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1 **Age matters: fate of soil organic matter during ageing of earthworm**
2 **casts produced by the anecic earthworm *Amyntas khami***

3

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26 **Abstract**

27 The fate of organic matter (OM) occluded in earthworm casts during the casts'
28 lifetime is poorly known. We collected casts produced by the anecic earthworm
29 *Amyntas khami* in tropical woodland in Northern Vietnam at different times after
30 their production. The aim of this study was to assess the (1) the effect of earthworms
31 on biogeochemical OM properties compared to control aggregates, separated from the
32 surrounding soil and (2) the fate of OM during cast ageing. We analysed five cast
33 ageing stages for elemental content, OM chemical composition by analytical pyrolysis
34 and stability by Rock-Eval thermal analysis. Moreover, we assessed the distribution
35 of organic carbon (OC) in density fractions.

36 Compared to control aggregates, fresh casts had higher OC values (5.3 vs. 2.6 %) and
37 increased OC in all density fractions. Cast OM had more lignin (1.1 vs. <0.1 %),
38 similar polysaccharide and N-compound contribution and was more thermally labile
39 than those of control aggregates. Changes in OM composition and content during cast
40 ageing appeared at the end of the casts' lifetime, when they were broken into small
41 sized aggregates (10-13 mm). At these latest stages, OC content decreased (1.5-fold);
42 along with OM in macro-aggregates (2.2-fold) and microaggregates (6.8-fold).
43 Reduced mineral-associated OC was also recorded (1.3-fold). During ageing, the
44 percentage of lignin decreased (5.5-fold) while the thermal stability of OM increased.
45 Most properties were still significantly different between the oldest ageing stage and
46 control aggregates. This study highlights that the impact of earthworms on OM
47 storage goes beyond cast disintegration and that there is a need to better understand
48 their influence on soil organic matter stabilisation processes and in particular the
49 formation and stability of organo-mineral associations.

50 **Key words:** soil, organic matter; aggregation; bioturbation; anecic earthworm cast

51 **1. Introduction**

52 Earthworms play an important role in the functioning of the soil system due to their
53 bioturbation activity and the production of casts with specific physical, chemical and
54 biological properties (Bottinelli et al., 2015; Van Groenigen et al., 2019). For
55 example, fresh earthworm casts are known as biogeochemical hotspots (Kuzyakov
56 and Blagodatskaya, 2015) and can be enriched in organic carbon (OC) and other
57 nutrients (Van Groenigen et al., 2019) as compared to the surrounding topsoil. In the
58 first hours after cast formation, plant-available nutrients may be released (Barois and
59 Lavelle, 1986), because casts contain a high proportion of fresh organic matter (OM)
60 (Guggenberger et al., 1996), which may be partly degraded shortly after their
61 incorporation into macro-aggregates. Conversely, the formation of microaggregates
62 (Bossuyt et al., 2005, 2004) and the association of OM to the mineral phase (Vidal et
63 al., 2019) within the earthworm gut and/or within macro-aggregates might hamper
64 OM mineralization in casts. During degradation of casts, the occluded OM (1) may
65 become available again for microbial decay and mineralization processes when
66 present in form of labile particulate organic matter (POM) or (2) may continue to
67 contribute to OM storage if strongly associated to the mineral phase and/or included
68 into stable microaggregates. There is little information about the time horizon of this
69 protection in the field, where casts can disintegrate through physical disturbance, e.g.
70 rainfall events. The very limited number of studies carried out under field conditions
71 showed contradictory results, with an increase (Decaëns et al., 1999) or a decrease
72 (McInerney et al., 2001) of OC in casts during ageing. Moreover, to the best of our
73 knowledge, the effect of cast ageing on SOM composition and stability has rarely
74 been assessed.

75 In North Vietnam, the anecic earthworm *Amyntas khami* (Megascolecidae
76 family) produces large casts on the soil surface. These casts are enriched in OC, lignin
77 and polysaccharides and reasonably water stable after their formation (Hong et al.,
78 2011; Jouquet et al., 2008). During our extensive field work, we noticed that they may
79 last for months to years. Thanks to their stability, casts from *A. khami* casts constitute
80 therefore an interesting model for understanding changes in OC content and OM
81 properties during cast ageing. Even if *A. khami* casts are rather stable after field
82 deposition, we hypothesized that the processes affecting occluded OM during the
83 casts' lifetime are similar to those affecting casts produced by other species. This
84 study aimed to evaluate the biogeochemical changes occurring during the casts'
85 lifetime. We collected surface earthworm casts of *A. khami* representing different
86 stages of ageing in the field and investigated their biogeochemical cast properties and
87 the distribution of OM in four different functional OM pools and compared them to
88 adjacent control aggregates without visible earthworm influence. We hypothesized
89 that (1) earthworm casts disintegrate after their formation due to disturbance events,
90 (2) occluded OM changes during this process and (3) OM characteristics become
91 similar to adjacent control aggregates at later ageing stages. Our specific objectives
92 were to investigate (1) the effect of earthworms on biogeochemical OM properties,
93 and (2) the fate of OM during cast ageing.

94

95 **2. Materials and methods**

96 *2.1. Study site*

97 Soil and earthworm casts were sampled at the M-Tropics long-term observatory
98 catchment located in the Dong Cao village in Northern Vietnam (20° 57'N, 105°
99 29'E). The climate in the region is subtropical humid with annual mean temperature of
100 20 °C and 1800 mm of rainfall. The dominant soil type is Acrisol (WRB)

101 (Podwojewski et al., 2008), characterized by an OC content of 2.5%, a clay content of
102 50% and a pH_{KCl} of 3.9. Woodlands, secondary forests, meadows and fallows are the
103 main land uses in the catchment. Surface earthworm activity in the catchment is high
104 with the anecic earthworm *Amyntas khami* being the main species depositing casts
105 on the soil surface. Samples were collected during the dry season in March 2017, in
106 woodland, where the quantity of casts deposited at the soil surface was on average
107 4400 g (oven dry weight) m^{-2} .

108

109 2.2. Soil sampling and preparation

110 Soil in woodland is covered by recent and old earthworm casts that form a typical
111 granular layer up to 5 cm thick. We compared casts of five contrasting stages of
112 ageing and adjacent control aggregates without visible earthworm activity (ctrl).
113 Sampling took place at five random locations in an area of 2500 m^2 . We sampled five
114 types of casts with contrasting stage of ageing differentiated by their humidity and
115 degradation features (Fig. 1). The aging stages comprising five different cast types
116 had been determined through detailed field observations: (1) cast-A: fresh casts,
117 known to be < 1 day; (2) cast-B: fresh casts < 1 week old; (3) cast-C: dry casts,
118 probably more than 1 month old; (4) cast-D: dry casts with marked signs of
119 degradation (greenish colour due to the presence algae with cracks at the surface),
120 older than cast-C. The size of casts (A to D) varied from 5 to 15 cm. They were
121 produced through the accumulation of faecal pellets deposited the one on the other.
122 After disintegration probably due to livestock trampling, human traffic, root growth
123 and raindrop impact or natural aggregate turnover, individual pellet of 1 cm diameter
124 are released: (5) cast-E of unknown age, consisting of small aggregates and
125 representing the last stage of degradation recognizable by naked eyes. Differentiation

126 of old casts and soil aggregates is an unresolved problem faced by many workers in
127 environments with high earthworm activity. In the present work, based on extensive
128 field observations, we considered that control aggregates had no round shape and
129 could in contrast to casts-E not be identified as earthworm casts by naked eyes. To
130 sample control aggregates, we collected soil aggregates (5-15 cm) from 2-7 cm depth
131 in the vicinity of the casts without visible earthworm influence.

132

133 The exact cast age was not very important for our study. We aimed at describing the
134 biogeochemical OM changes occurring during ageing, and therefore sampled an age
135 gradient of casts based on morphological cast properties determined through extensive
136 fieldwork. During ageing, these stages follow one another, at varying timescales
137 depending on the actual lifetime of the cast, which is depending on climatic
138 conditions and the occurrence of disturbance events.

139

140 In total, 30 samples were collected: (5 types of casts + 1 control) \times 5 replicates. To
141 work on samples with similar physical properties, we air-dried and broke up manually
142 the earthworm casts and ctrl samples into aggregates between 10 and 13 mm.

143

144 *2.3. Physical fractionation of and isolation of particulate organic matter (POM)*

145 Before starting fractionation, the samples were hydrated for 2 h. Thereafter, they were
146 automatically shaken in water and wet-sieved at 2 mm for 3 h at 140 rotations per
147 minute. The material $< 2\text{mm}$ was filtered and the soil remaining on the filter paper
148 was air-dried for two days. The dry material $< 2\text{mm}$ was subjected to density
149 fractionation using SPT (Na-polytungstate) with a density of $1.67\text{ g}\cdot\text{cm}^{-3}$ following
150 the method suggested by Cerli et al. (2012). We isolated four density fractions: free

151 particulate organic matter (fPOM), two occluded particular organic matter fractions
152 (oPOM) and the remaining heavy fraction (HF). Briefly, 10 g of sieved soil or casts
153 were mixed with 67.2 ml Na-polytungstate solution with a density of $1.67 \text{ g}\cdot\text{cm}^{-3}$ and
154 the fPOM fraction was recovered by filtration after gently shaking and centrifuging
155 (3,500 rpm for 30 min). The fraction was washed with distilled water and freeze-
156 dried. The remaining soil in the polytungstate solution was treated with
157 ultrasonication (40 J ml^{-1}) to disperse macro-aggregates and release the occluded
158 particulate organic matter (oPOM) fraction. After recovery of this fraction by
159 centrifugation and filtration, the procedure was repeated with stronger ultrasonication
160 (400 J ml^{-1}) to completely disperse the samples and to recover POM occluded in
161 micro-aggregates. The remaining material represented mineral-associated OC of the
162 heavy fraction. All fractions were freeze-dried, weighted, crushed at $200\mu\text{m}$ and
163 analyzed for OC and N content. To determine the contribution of the four OM pools
164 isolated by density fractionation to the total SOC content, we calculated the mass of
165 OC present in the fractions. We considered that fPOM and oPOM ($40 \text{ J}\cdot\text{ml}^{-1}$)
166 indicated POM stored in macro-aggregates whereas oPOM ($400 \text{ J}\cdot\text{ml}^{-1}$) indicated
167 POM stored in micro-aggregates. HF represented the OM associated to the mineral
168 phase. Total OC and N of bulk samples and physical fractions were measured using
169 an elemental analyser (Thermo Flash HT, Thermo Fisher Scientific Inc., Waltham,
170 USA). The mean OC recovery after the density fractionation procedure was 92%.

171

172 *2.4. Molecular composition of SOM: analytical pyrolysis*

173 Bulk samples were subjected to analytical pyrolysis to investigate the molecular
174 composition of their OM. The analyses were carried out with a pyrolysis unit (GSG
175 Curie point Pyrolyser 1040 PSC) coupled to a gas chromatograph (Hewlett Packard

176 HP 5890) and a mass spectrometer (Hewlett Packard HP 5889; electron energy 70
177 eV). Briefly, 0.5–1 mg of sample were loaded in tubular ferromagnetic wires,
178 inductively heated to their Curiepoint temperature of 650 °C in 0.15 sec in the
179 pyrolysis unit. The pyrolysis products were transferred to the GC system operating in
180 spitless mode with He as carrier gas (flow 1 ml/min). They were separated on a 60 m
181 fused silica capillary column with a wax stationary phase (SolGelWax, SGE, 0.32 mm
182 i.d., film thickness 0.5 µm) before being detected using quadrupole mass
183 spectrometry. The temperature of the GC oven was programmed from 30 °C to
184 280 °C at 2 °C/min. The final temperature was hold for 15 min. Pyrolysis releases a
185 suite of pyrolysis products. We chose a polar column, because we were interested in
186 the relative quantification of polysaccharide-derived, lignin-derived and N-containing
187 pyrolysis products as main SOM compounds from the GC traces. Such compounds
188 were found to yield broad peaks when separated on a non-polar column (Dignac et al.,
189 2006). We quantified the relative contribution of these compounds after their
190 identification using the total ion chromatogram.

191

192 *2.5. Thermal analysis: Rock-Eval 6*

193 We used a Rock-Eval 6 turbo device (Behar et al., 2001) for the thermal analysis of
194 bulk samples. We adapted the procedure developed for the analysis of SOM proposed
195 by Disnar et al. (2003) as specified in Soucémarianadin et al. (2018). Briefly, about 60
196 mg of ground sample were subjected to two consecutive thermal treatments: first,
197 samples were transferred to a pyrolysis oven (under N₂ atmosphere) then to a
198 combustion oven (under laboratory air atmosphere). Effluents released during the
199 pyrolysis contained mostly hydrocarbons or HC. They were detected and quantified
200 by a flame ionization detector; during both the pyrolysis and oxidation stages. CO and

201 CO₂ were detected with an infrared detector. We determined two standard parameters:
202 the hydrogen index (HI) and the oxygen index (OI_{RE6}). HI is defined as the amount of
203 pyrolyzed HC relative to the total OC content of the sample and shows the relative
204 enrichment (high HI) or depletion (low HI) of OC in hydrogen-rich moieties. OI_{RE6} is
205 defined as the amount of oxygen present as CO and CO₂ during the pyrolysis stage
206 divided by the total OC content of the sample (Lafargue et al., 1998). The OI_{RE6}
207 describes the relative oxidation status of OC. Other indexes proposed by Sebag et al.
208 (2016) were calculated by integrating the pyrolysis curve between predefined
209 temperature thresholds to describe the OM thermal stability. The R-index, which
210 ranges from 0 to 1, corresponds to the relative contribution of thermally stable OM
211 with refractory compounds released at higher temperature. The I-index provides
212 information on the preservation of thermally labile immature OM, with compounds
213 released at lower temperatures (Sebag et al., 2016). We also calculated T_{50_CO2_PYR}
214 and T_{50_CO2_OX} (Cécillon et al., 2018), which are the temperature at which 50% of the
215 CO₂ are released during pyrolysis and combustion.

216

217 2.6. *Statistical analysis*

218 One-way ANOVAs were used to test the effect of earthworm activity on soil
219 properties. We compared casts of different degradation stages and ctrl samples (n =
220 5). Prior to running ANOVAs, data were tested for homogeneity of variances and
221 normality and log-transformed when required. LSD post hoc multiple comparison
222 tests were used if the effects were significant. Differences among treatments were
223 declared significant at the 0.05 probability level. We also performed between-class
224 analyses (BCA) and Monte Carlo permutation tests (1000 permutations) to exploit the
225 information of the (i) pyrolysis compounds, (ii) Rock-Eval 6 thermal stability

226 parameters and (iii) all properties characterizing OM in casts. All statistical
227 calculations and plots were executed with R 3.12 statistical package for Windows
228 (<http://www.r-project.org>) using “car”, “ade4” and “ggplot2” packages.

229

230 **3. Results**

231 *3.1. OC content of bulk samples and density fractions*

232 The OC content of cast-A was twice as high as those of ctrl samples, and gradually
233 decreased during cast ageing (**Table 1**). All cast types showed a significantly higher
234 OC content than samples from ctrl samples, while their C/N ratios were similar
235 (**Table 1**).

236 The OC allocation to specific pools changed between casts and ctrl samples
237 (**Table 1**). In all samples, the vast majority of OC, representing between 83 and 94%
238 was found in the mineral-associated fraction. Between 5 and 12% of OC was
239 occluded in macro-aggregates and up to 5% of OC was present in micro-aggregates.

240 It was interesting to note that earthworm activity increased the total amount of
241 OC in the mineral-associated fraction of fresh casts (cast-A versus ctrl), but that this
242 OC was also decreasing during cast ageing (from cast-A to cast-E). We observed that
243 total OC storage decreased from casts-A to -E by 69% in the microaggregate fraction,
244 by 50% in the macro-aggregate fraction and by 29% in the mineral-associated
245 fraction. Despite this decrease, the latter fraction was the only one, which showed
246 significantly higher OC amounts in cast-E as compared to ctrl samples. Overall, the
247 C/N ratio was lowest in the mineral-associated fraction, highest in micro-aggregates
248 and intermediate in macro-aggregates (**Table 1**). Cast-A showed higher C/N ratio than
249 ctrl samples, for macro-aggregate and mineral-associated OM, lower C/N ratios in
250 macro-aggregates and slightly lower C/N ratios in the mineral-associated fraction as

251 compared to ctrl aggregates. From cast-A to -E, the C/N ratio was quite stable and
252 significantly different than for ctrl aggregates.

253

254 *3.2. Molecular composition of bulk samples*

255 Pyrolysis products showing significant differences among samples and their possible
256 origin are shown in **Table 2**. All pyrograms were characterised by contribution of N-
257 containing products, which represented between 30 and 34% of the Total Ion
258 Chromatogram (TIC). Polysaccharide-derived pyrolysis products contributed between
259 13 and 17% to the TIC, and most compounds did not show any significant differences
260 among cast ageing stages and/or ctrl. Furfural was the only compound, which showed
261 a higher contribution in casts than ctrl (**Table 2**). The most significant peaks,
262 representing 50 to 60% to the TIC were attributed to compounds of unspecific origin.
263 Lignin-derived compounds contributed less than 1–2% to the TIC and were identified
264 in cast samples only (**Table 2**).

265

266 Cast-A was characterized by higher percentage of lignin, lower percentage of
267 unspecific compounds and similar percentages of polysaccharides and N-containing
268 compounds in comparison to ctrl aggregates. The percentage of lignin decreased from
269 cast-A to cast-E, which showed similar values than ctrl aggregates. The percentage of
270 unspecific compounds was stable from cast-A to cast-E and significantly different to
271 ctrl aggregates (**Table 2**). The PCA of pyrolysis products showed that the cast ageing
272 stages were differentiated into three groups: (1) cast-A to cast-D, (2) cast-E and (3)
273 ctrl on the first and second axis, which explained 91% of the total variability. Cast-A
274 was characterized by higher contribution of P5, U9, U6, L1, N10 and lower
275 contribution of U1, U7 and N2 than ctrl samples. From cast-A to cast-E, the

276 percentage of P5 decreased. Cast-E samples were significantly different from ctrl for
277 all the properties except for P5 and N8.

278

279 3.3. *Thermal stability of OM in bulk samples*

280 All variables were significantly influenced by the presence of earthworms and ageing
281 of casts (**Table 3**). The HI, OI_{RE6} and T_{50_CO2_OX} were more sensitive than I-index, R-
282 index and T_{50_CO2_PYR} according to the F-values. BCA of the thermal stability
283 parameters (**Fig. 3**) separated samples into 3 groups: (1) cast-A to -D, (2) cast-E and
284 (3) ctrl aggregates on the first and second axis, which explained 93% of the total
285 variability. Cast-A was characterized by higher values of HI, and lower values of
286 OI_{RE6}, R-index, T_{50_CO2_PYR} and T_{50_CO2_OX} than ctrl samples. Conversely, the I-index
287 was similar in cast-A and ctrl aggregates. From cast-A to cast-E, HI and I-indexes
288 decreased whereas OI_{RE6}, the R-index and T_{50_CO2_OX} increased. In cast-E samples,
289 HI, OI_{RE6}, I-index and T_{50_CO2_OX} were still significantly different from ctrl
290 aggregates.

291

292 **4. Discussion**

293 4.1. *Earthworm-induced changes in OM properties during cast formation*

294 4.1.1 *Quantitative changes of organic matter pools*

295 The OC content of cast-A doubled as compared to ctrl aggregates, which is in
296 agreement with many other studies concerning the influence of earthworms on soil
297 properties (reviewed by Van Groenigen et al., 2019). Our results indicated that this
298 was due to greater amounts of OC associated to the mineral fraction, which increased
299 by 50% through earthworm activity. A high proportion of mineral-associated OC in
300 earthworm casts is in agreement with results of Vidal et al. (2019), who showed that

301 90% of the OC in casts was associated to the mineral fraction. The presence of a high
302 amount of this material in cast-A could indicate that organo-mineral associations were
303 formed within the earthworm gut (LeMer et al., 2020). Although such a process has
304 been hypothesised for long (Shipitalo and Protz, 1989), to the best of our knowledge
305 this study is among the first to provide quantitative evidence of organo-mineral
306 formation through earthworm activity under field conditions. The amount of OC in
307 macro- and micro-aggregates contributed together for only 16% of the total OC. OM
308 protection by occlusion in macro- and micro-aggregates is a well-known phenomenon
309 and is generally assumed to be the most important process stabilizing OM following
310 earthworm activity (Bossuyt et al., 2004, 2005). Our data indicate, that physico-
311 chemical protection of OM in earthworm casts may at least be equally important. A
312 recent laboratory study showed that such protection may affect completely degraded
313 as well as fresh OM compounds (Barthod et al., 2020).

314

315 *4.1.2 Qualitative changes of OM during cast ageing*

316 Cast-A had higher percentages of lignin than ctrl aggregates showing that anecic
317 species like *A. Khami* ingest a mixture of soil and fresh plant debris (Guggenberger et
318 al., 1996; Hong et al., 2011). This is also supported by the molecular composition,
319 which indicated the higher contribution of furfural, a polysaccharide compound, in
320 cast-A than ctrl aggregates. The rather undecomposed nature of OM in cast-A is
321 supported by the Rock-Eval 6 thermal stability parameters. Cast-A had a higher HI
322 and I-index and lower OI_{RE6}, R-index, T_{50_CO2_OX} and T_{50_CO2_PYR} than ctrl aggregates.
323 These parameters are all indicative of fresh, easy decomposable OM compounds
324 with a low thermal stability (Cécillon et al., 2018; Schomburg et al., 2018; Sebag et
325 al., 2016; Soucémarianadin et al., 2019). Our result is in agreement with a study

326 showing that OM in casts formed by an anecic *Martiodrilus* species is more labile
327 than in ctrl aggregates (Guggenberger et al., 1996). Conversely, Schomburg et al.
328 (2018) showed in experimental conditions that the belowground casting activity of the
329 endogeic species *Allolobophora chlorotica* lead to the decrease in decomposable OM
330 compounds and to the increase of its thermal stability in soil aggregates. These
331 contradictory results are most probably related to the different feeding activity of
332 anecic and endogeic species.

333

334 4.2. Changes in OM storage and quality during cast ageing in the field

335 The OC content of casts remained similar from cast-A to -C and decreased thereafter.
336 In contrast, the molecular composition of cast changes in the first ageing stages. From
337 cast-A to -C the contribution of lignin and N-containing compounds increased
338 (significant for L1 and N10). It is likely that the lower porosity of casts as compared
339 to ctrl samples (Bottinelli et al., 2012) prevented the mineralization of OM and thus
340 led to transformation of OM compounds. A similar explanation was suggested by
341 Martin (1991), who found no change of OC content in casts during a laboratory
342 incubation of 420 days. Changes in chemical composition were similar to those
343 observed upon microbial decomposition of crop residues in soil (Franchini et al.,
344 2002) and may reflect microbial use of easily decomposable polysaccharides leading
345 to selective preservation of lignin and simultaneously accumulation of N-containing
346 compounds of microbial origin (Kögel-Knabner and Rumpel, 2018). The chemical
347 changes in the first three cast ageing stages may thus be related to an intense activity
348 of micro-organisms occurring in earthworm casts (Aira et al., 2005; Tiunov and
349 Scheu, 2000).

350

351 Most significant changes appeared in later cast ageing stages, and in particular when
352 casts were broken into small-sized aggregates (cast-E). Changes from casts-A to -E
353 included gradual OC loss from all physical fractions, representing a total loss of 32%
354 of OC. Similar observations were made by McInerney et al. (2001), who studied
355 ageing of surface casts produced by the epi-anecic *L. terrestris* and found that OM
356 decreased by 15% 2.5 years after field exposure. Our results are however in contrast
357 to a study by Decaëns et al. (1999), who found a doubling of the OC content in casts
358 produced by the anecic *M. carimaguensis* after 330 days. These contrasting results
359 may indicate that OC dynamics in casts may be influenced differently by different
360 earthworm species and possibly also by their environment (i.e., climate, vegetation
361 and soil properties). Our results indicated that OC associated to micro-aggregates was
362 lost after cast disintegration along with a high proportion of presumably stable OC
363 associated to the mineral fraction. This could be related to enhanced decomposition
364 after exposure to microbial activity but may also be due to loss of small particles
365 following rainfall events (Barthes and Roose, 2002). Although presumably stable OC
366 associated with the mineral fraction was lost during ageing, the bulk OC was
367 characterized by a higher thermal stability and less labile plant material after ageing
368 (cast-A to -E) and higher contribution of N-containing and unspecific compounds,
369 which generally accumulates during degradation and stabilization processes of OM in
370 soils (Kögel-Knabner and Rumpel, 2018).

371

372 To summarize the impact of earthworms on OM properties, we performed a between-
373 class analysis (BCA) (**Fig. 4**). Our analysis indicated that OM properties were
374 changing when casts were broken into small aggregates (10-13 mm) (cast-E). The
375 latest ageing stage, however, kept specific properties significantly different from ctrl

376 samples, and was not on the trajectory towards ctrl aggregates on the BCA plan. As
377 we hypothesized that eventually cast properties should become similar to those of ctrl
378 aggregates, further studies are needed to investigate the timescale at which this
379 happens and what processes are involved. Such information is important in the
380 studied ecosystem because of the large quantity of soil and OC excreted by *A. Khami*
381 as casts. Based on a soil bulk density of $1.0 \text{ g}\cdot\text{cm}^{-3}$, the total quantity of OC in casts
382 represented 6 to 9% of the topsoil (10 cm depth) depending if casts were degraded
383 (cast-E) or recent (cast-A). We therefore suggest that the processes leading to their
384 ageing are important in terms of SOC dynamics, through changing SOC chemical
385 composition and its distribution in stabilized SOM pools.

386

387 **Conclusions**

388 In this study we investigated the fate of SOM during cast ageing. The anecic
389 behaviour of the earthworm *A. khami* leads to the incorporation of fresh OM in their
390 casts. In addition, increased occlusion of OM in micro-aggregates and higher amounts
391 of OM associated to mineral surfaces in casts as compared to ctrl suggested a high
392 potential for OC storage. Our results indicated gradual changes in OM chemical
393 composition and increased thermal stability during ageing. In addition, ageing led to
394 disintegration of aggregates and the decrease of OM in all physical fractions. Even
395 OM associated to the mineral phase containing presumably large amounts of stable
396 OC showed strong dynamics. Our results thus suggest that earthworm activity
397 accelerates SOM dynamics in stabilized pools. Since casts presenting the ultimate
398 degradation stage recognized by the naked eye kept specific OM properties different
399 from the ctrl aggregates, more studies are needed to understand the time horizons and
400 ecological mechanisms affecting SOC transformation in the last cast ageing stages.

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405

406 **References**

407 Aira, M., Monroy, F., Domínguez, J., 2005. Ageing effects on nitrogen dynamics and
408 enzyme activities in casts of *Aporrectodea caliginosa* (Lumbricidae).
409 *Pedobiologia* 49, 467–473. doi:10.1016/j.pedobi.2005.07.003

410 Barois, I., Lavelle, P., 1986. Changes in respiration rate and some physicochemical
411 properties of a tropical soil during transit through *Pontoscolex corethrurus*
412 (glossoscolecidae, oligochaeta). *Soil Biology and Biochemistry* 18, 539–541.
413 doi:10.1016/0038-0717(86)90012-X

414 Barthès, B. and Roose, E., 2002. Aggregate stability as an indicator of soil
415 susceptibility to runoff and erosion : validation at several levels. *Catena*, 47, 133-
416 149. doi: 10.1016/S0341-8162(01)00180-1

417 Barthod, J., Dignac, M.-F., Mer, G. Le, Bottinelli, N., Watteau, F., Kögel-Knabner, I.,
418 Rumpel, C., 2020. How do earthworms affect organic matter decomposition in
419 the presence of clay-sized minerals? *Soil Biology and Biochemistry* 107730.
420 doi:https://doi.org/10.1016/j.soilbio.2020.107730

421 Behar, F., Beaumont, V., De B. Penteadó, H.L., 2001. Rock-Eval 6 Technology:
422 Performances and Developments. *Oil & Gas Science and Technology* 56, 111–
423 134. doi:10.2516/ogst:2001013

424 Bossuyt, H., Deneff, K., Six, J., Frey, S.D., Merckx, R., Paustian, K., 2001. Influence
425 of microbial populations and residue quality on aggregate stability. *Applied Soil*

426 Ecology 16, 195–208. doi:10.1016/S0929-1393(00)00116-5

427 Bossuyt, H., Six, J., Hendrix, P.F., 2005. Protection of soil carbon by microaggregates
428 within earthworm casts. *Soil Biology and Biochemistry* 37, 251–258.
429 doi:10.1016/j.soilbio.2004.07.035

430 Bossuyt, H., Six, J., Hendrix, P.F., 2004. Rapid incorporation of carbon from fresh
431 residues into newly formed stable microaggregates within earthworm casts.
432 *European Journal of Soil Science* 55, 393–399. doi:10.1111/j.1351-
433 0754.2004.00603.x

434 Bottinelli, N., Jouquet, P., Capowiez, Y., Podwojewski, P., Grimaldi, M., Peng, X.,
435 2015. Why is the influence of soil macrofauna on soil structure only considered
436 by soil ecologists? *Soil and Tillage Research* 146, 118–124.
437 doi:10.1016/j.still.2014.01.007

438 Bottinelli, N., Jouquet, P., Tran, T.D., Hallaire, V., 2012. Morphological
439 characterisation of weathered earthworm casts by 2D-image analysis. *Biology
440 and Fertility of Soils* 48, 845–849. doi:10.1007/s00374-012-0674-5

441 Cécillon, L., Baudin, F., Chenu, C., Houot, S., Jolivet, R., Kätterer, T., Lutfalla, S.,
442 Macdonald, A., Van Oort, F., Plante, A.F., Savignac, F., Soucémariadin, L.N.,
443 Barré, P., 2018. A model based on Rock-Eval thermal analysis to quantify the
444 size of the centennially persistent organic carbon pool in temperate soils.
445 *Biogeosciences* 15, 2835–2849. doi:10.5194/bg-15-2835-2018

446 Cerli, C., Celi, L., Kalbitz, K., Guggenberger, G., Kaiser, K., 2012. Separation of light
447 and heavy organic matter fractions in soil - Testing for proper density cut-off and
448 dispersion level. *Geoderma* 170, 403–416. doi:10.1016/j.geoderma.2011.10.009

449 Decaëns, T., Rangel, A.F., Asakawa, N., Thomas, R.J., 1999. Carbon and nitrogen
450 dynamics in ageing earthworm casts in grasslands of the eastern plains of

451 Colombia. *Biology and Fertility of Soils* 30, 20–28. doi:10.1007/s003740050582

452 Dignac, M.F., Houot, S., Derenne, S., 2006. How the polarity of the separation
453 column may influence the characterization of compost organic matter by
454 pyrolysis-GC/MS. *Journal of Analytical and Applied Pyrolysis* 75, 128–139.
455 doi:10.1016/j.jaap.2005.05.001

456 Disnar, J.R., Guillet, B., Keravis, D., Di-Giovanni, C., Sebag, D., 2003. Soil organic
457 matter (SOM) characterization by Rock-Eval pyrolysis: Scope and limitations.
458 *Organic Geochemistry* 34, 327–343. doi:10.1016/S0146-6380(02)00239-5

459 Franchini, J.C., Gonzalez-Vila, F.J., Rodriguez, J., 2002. Decomposition of plant
460 residues used in no-tillage systems as revealed by flash pyrolysis. *Journal of*
461 *Analytical and Applied Pyrolysis* 62, 35–43. doi:10.1016/S0165-2370(00)00210-
462 2

463 Guggenberger, G., Thomas, R.J., Zech, W., 1996. Soil organic matter within
464 earthworm casts of an anecic-endogeic tropical pasture community, Colombia.
465 *Applied Soil Ecology* 3, 263–274. doi:10.1016/0929-1393(95)00081-X

466 Hong, H.N., Rumpel, C., Henry des Tureaux, T., Bardoux, G., Billou, D., Tran Duc,
467 T., Jouquet, P., 2011. How do earthworms influence organic matter quantity and
468 quality in tropical soils? *Soil Biology and Biochemistry* 43, 223–230.
469 doi:10.1016/j.soilbio.2010.09.033

470 Jouquet, P., Podwojewski, P., Bottinelli, N., Mathieu, J., Ricoy, M., Orange, D., Tran,
471 T.D., Valentin, C., 2008. Above-ground earthworm casts affect water runoff and
472 soil erosion in Northern Vietnam. *Catena* 74, 13–21.
473 doi:10.1016/j.catena.2007.12.006

474 Kögel-Knabner, I., Rumpel, C., 2018. Advances in Molecular Approaches for
475 Understanding Soil Organic Matter Composition, Origin, and Turnover: A

476 Historical Overview. *Advances in Agronomy* 149, 1–48.
477 doi:10.1016/bs.agron.2018.01.003

478 Kuzyakov, Y., Blagodatskaya, E., 2015. Microbial hotspots and hot moments in soil:
479 Concept & review. *Soil Biology and Biochemistry* 83, 184–199.
480 doi:10.1016/j.soilbio.2015.01.025

481 McInerney, M., Little, D.J., Bolger, T., 2001. Effect of earthworm cast formation on
482 the stabilization of organic matter in fine soil fractions. *European Journal of Soil*
483 *Biology* 37, 251–254. doi:10.1016/S1164-5563(01)01092-5

484 Podwojewski, P., Orange, D., Jouquet, P., Valentin, C., Nguyen, V.T., Janeau, J.L.,
485 Tran, D.T., 2008. Land-use impacts on surface runoff and soil detachment within
486 agricultural sloping lands in Northern Vietnam. *Catena* 74, 109–118.
487 doi:10.1016/j.catena.2008.03.013

488 Schomburg, A., Verrecchia, E.P., Guenat, C., Brunner, P., Sebag, D., Le Bayon, R.C.,
489 2018. Rock-Eval pyrolysis discriminates soil macro-aggregates formed by plants
490 and earthworms. *Soil Biology and Biochemistry* 117, 117–124.
491 doi:10.1016/j.soilbio.2017.11.010

492 Sebag, D., Verrecchia, E.P., Cécillon, L., Adatte, T., Albrecht, R., Aubert, M.,
493 Bureau, F., Cailleau, G., Copard, Y., Decaens, T., Disnar, J.R., Hetényi, M.,
494 Nyilas, T., Trombino, L., 2016. Dynamics of soil organic matter based on new
495 Rock-Eval indices. *Geoderma* 284, 185–203.
496 doi:10.1016/j.geoderma.2016.08.025

497 Shipitalo, M.J., Protz, R., 1989. Chemistry and micromorphology of aggregation in
498 earthworm casts. *Geoderma* 45, 357–374. doi:10.1016/0016-7061(89)90016-5

499 Soucémariadin, L., Cécillon, L., Chenu, C., Baudin, F., Nicolas, M., Girardin, C.,
500 Barré, P., 2018. Is Rock-Eval 6 thermal analysis a good indicator of soil organic

501 carbon lability? – A method-comparison study in forest soils. *Soil Biology and*
502 *Biochemistry* 117, 108–116. doi:10.1016/j.soilbio.2017.10.025

503 Soucémarianadin, L., Cécillon, L., Chenu, C., Baudin, F., Nicolas, M., Girardin, C.,
504 Delahaie, A., Barré, P., 2019. Heterogeneity of the chemical composition and
505 thermal stability of particulate organic matter in French forest soils. *Geoderma*
506 342, 65–74. doi:10.1016/j.geoderma.2019.02.008

507 Tiunov, A. V., Scheu, S., 2000. Microbial biomass, biovolume and respiration in
508 *Lumbricus terrestris* L. cast material of different age. *Soil Biology and*
509 *Biochemistry* 32, 265–275. doi:10.1016/S0038-0717(99)00165-0

510 Van Groenigen, J.W., Van Groenigen, K.J., Koopmans, G.F., Stokkermans, L., Vos,
511 H.M.J., Lubbers, I.M., 2019. How fertile are earthworm casts? A meta-analysis.
512 *Geoderma* 338, 525–535. doi:10.1016/j.geoderma.2018.11.001

513 Vidal, A., Watteau, F., Remusat, L., Mueller, C.W., Nguyen Tu, T.T., Buegger, F.,
514 Derenne, S., Quenea, K., 2019. Earthworm cast formation and development: A
515 shift from plant litter to mineral associated organic matter. *Frontiers in*
516 *Environmental Science* 7, 55. doi:10.3389/fenvs.2019.00055

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526 **Figure caption**

527 **Figure 1:** Earthworm cast ageing stages. Cast-A: fresh casts, known to be < 1 day;
528 cast-B: fresh casts < 1 week old; cast-C: dry cast and probably more than 1 month old;
529 cast-D: dry cast with marked signs of degradation (greenish colour due to the
530 presence algae with cracks at the surface), older than cast-C; cast-E: casts broken in
531 small aggregates (10-13 mm) representing the last state of degradation recognizable
532 by naked eyes possibly more than 1 year old.

533

534 **Figure 2:** Biplot showing the between components analysis (BCA) from variables
535 describing the chemical composition OM for for the five cast ageing stages and for
536 control aggregates (Ctrl) presenting no recent earthworm activity. Only significant
537 compounds (Table 2) were included into the analysis. Variables are L1: 2-Methoxy-
538 phenol (guaiacol), N2: Pyridine, N8: Benzonitrile, N10: Benzenepropanenitrile, U1:
539 Benzene, U6: Acetic acid, U7: Naphtalene, U9: 4-Methyl-phenol/2- oder 3-Methyl-
540 phenol.

541

542 **Figure 3:** Biplot showing the between components analysis (BCA) from variables
543 describing the Rock-Eval 6 thermal stability parameters for the five cast ageing stages
544 and for control aggregates (Ctrl) presenting no recent earthworm activity. Variables
545 are HI for hydrogen index, OI_{RE6} for oxygen index, R-index for refractory index, I-
546 index for immature index, T_{50_CO2_PYR} corresponding to the temperature at which 50%
547 of the CO₂ resulting from organic matter pyrolysis had evolved and T_{50_CO2_OX}
548 corresponding to the temperature at which 50% of the CO₂ resulting from organic
549 matter oxidation had evolved.

550 **Figure 4:** Biplot showing the between components analysis (BCA) from variables
551 describing OM for for the five cast ageing stages and for control aggregates (Ctrl)
552 presenting no recent earthworm activity. Variables described the OM composition in
553 purple (lignin, N-containing, polysaccharides and unspecific), the Rock-Eval 6
554 thermal stability parameters in blue (HI for hydrogen index, OI_{RE6} for oxygen index,
555 R-index for refractory index, I-index for immature index, T_{50_CO2_PYR} corresponding
556 to the temperature at which 50% of the CO₂ resulting from organic matter pyrolysis
557 had evolved and T_{50_CO2_OX} corresponding to the temperature at which 50% of the
558 CO₂ resulting from organic matter oxidation had evolved), the OC content and C/N
559 ratio of the whole aggregate and the three physical fractions (macroaggregate (macro),
560 microaggregates (micro) and mineral-associated (min)).

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569 **Table 1:** Average values and standard deviation (n=5) of OC content and C/N ratio of the bulk samples, and distribution of OC and the C/N ratio
 570 in the three physical fractions for the five cast ageing stages and for control aggregates (Ctrl) presenting no recent earthworm activity. Different
 571 letters indicate statistically significantly differences (*** p < 0.001; ** p < 0.01; * p < 0.05 and ns for p > 0.05).

Properties	Ctrl	Cast-A	Cast-B	Cast-C	Cast-D	Cast-E	F and p-values
Bulk aggregates							
OC (mg g ⁻¹)	25.9 (4.2) d	52.6 (6.6) a	49.5 (4.1) ab	54.4 (2.0) a	44.8 (3.8) b	35.9 (4.4) c	31.2 ***
C/N	12 (0.4)	12.3 (0.5)	12.7 (1.1)	12.4 (0.8)	12.1 (0.8)	12.1 (0.4)	1.4 ns
Physical fractions							
OC-macro-aggregates (% of bulk)	5.9 (2.3)	11.9 (4.4)	9.8 (4.0)	11.4 (5.0)	8.6 (4.3)	7.7 (2.5)	1.5 ns
OC-micro-aggregates (% of bulk)	0.0 (0.0) c	4.5 (3.4) a	4.9 (1.5) a	4.7 (2.6) a	2.8 (2.6) ab	1.0 (1.3) bc	6.1 ***
OC-mineral-associated (% of bulk)	94.1 (2.3) a	83.6 (7.7) bc	85.2 (4.4) bc	83.9 (6.9) c	88.6 (5.4) abc	91.3 (3.3) ab	2.8 *
OC-macro-aggregates (mg)	15.2 (5.5) c	62.6 (29.8) a	48.6 (19.5) ab	61.8 (28.9) a	38.4 (21.8) abc	27.9 (9.2) bc	5.9 **
OC-micro-aggregates (mg)	0.0 (0.0) b	23.7 (2.1) a	24.4 (6.8) a	25.6 (14.2) a	12.7 (11.9) ab	3.5 (4.9) b	6.5 ***
OC-mineral-associated (mg)	244.4 (43.5) d	439.8 (30.8) ab	421.6 (48.3) ab	456.7 (35.9) a	396.9 (33.0) b	328.8 (44.9) c	22.2 ***
C/N-macro-aggregates	23.9 (4.2) a	16.4 (1.6) bc	15.0 (2.2) c	14.7 (2.5) c	15.5 (1.5) c	18.4 (1.6) b	14.4 ***
C/N-micro-aggregates	-	18.4 (6.5)	17.5 (3.8)	18.1 (7.2)	18.4 (4.8)	-	0.7 ns
C/N-mineral-associated	8.9 (0.4) c	9.7 (0.5) b	9.6 (0.5) b	10.2 (0.3) a	9.6 (0.4) b	9.6 (0.4) b	5.7 **

572
 573 Cast-A: fresh casts, known to be < 1 day; cast-B: fresh casts < 1 week old; cast-C: dry cast and probably more than 1 month old; cast-D: dry cast with marked signs of
 574 degradation (greenish colour due to the presence algae with cracks at the surface), older than cast-C; cast-E: casts broken in small aggregates (10-13 mm) representing the last
 575 state of degradation recognizable by naked eyes.

576 **Table 2:** Average values and standard deviation (n = 5) for the five cast ageing stages and for control aggregates (Ctrl) presenting no recent
 577 earthworm activity. Different letters indicate statistically significant differences (*** p < 0.001; ** p < 0.01; * p < 0.05 and ns for p > 0.05).

Origin	Code	Ctrl	Cast-A	Cast-B	Cast-C	Cast-D	Cast-E	F and p-values
Lignin (%)	L	0.0 (0.0) d	1.8 (0.5) b	0.8 (0.1) c	1.8 (0.6) a	0.5 (0.1) c	0.2 (0.3) d	28.6 ***
2-Methoxy-phenol	L1	0 (0.0) d	0.4 (0.2) bc	0.5 (0.1) b	0.9 (0.3) a	0.5 (0.1) b	0.2 (0.3) cd	11.8***
N-containing (%)	N	31.9 (3.1)	33.4 (3.1)	37.1 (5.4)	33.6 (2.7)	33.6 (1.9)	38.6 (5.6)	2.3 ns
Pyridine	N2	6.5 (1.8) a	4.5 (0.4) bc	4.5 (0.3) bc	4.3 (0.7) c	5.3 (0.4) b	5.1 (1.2) bc	6.9 ***
Benzonitrile	N8	4.8 (1.4) a	4.8 (0.9) ab	3.6 (0.3) abc	2.6 (1.3) c	4.0 (0.3) ab	3.3 (1.5) bc	3.1 *
Benzenepropanenitrile	N10	0.3 (0.2) b	0.9 (0.2) b	0.8 (0.1) b	2.4 (2.3) a	0.9 (0.1) b	0.8 (0.1) b	2.9 *
Polysaccharides (%)	P	10.8 (3.4)	14.5 (4.9)	12.6 (0.9)	16.6 (3.9)	16.4 (3.0)	13.5 (1.9)	2.4 ns
Furfural	P5	1.5 (1.4) c	3.4 (1.4) ab	2.8 (0.5) abc	2.4 (0.8) bc	3.9 (1.2) a	2.0 (0.2) bc	3.4 *
Unspecific (%)	U	57.2 (5.9) a	50.3 (2.8) b	49.6 (4.7) b	45.4 (6.4) b	49.5 (2.5) b	47.7 (6.9) b	2.9 *
Benzene	U1	17.1 (6.4) a	8.8 (2.4) b	7.8 (1.0) b	5.5 (1.5) b	8.1 (1.1) b	8.1 (3.3) b	8.1 ***
Acetic acid	U6	0.0 (0.0) c	1.5 (1.0) ab	1.8 (0.2) ab	2.3 (0.9) a	1.6 (0.7) ab	0.9 (0.9) bc	6.1 ***
Naphtalene	U7	7.6 (1.7) a	3.9 (0.8) b	3.5 (0.4) bc	2.5 (1.0) c	3.8 (0.4) bc	3.6 (1.6) bc	11.8 ***
4,3 or2-Methyl-phenol	U9	3.4 (2.1) b	7.0 (2.6) a	8.1 (0.7) a	7.5 (3.3) a	6.5 (0.8) a	6.2 (1.4) a	4.9 **

578

579 Cast-A: fresh casts, known to be < 1 day; cast-B: fresh casts < 1 week old; cast-C: dry cast and probably more than 1 month old; cast-D: dry cast with marked signs of
 580 degradation (greenish colour due to the presence algae with cracks at the surface), older than cast-C; cast-E: casts broken in small aggregates (10-13 mm) representing the last
 581 state of degradation recognizable by naked eyes.

582 **Table 3:** Average values and standard deviation (n = 5) of Rock-Eval 6 thermal stability parameters for the five cast ageing stages and for
 583 control aggregates (Ctrl) presenting no recent earthworm activity. Different letters indicate are statistically significant differences. (***) p <
 584 0.001; ** p < 0.01; * p < 0.05 and ns for p > 0.05).

Properties	Ctrl	Cast-A	Cast-B	Cast-C	Cast-D	Cast-E	F and p-values
HI (mg HC·g ⁻¹ OC)	154 (8) d	185 (5) ab	179 (6) bc	190 (12) a	176 (8) bc	169 (13) c	12.1***
OI _{RE6} (mg O ₂ ·g ⁻¹ OC)	375 (47) a	236 (15) cd	241(10) cd	229 (4) d	2589 (9) bc	284 (30) b	30.1 ***
I-index	0.07 (0.03) a	0.08 (0.01) a	0.07 (0.01) a	0.08 (0.02) a	0.08 (0.01) a	0.04 (0.01) b	2.8*
R-index	0.62 (0.02) a	0.61 (0.01) b	0.61 (0.01) b	0.60 (0.01) b	0.61 (0.01) b	0.63 (0.01) a	4.3**
T _{50_CO2_PYR}	402 (1) a	398 (2) bc	399 (1) bc	396 (4) c	400 (1) ab	400 (1) ab	4.9 **
T _{50_CO2_OX}	395 (4) a	382 (2) c	386 (2) b	387 (4) b	386 (2) b	388 (2) b	11.6 ***

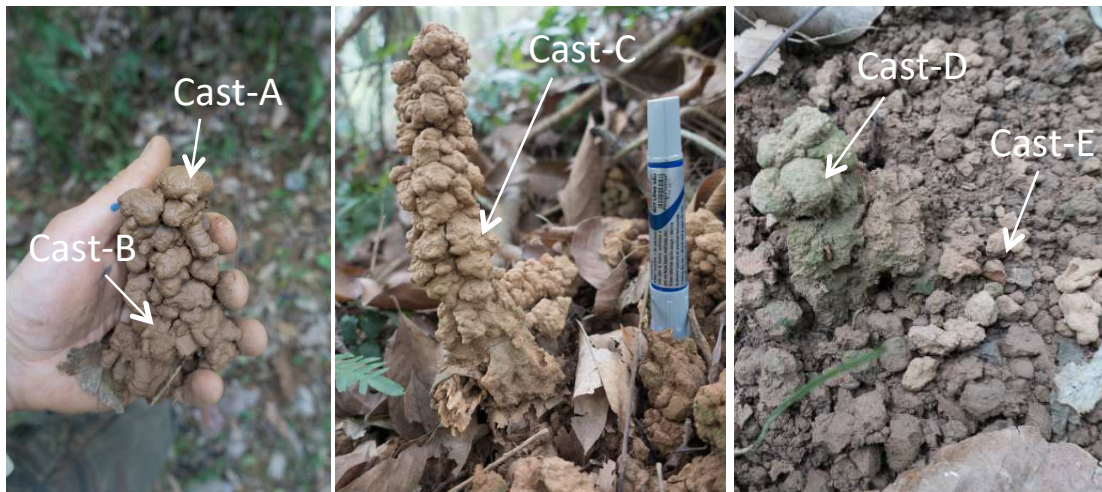
585
 586 Cast-A: fresh casts, known to be < 1 day; cast-B: fresh casts < 1 week old; cast-C: dry cast and probably more than 1 month old; cast-D: dry cast with marked signs of
 587 degradation (greenish colour due to the presence algae with cracks at the surface), older than cast-C; cast-E: casts broken in small aggregates (10-13 mm) representing the last
 588 state of degradation recognizable by naked eyes.

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Ageing of earthworm casts

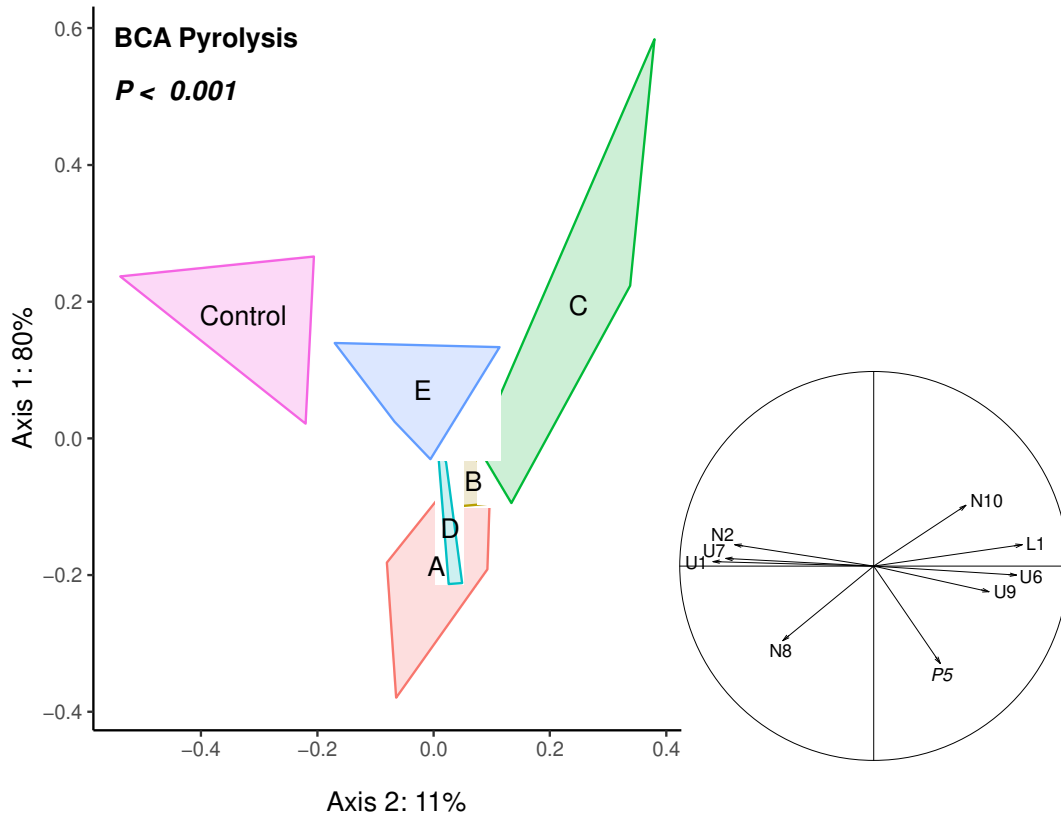


592

593 **Figure 1:** Earthworm cast ageing stages. Cast-A: fresh casts, known to be < 1 day;
594 cast-B: fresh casts < 1 week old; cast-C: dry cast and probably more than 1 month old;
595 cast-D: dry cast with marked signs of degradation (greenish colour due to the
596 presence algae with cracks at the surface), older than cast-C; cast-E: casts broken in
597 small aggregates (10-13 mm) representing the last state of degradation recognizable
598 by naked eyes possibly older than 1 year.

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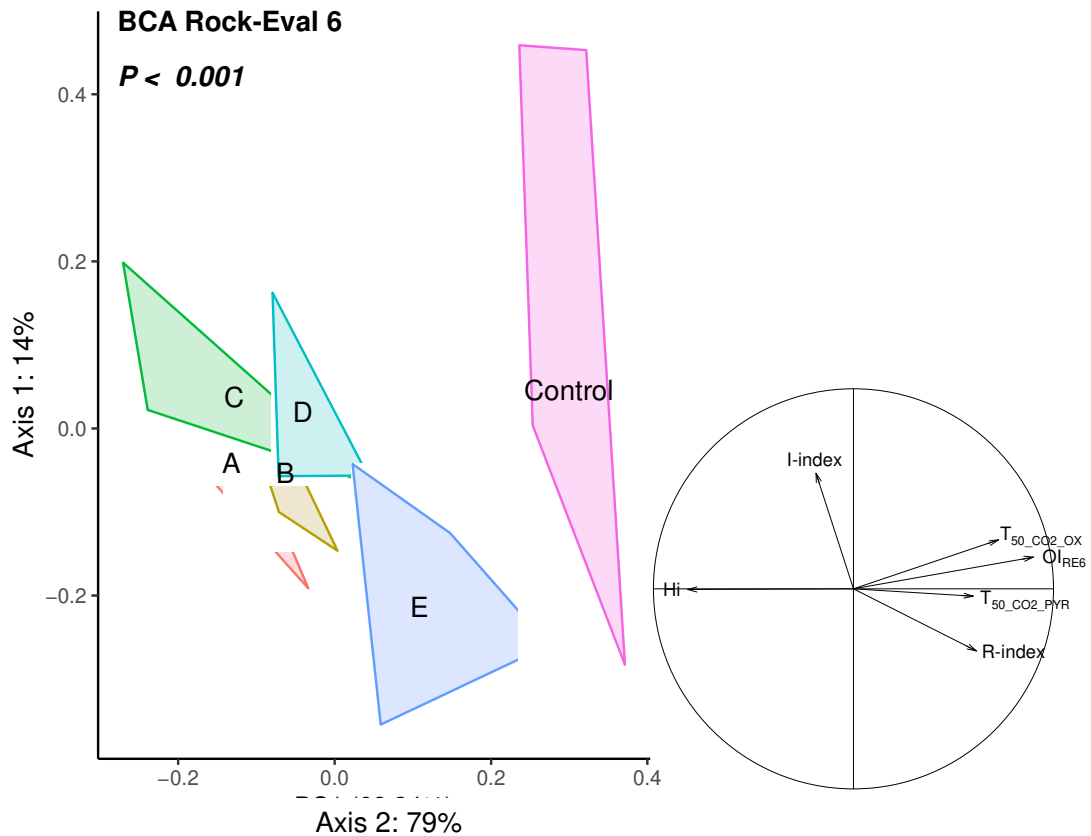


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602 Cast-A: fresh casts, known to be < 1 day; cast-B: fresh casts < 1 week old; cast-C: dry cast and
 603 probably more than 1 month old; cast-D: dry cast with marked signs of degradation (greenish colour
 604 due to the presence algae with cracks at the surface), older than cast-C; cast-E: casts broken in small
 605 aggregates (10-13 mm) representing the last state of degradation recognizable by naked eyes.
 606

607 **Figure 2:** Biplot showing the between components analysis (BCA) from variables
 608 describing the chemical composition OM for for the five cast ageing stages and for
 609 control aggregates (Ctrl) presenting no recent earthworm activity. Only significant
 610 compounds (Table 2) were included into the analysis. Variables are L1: 2-Methoxy-
 611 phenol (guaiacol), N2: Pyridine, N8: Benzonitrile, N10: Benzenepropanenitrile, U1:
 612 Benzene, U6: Acetic acid, U7: Naphtalene, U9: 4-Methyl-phenol/2- oder 3-Methyl-
 613 phenol.

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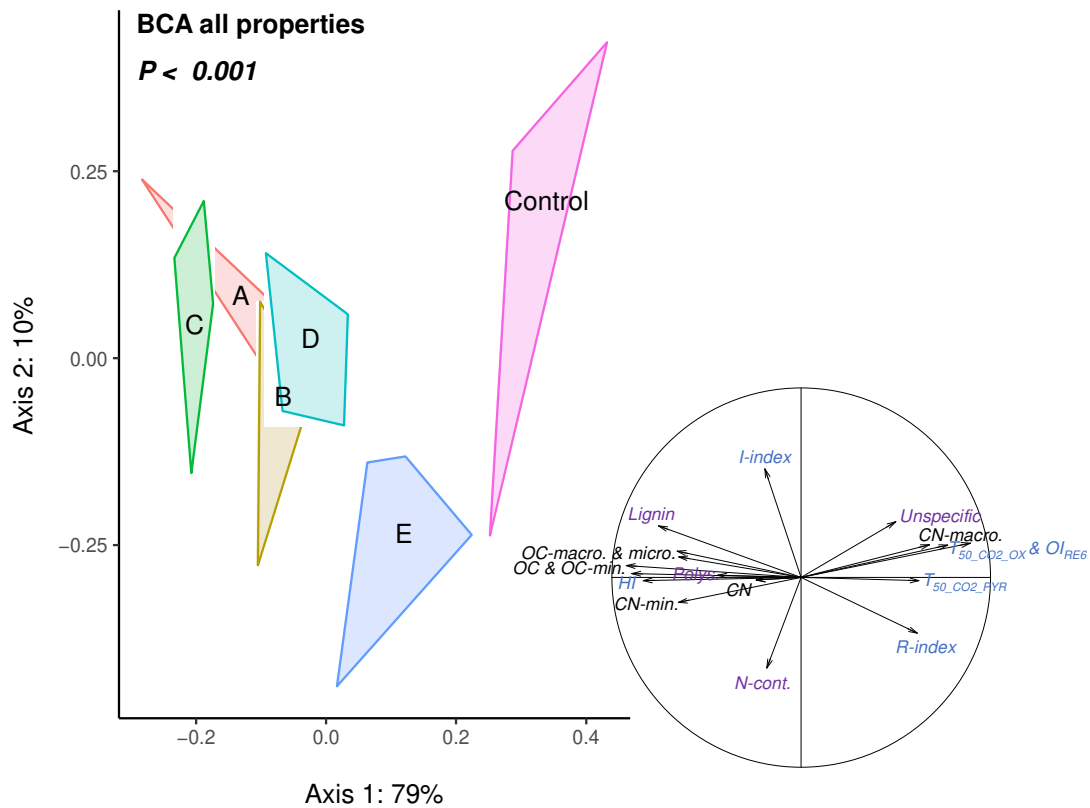


615

616 Cast-A: fresh casts, known to be < 1 day; cast-B: fresh casts < 1 week old; cast-C: dry cast and
 617 probably more than 1 month old; cast-D: dry cast with marked signs of degradation (greenish colour
 618 due to the presence algae with cracks at the surface), older than cast-C; cast-E: casts broken in small
 619 aggregates (10-13 mm) representing the last state of degradation recognizable by naked eyes.

620

621 **Figure 3:** Biplot showing the between components analysis (BCA) from variables
 622 describing the Rock-Eval 6 thermal stability parameters for the five cast ageing stages
 623 and for control aggregates (Ctrl) presenting no recent earthworm activity. Variables
 624 are hydrogen index (HI), oxygen index (OI_{RE6}), refractory index (R-index), immature
 625 index (I-index), and the temperature at which 50% of the CO_2 resulting from organic
 626 matter pyrolysis had evolved during pyrolysis and oxidation ($T_{50_CO2_PYR}$ and
 627 $T_{50_CO2_OX}$).



628

629 Cast-A: fresh casts, known to be < 1 day; cast-B: fresh casts < 1 week old; cast-C: dry cast and
 630 probably more than 1 month old; cast-D: dry cast with marked signs of degradation (greenish colour
 631 due to the presence algae with cracks at the surface), older than cast-C; cast-E: casts broken in small
 632 aggregates (10-13 mm) representing the last state of degradation recognizable by naked eyes.
 633

634 **Figure 4:** Biplot showing the between components analysis (BCA) from variables
 635 describing OM for for the five cast ageing stages and for control aggregates (Ctrl)
 636 presenting no recent earthworm activity. Variables in purple describe the OM
 637 composition (lignin, N-containing, polysaccharides and unspecific), those in blue the
 638 Rock-Eval 6 thermal stability parameters and those in black the OC content and C/N
 639 ratio of the whole aggregate and the three physical fractions (macro-aggregate
 640 (macro), micro-aggregates (micro) and mineral-associated (min)).

641

642 **Supplementary material**

643 **Table S1:** Average values (n = 5) for the five cast ageing stages and for control aggregates (Ctrl) presenting no recent earthworm activity. cast-
 644 A: fresh casts, assumed to be < 1 day and cast-B < 1 week old; cast-C: dry cast with a rounded shape and assumed to be more than 1 month old;
 645 cast-D: dry cast with visible signs of degradation (greenish colour with cracks at the surface); cast-E: broken casts in small aggregates
 646 representing the last state of degradation. Values bearing different letters are statistically significantly different. The numbers of stars for the
 647 significance level indicated the p-value range (*** p < 0.001; ** p < 0.01; * p < 0.05 and ns for p > 0.05).

Origin	Code	Cast-A	Cast-B	Cast-C	Cast-D	Cast-E	Ctrl	F and p-values
Lignin (%)	L	1.8 b	0.8 c	1.8 a	0.5 c	0.2 d	0.0 d	28.6 ***
2-Methoxy-phenol (guaiacol)	L1	0.4 bc	0.5 b	0.9 a	0.5 b	0.2 cd	0 d	11.8***
2-Methoxy-4-(1-propenyl)-phenol (isoeugenol)	L2	0.3	0.3	0.9	0.0	0.0	0.0	1.8 ns
1-(4-Hydroxy-3-methoxyphenyl)-ethanone	L3	1.1	0.0	0.0	0.0	0.0	0.0	1.6 ns
N-containing (%)	N	33.4	37.1	36.2	33.6	38.6	31.9	2.3 ns
1_Methyl-1H-pyrrole	N1	1.6	1.5	1.9	1.9	5.2	2.0	0.9 ns
Pyridine	N2	4.5 bc	4.5 bc	4.3 c	5.3 b	5.1 bc	6.5 a	6.9 ***
2-Methyl-pyridine	N3	1.4	1.2	1.6	1.7	1.6	1.7	1.9 ns
3-Methyl-pyridine	N4	2.1	2.1	4.6	2.4	2.5	2.5	1.8 ns
1H-Pyrrole /Pyrrole	N5	2.3	2.0	2.5	2.5	1.7	2.0	1.5 ns
3-Methyl-1H-pyrrole /2-Methyl-1H-pyrrole	N6	1.2	1.1	2.2	1.3	1.9	1.1	1.9 ns
3-Methyl-1H-pyrrole	N7	1.4	1.2	1.4	1.4	2.0	1.0	1.5 ns
Benzonitrile	N8	4.8 ab	3.6 abc	2.6 c	4.0 ab	3.3 bc	4.8 a	3.1 *
Benzeneacetonitrile	N9	0.6	2.2	0.0	0.0	0.0	0.0	0.9 ns

Benzenepropanenitrile	N10	0.9 b	0.8 b	2.4 a	0.9 b	0.8 b	0.3 b	2.9 *
1H-Indole /Indole	N11	6.2	8.5	6.1	5.4	6.0	4.3	2.2 ns
3-Methyl-1H-indole	N12	6.6	8.5	6.6	6.9	8.4	5.7	1.1 ns
Polysaccharides (%)	P	14.5	12.6	16.6	16.4	13.5	10.8	2.4 ns
2-Methyl-Furan	P1	1.9	1.8	4.3	2.3	2.5	2.3	1.1 ns
1-Heptyl-2-methyl-cyclopropane	P2	0.5	0.3	0.4	0.4	0.9	0.1	1.2 ns
2-Cyclopentene-1-one	P3	3.5	3.0	2.4	3.5	2.7	2.5	1.6 ns
2-Methyl-2-cyclopentene-1-one	P4	1.7	1.6	2.7	2.2	1.9	1.9	0.7 ns
Furfural	P5	3.4 ab	2.8 abc	2.4 bc	3.9 a	2.0 bc	1.5 c	3.4 *
3-Methyl-2-cyclopentene-1-one	P6	1.8	1.5	1.8	2.0	1.6	1.3	1.6 ns
2,3-Dimethyl-cyclopenten-1-one	P7	0.8	0.7	1.4	0.9	1.0	0.7	2.4 ns
5-Methyl-2-furancarboxaldehyde	P8	1.0	0.8	1.3	1.3	0.9	0.5	1.6 ns
Unspecific (%)	U	50.3 b	49.6 b	45.4 b	49.5 b	47.7 b	57.2 a	2.9 *
Benzene	U1	8.8 b	7.8 b	5.5 b	8.1 b	8.1 b	17.1 a	8.1 ***
Toluene	U2	9.4	7.8	8.5	9.0	7.2	9.6	0.9 ns
Ethylbenzene	U3	1.5	1.3	1.6	1.5	1.4	1.4	0.6 ns
1,3-Dimethyl-benzene	U4	1.2	1.1	2.9	1.3	4.6	1.1	0.9 ns
Styrene	U5	2.2	1.7	1.6	2.1	1.8	2.4	2.4 ns
Acetic acid	U6	1.5 ab	1.8 ab	2.3 a	1.6 ab	0.9 bc	0.0 c	6.1 ***
Naphtalene	U7	3.9 b	3.5 bc	2.5 c	3.8 bc	3.6 bc	7.6 a	11.8 ***
Phenol	U8	8.7	8.5	7.0	9.4	8.0	9.0	0.3 ns
4-Methyl-phenol/2- oder 3-Methyl-phenol	U9	7.0 a	8.1 a	7.5 a	6.5 a	6.2 a	3.4 b	4.9 **
3-Ethyl-phenol	U10	1.7	2.4	1.9	2.2	1.9	1.2	2.6 ns
Fluorene	U11	4.5	5.7	4.1	4.0	4.0	4.6	0.8 ns

Cast ageing stages



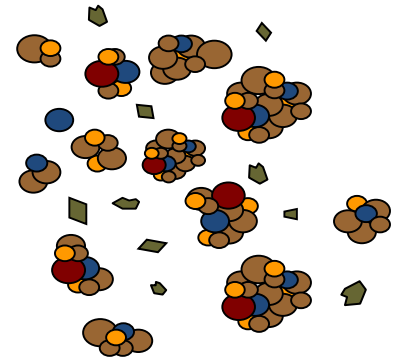
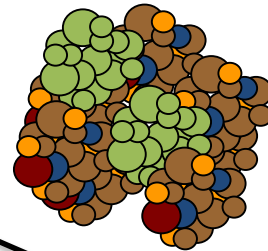
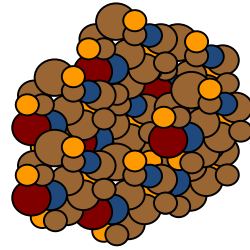
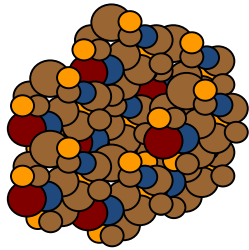
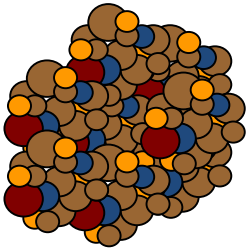
A

B

C

D

E



Anecic species



Stress
(rainfall, animal
trampling....)

oPOM 

Microagg 

MOM 