active $CaCO_3$ found in EW and P + EW treatments indicates that earthworms might ingest Ca^{2+} and/or $CaCO_3$ from the bulk soil, produce calcium carbonate granules and incorporate them into the soil aggregates. Similar results have already been reported in Canti (2009) and Garcia-Montero et al. (2013). The reasons for the excretion of calcium carbonate have not been clarified, so far. Recent discussions focus on a mechanism that might regulate the pH of blood and tissue fluids or incidentally neutralizes the gut system (Canti and Piearce, 2003; Coleman et al., 2004; Briones et al., 2008).

4.4. Plant + Earthworm signature

In the P + EW treatment, TOC content slightly increased whereas MINC did not show any significant changes compared to the CT treatments. HI, I-Index and R-Index values ranged exactly between the values of P and EW treatments. These findings represent the combined signature of plants and earthworms on the macro-aggregates that might be either a mixed signature of sampled aggregates or indicate interaction of both ecosystem engineers during the formation of soil macro-aggregates. Previous studies have already shown that earthworms have a preferential feeding behaviour by consuming the Corg produced by plants through root exudates, as root exudates are more easily consumable by earthworms (Decaëns et al., 2001; Curry and Schmidt, 2007). Recent investigations emphasize that earthworms consume significant amounts of root derived OM that is incorporated into soil aggregates (Gilbert et al., 2014; Sanchez-de Leon et al., 2014). Beneficial effects are however reciprocal since plants take advantage of the earthworms' activity in the soil in the same way (Le Bayon et al., 2017). Earthworms are known to mobilise nitrogen, phosphorus, and exchangeable nutrients that are easier accessible for plants (Brown et al., 2000; Shipitalo and Le Bayon, 2004; Le Bayon & Binet et al., 2006; Le Bayon et al., 2011). Therefore, plants roots preferentially colonise earthworm structures (casts, burrow linings) to get access to the nutrient hotspot (Spiers et al., 1986; Zaller and Arnone, 1999). As a consequence, our results are an indicator that the interactions of both studied soil engineers, not only have positive effects on their growth or survival rate but also contribute concomitantly to the formation of soil macro-aggregates. Similar results were shown by Zangerlé et al. (2011), who recovered NIRS signatures of these two soil engineers in aggregates.

4.5. *OM thermal stability*

The R-index and I-index have been proved to be powerful indicators for the assessment of the thermal stability of soil organic matter. These indices point to functional allocations of field samples corresponding to specific soil horizons (Sebag et al., 2016). Additionally, the HI was proposed as another indicator for the analysis of OM thermal stability (Gregorich et al., 2015; Barré et al., 2016). Results for the thermal stability obtained by the R-index and HI in this study are indeed comparable. However, HI values have a tendency to show greater differences between the signatures of the treatments and make them statistically significant. Compared to the R-index, HI is strongly controlled by the chemical composition of OM leading to increasing variances of the obtained values (Gregorich et al., 2015; Barré et al., 2016).

Differences in the values of treatments in this study also suggest that these indices are suitable to assess thermal stability of OM in soil macro-aggregates. Thus, the values found in the CT treatments represent the Rock-Eval signature of the transported sediment layer, which can be used as an initial value for the mesocosm experiment. CT treatments showed a certain variation, despite a thorough mixing of sediment with added plant leaves. Once a plant is added to the system, the thermal stability of organic matter in soil macro-aggregates decreases with a simultaneous increase of the degree of preservation of fresh organic matter. Plant root exudates consist of easily decomposable organic matter, belonging to labile OM pools in soil due to the rapid turnover rate. However, even thermally labile OM can be efficiently protected by mineral matter against microbial decay through occlusion into aggregates that can extend its residence time in soil (Schmidt et al., 2011). SOM thermal stability does not seem to be improved regarding EW and P + W except when using HI values. In general, the association of mineral and organic compounds is facilitated while passing through earthworms' digestive tract (Lavelle, 1988). As already mentioned above, recent findings indicate that, during this process, SOM is rather physically protected than modified regarding its chemical composition (Angst et al., 2017). Mechanical or biological breakdown and re-formation of aggregates hinder an effective stabilisation of OM in soil aggregates and thus decrease its long-term persistence. In a short term (20 days of incubation), Bossuyt et al. (2005) found that organic carbon was not protected in macro-aggregates in the presence of earthworms. During longer periods, the endogeic earthworm *A. caliginosa* was shown to stabilise significant amounts of organic carbon in micro-aggregates agglutinated to macro-aggregates (Bossuyt et al., 2005). Considering only the HI values, our studied species *A. chlorotica* might increase the thermal stability of OM in macro-aggregates even over short periods. On the other hand, *A. chlorotica* is in fact considered as a pioneer species in young dynamic ecosystems such as floodplains, but was shown to decrease the structural stability and increase soil compaction in a microcosm experiment (Milleret et al., 2009a). The signature must therefore be considered as a species-specific signature for *A. chlorotica* and thus cannot be readily projected to other earthworm species. However, Milleret et al. (2009a) highlighted that the effect of *A. chlorotica* was reversed when adding arbuscular mycorrhizal fungi and turned positive when also adding plants to their mesocosms. Nevertheless, the combination of plants and earthworms did not improve OM thermal stability of macro-aggregates in our study, regardless of whether the OM signature obtained from R-index and HI represents a mixture or a combined effect of both engineers. In contrast, according to Schmidt et al. (2011), the incorporation and sequestration of SOM is a combination of interacting physical, chemical, and biological parameters within an ecosystem. Therefore, it was actually expected that the interaction between plants and earthworms would have positive effects on soil structuration and improve the thermal stability SOM.

4.6. *Limitations*

This study highlights the applicability of Rock-Eval pyrolysis to identify soil macro-aggregates according to their biological origin, based on specially selected parameters for the experimental setup. It is important to mention that the absolute numbers obtained from Rock-Eval analyses depend on initial conditions characterizing bulk soil or sediment and thus cannot be generalized or directly compared to other studies. The results obtained in our study thus reflect a mixture of pre-existing macro-aggregates and aggregates that were modified or newly formed by ecosystem engineers during the incubation experiment. The amount and stabilisation degree of SOM determines the starting conditions from which modifications of ecosystem engineers can be considered as relative deviations and may vary from strong to weak. Therefore, a general valid factor describing the degree of modification of organic signatures in soil macro-aggregates cannot be defined. In our study, the initial range of OM values is provided by the sediment mixed with litter in CT treatment because the sediment was not subject to any (macro-)biological modifications during the incubation period. Biogenic signatures led only to slight changes of absolute OM values in aggregates, but were grouped according to their respective treatments with low deviations leading to distinct OM signatures. Low initial OM contents in the bulk sediment might have been advantageous in order to allow visualisation of biogenic modifications in aggregates. Slight changes in OM composition in treatments could disappear with a strong OM signal of the bulk sediment. The use of OM poor sediments might be more appropriate to test biogenic modifications in soil aggregates, despite the fact that no application of OM rich sediments has been evaluated in this context so far.

Although the signatures for each treatment seem to be comprehensible regarding the behaviour of plants and earthworms during soil aggregation, it should be noted that only one specific plant and earthworm species was tested in this study. It can be assumed that signatures for different plant and earthworm species might show certain variability due to the varying composition of root exudates or digestive flora. The P + EW signature is especially suspected to vary on a larger scale because different earthworm species allocated to other ecological categories were shown to interact to a lesser extent with plants or contribute less to SOM incorporation and to soil aggregation (Bouché, 1972; Fragoso and Lavelle, 1995; Lavelle et al., 1997). Endogeics including the studied species *A. chlorotica* are most efficient in soil structuration and show high interaction with plants (Milleret et al., 2009a; Fonte et al., 2012). Epigeic earthworms primarily feed on OM in the topsoil whereas anecic earthworm burrowing activity is limited to a few galleries in deeper soils. The P + EW signature might therefore be less marked by earthworms when testing epigeics or anecics with plants in a mesocosm treatment. Further tests with different species are therefore mandatory in order to validate the signatures presented in this study.

5. Conclusion

This study presents the first application of Rock-Eval pyrolysis to aggregates formed by soil engineering organisms under controlled conditions. Our results show the potential of Rock-Eval pyrolysis to identify organismal-specific OM signatures and thus to distinguish soil macro-aggregates by their origin. In contrast to the methods applied for the identification of aggregate origin so far, Rock-Eval pyrolysis provides further information on SOM bulk chemistry. Using standard values and calculated indices, quantitative and qualitative assumptions can be formulated regarding SOM in aggregates. Indices for refractory and immature OM have shown their potential for the determination of SOM thermal stability in aggregates. During a short incubation period, our studied organisms did not significantly improve SOM thermal stability in soil macro-aggregates. SOM might be physically protected in soil aggregates rather than chemically bonded in short term. However, only one single plant and earthworm species was tested in this study, so signatures must be considered as species-specific. In order to validate and generalise the results, further experiments including different plant and earthworm species are required.

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