



**HAL**  
open science

## Soil aggregation, ecosystem engineers and the C cycle

Patrick Lavelle, Alister Spain, Steven Fonte, Jose Camilo Bedano, Eric Blanchart, Victor Galindo, Michel Grimaldi, Juan Jose Jimenez, Elena Velasquez, Anne Zangerlé

### ► To cite this version:

Patrick Lavelle, Alister Spain, Steven Fonte, Jose Camilo Bedano, Eric Blanchart, et al.. Soil aggregation, ecosystem engineers and the C cycle. *Acta Oecologica*, 2020, 105, pp.103561 -. 10.1016/j.actao.2020.103561 . hal-03490669

**HAL Id: hal-03490669**

**<https://hal.science/hal-03490669v1>**

Submitted on 20 May 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 Soil aggregation, ecosystem engineers and the C cycle

2

3 Patrick Lavelle<sup>1</sup>, Alister Spain<sup>2</sup>, Steven Fonte<sup>3</sup>, Jose Camilo Bedano<sup>4</sup>, Eric Blanchart<sup>5</sup>, Victor

4 Galindo<sup>6</sup>, Michel Grimaldi<sup>7</sup>, Juan Jose Jimenez<sup>8</sup>, Elena Velasquez<sup>9</sup>, Anne Zangerlé<sup>10</sup>

5 1. Université Paris Sorbonne, IEES-BIODIS, Paris, France

6 2. The University of Western Australia, Perth, Australia

7 3. Colorado State University, Fort Collins, USA

8 4. National University of Río Cuarto, Córdoba, Argentina

9 5. IRD, UMR Eco&Sols, Montpellier, France

10 6. Universidad del Valle, Cali, Colombia

11 7. Centre IRD, IEES, Centre IRD, Bondy, France

12 8. Instituto Pirenaico de Ecología-CSIC, Jaca, Huesca, Spain

13 9. Universidad Nacional de Colombia, Palmira, Colombia

14 10. Ministry of Agriculture, Viticulture and Rural Development. ASTA, Luxemburg

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32 HIGHLIGHTS

33

34 1. Invertebrates and roots are essential drivers of soil aggregation, but often overlooked.

35

36 2. Manual separation and NIR spectroscopy allow classifying macro-aggregates according  
37 to the physical or biogenic agents that produced them.

38

39 3. A simple field method is proposed to measure in situ rates of production and losses in  
40 the different macro-aggregate pools.

41

42 4. Stocks, inputs and losses of C in aggregate pools characterize their contribution to C  
43 cycling and conservation.

44

45 5. This simple and robust approach can support the identification of management  
46 practices that best store and conserve C in soils

47

48

49

50

51 **Abstract:** Soil aggregation and its effects on soil C storage have been addressed in  
52 thousands of research articles over the last 40 years. Research has been mostly focussed  
53 on the resistance of aggregates to mechanical disruption and the role of organic matter in  
54 aggregate stabilization. On the other hand, relatively little attention has been paid to  
55 identifying the microbial, plant root and macro-invertebrate actors and physical processes  
56 that continuously create and destroy aggregates. The sum and dynamics of these  
57 processes determines the ability of soils to store and conserve C. Understanding the  
58 interactions between aggregation dynamics and C transformations in soils therefore  
59 requires a precise identification of the agents that produced aggregates and knowledge of  
60 the rates of formation and persistence in the pools thus identified.

61 We propose to separate macro-aggregated components of different, physicogenic and  
62 biogenic origins from non-macro-aggregated soil on a morphological basis, using a simple  
63 visual technique. The specific biological or physico-chemical agent which produced each  
64 individual macro-aggregate can then be determined using Near Infrared Spectrometry  
65 (NIRS). A general description of the distribution and quality of organic matter among the

66 different groups of macro-aggregates can be made. Simple soil re-aggregation or dis-  
67 aggregation test conducted in field conditions further measure the production of different  
68 macro-aggregates with time and their mean residence times in the studied soil.  
69 Respirometry measurements on each recognized category of macro-aggregates evaluate  
70 the respective C losses through respiration. The methods described here will allow the  
71 dominant pathways of C flow at a given site to be characterized and possible management  
72 options to increase C storage identified. We finally discuss the different assumptions made  
73 to build this simple model and offer ways to test the methodology under field conditions.

74

75

76

## 77 1. Introduction

78

79 While improved soil management offers one of many promising approaches for climate  
80 change mitigation, soil scientists and technicians are increasingly faced with the challenge  
81 of proposing and testing viable strategies to enhance C sequestration in soils (Altieri et al.,  
82 2015; Poeplau and Don, 2015; Paustian et al., 2016; Powlson et al., 2016; Smith et al., 2016;  
83 Bedano et al., 2019). As a major C reservoir, soil organic matter on Earth is estimated to be  
84 1500 to 2400 Gt C, that is more than twice the mass of the C contained in the atmosphere  
85 (860 Gt). In addition, soils currently sequester an additional  $3.2 \pm 0.7$  Gt C annually,  
86 equivalent to 29.4% of the annual emissions from fossil fuel burning. However, this positive  
87 contribution is offset each year by  $1.5 \pm 0.7$  Gt C emitted as a consequence of land use  
88 change and soil degradation (Le Quéré et al. 2018). We currently do not know whether  
89 soils will continue to store C as climate change accelerates, or lose it -as land degradation  
90 continues- and this represents a major uncertainty for climate change projections  
91 (Carvalhais et al. 2014). The international 4 per 1000 initiative proposes offsetting  
92 anthropogenic emissions by storing every year an extra 0.4 % in agricultural soils through  
93 adequate management options (Minasny et al., 2017). Such a challenge will require precise  
94 tools for implementation and monitoring at plot and farm scales. We argue that our

95 understanding of the mechanisms involved in the stabilization of C in soils and ways to  
96 harness our knowledge under field conditions need to be improved.

97

### 98 The Importance of physical protection for organic matter conservation

99 Three processes allow organic matter conservation in soils: physical protection in  
100 aggregated structures, the association of transformed organic matter to mineral particles in  
101 organo-mineral complexes and chemical recalcitrance (Six et al., 2004). We argue that  
102 these processes are not independent and soil aggregation is expected to be a key process  
103 in facilitating all three (Amezketta, 1999; Sollins et al., 1996; Feller and Beare, 1997; Six et  
104 al., 2000a, 2002a and b; 2004; Von Lutzow et al., 2006). Physical protection is likely a first  
105 step in conservation, that slows down the mineralization process by isolation of microbes  
106 from their organic substrates and/or limits water and oxygen supplies; Kuzyakov et al.,  
107 2015; Negassa et al., 2015; Keiluweit et al., 2016). Suitable conditions are then created for  
108 the other two processes to occur and a great part of SOM storage in soils may be in the  
109 form of organo-mineral complexes (Cotrufo et al., 2019). Organic matter accumulation has  
110 positive feed backs on in turn is widely acknowledged as an essential component for  
111 aggregate formation and stabilization (Amezketta, 1999; Six et al., 2004; Abiven et al, 2009;  
112 Fultz et al., 2013; Gumus and Seker, 2015; Zhu et al., 2017).

113 However, a variable proportion of the leaf and root litter Carbon may have been  
114 mineralized before these complexes are formed. It depends on the suite of initial  
115 decomposition processes that associate physical processes like comminution, transfers in  
116 the soil profile, inclusion in aggregate structures and chemical transformations associated  
117 to digestion and humification (Lavelle and Spain, 2001). In determined conditions of  
118 climate, soil and plant cover, the stabilization of C in soils, in particulate (POM) or mineral  
119 associated (MAOM) forms thus depends to a great extent on the efficiency of physical  
120 protection of organic matter against decay at macro- or microsite scales (Parton et al.,  
121 1988, Lavelle, 2002; Jimenez and Lal., 2006; Kuzyakov et al., 2015).

122 The long admitted alternative option for accumulation in soils of C pools made recalcitrant  
123 because of their chemical compositions is now strongly challenged (Lehmann et al., 2015).

124 Priming effects triggered by water-soluble organic compounds migrating down the soil  
125 profile or released by roots and invertebrate soil ecosystem engineers actually allow the  
126 mineralization of substrates reputed to be highly recalcitrant (Martin et al., 1992; Trigo et  
127 al., 1999; Fontaine et al., 2007; Lehmann et al., 2015).

128 There is a relative dearth of knowledge, however, of the diversity of ways aggregates are  
129 formed and the consequences this has on the amounts and quality of C concentrated and  
130 conserved inside these structures.

131

132 What are soil aggregates??

133 Aggregates defined as “soil specific entities built from mineral and organic compounds with  
134 stronger bonds between building blocks than with neighbouring particles”.....“have a size,  
135 form and stability that is typical for individual soils depending on parent material and  
136 texture, climate and vegetation, biological activity and management” (Yudina and  
137 Kuzyakov, 2018). Their classification and evaluation are still largely determined by the  
138 method used to view or isolate them.

139 Non-destructive viewing techniques require rather sophisticated approaches such as  
140 tomography or the realization and image analysis of soil thin sections (Tracy et al., 2015;  
141 Wang et al., 2016; Zhao et al., 2017; Scarciglia and Barca, 2017; Gutierrez-Castorena et al.,  
142 2018). Isolating techniques separate aggregates according to their resistance to physical  
143 rupture following dry or wet sieving or slaking in water or other liquid substrates (Elliott,  
144 1986; Le Bissonnais, 1996). Isolating may provide different results from viewing techniques  
145 since the energy used to isolate may determine the size of elements separated (Ashman et  
146 al., 2003; Kravchenko et al., 2018).

147 Separation of macroaggregates according to their morphology is a simple method that  
148 associates viewing and isolating approaches (Topoliantz et al., 2000; Velasquez et al.,  
149 2007a). The major advantage is that it does not require sophisticated equipment and thus  
150 can be applied by non-scientific operators, school children, students, technicians and  
151 farmers to discover and quantify soil macro-aggregation.

152

153 Importance and diversity of macro-aggregates in natural soils

154 A wide range of possible mechanisms for aggregate formation exists, from the close  
155 association of mineral and/or organic particles in microaggregates, at microsite scales of a  
156 few tens of microns, to the production of highly organized long-lasting macro-aggregates  
157 made by soil ecosystem engineers such as ants, termites and earthworms (Lavelle et al.,  
158 1997; Tötsche et al., 2018, Zanella et al., 2018; Yudina and Kuzyakov, 2019). Aggregates  
159 frequently have a hierarchical organization and it is widely thought that microaggregates of  
160 a size < 250  $\mu\text{m}$ , the most stable elements, are formed inside macroaggregates as part of  
161 their stabilization process (Six et al., 2014).

162 Many types of macro-aggregates of physicogenic or biogenic origin can be distinguished  
163 (Velasquez et al., 2007a; Zanella et al., 2018; Yudina and Kuzyakov, 2019). The simple visual  
164 separation of biogenic aggregates produced by roots and macroinvertebrate shows their  
165 importance in surface soil horizons: in natural soils of temperate (Pulleman et al., 2004) or  
166 tropical regions (Velasquez and Lavelle, 2019; Grimaldi et al., 2014), aggregates of biogenic  
167 origin, mainly produced by earthworm activities often represent 40 to 60% of the soil  
168 weight in the upper 15 cm of soil.

169

170 Energy cost of forming aggregates

171 When considering the effects of soil aggregation on the C cycle, the release of C by  
172 respiratory activities during their construction is often overlooked. It may be an important  
173 element to consider when designing management options aimed at storing C in soils. For  
174 example, populations of the earthworm *Reginaldia omodeoi* in savannas of Ivory Coast  
175 ingest every year an estimated 850 Mg eq. dry soil and transform it into casts that are  
176 compact and highly resistant macroaggregates with a rather complex general structure  
177 (Lavelle, 1978; Blanchart, 1993; Blanchart et al., 1997). The mechanical work required to  
178 burrow, ingest soil particles, take them through the gut and release them as casts is  
179 allowed by the assimilation of 9% of the C contained in the ingested soil, that is 1.2 Mg C  
180  $\text{ha}^{-1}\text{yr}^{-1}$  emitted as  $\text{CO}_2$  through earthworm respiration (Lavelle, 1978). Energy cost derived

181 from C mineralization is expected to be lower for macro-aggregates formed by fungal  
182 entanglement of particles since hyphae likely grow in the connected porous space of the  
183 soil and do not spend energy moving particles. Data to address this point however, are  
184 lacking. The creation of physicogenic aggregates by processes like wetting/drying or  
185 freeze/thaw alternances apparently has no direct C cost.

186

#### 187 Temporal stability and turnover time of aggregates

188 Aggregates experience dynamic processes of creation, stabilization, ageing, destabilization  
189 and disruption (Marquez et al., 2019). A precise description of their spatio-temporal  
190 dynamics and turnover is therefore an obligate step in evaluating the effect of aggregation  
191 on C storage and conservation in soils. Assuming that C mineralization is either decreased  
192 or stopped in aggregated structures, we need to know how much C is protected in these  
193 structures, to what extent, in which forms and for how long time.

194 We actually know little of the temporal stability of aggregates of different origins and thus  
195 how long these structures will conserve C before they collapse. Macro-aggregates formed  
196 by entanglement of soil particles, by fine roots or mycorrhizal and other fungal hyphae,  
197 generally have rather short life times, from a few days to a few months (Plante and Mc Gill,  
198 2002 ; De Gryze et al., 2005, Segoli et al., 2013); they tend to disaggregate when the fungal  
199 hyphae or root disappear and/or organic binding agents are mineralized. On the other  
200 hand, casts of the African endogeic species *Reginaldia omodeoi* can remain intact for long  
201 periods of time – from 20 to 28 months - depending on soil texture and activity of  
202 decompacting earthworm populations that feed on these structures and disperse them  
203 (Blanchart et al., 1993b). Stabilized macro-aggregates are actually estimated to last up to  
204 10 or 20 years if no disturbed (Lobe et al., 2011, Koesters et al., 2013; Marquez et al.,  
205 2019).

206 Many studies have monitored the build-up or decrease of aggregation as a result of  
207 experimental conditions or changes in management options (Beare et al, 1994; Bronick et  
208 al., 2005; Calonego and Rosolem, 2008). However, the direct measurement of aggregate  
209 turnover under steady state conditions is difficult. It has only been achieved, to our



210 knowledge, using labelling with very specific rare earth or isotopic tracers (Plante and Mc  
211 Gill., 2002; De Gryze et al., 2005), tomography (Roose et al., 2016) or by using rather  
212 complex mechanistic simulation models (Lavelle and Meyer, 1983, Martin and Lavelle,  
213 1992; Segoli et al., 2013; Marquez et al., 2019). These approaches can help with  
214 understanding aggregate turnover dynamics, but also have a number of limitations.

215

## 216 A field-based conceptual model and methodological approach

217 The rarity and diversity of attempts to describe aggregate life histories and dynamics is a  
218 clear indication that the problem is complex. Actually, no simple conceptual or  
219 methodological approach have been proposed so far. While much is known on the  
220 aggregation process, its diversity and importance, we argue that a few critical elements are  
221 still lacking to allow us to evaluate and accurately model the effect of soil aggregation  
222 dynamics on C conservation. This exists as a serious impediment to identify management  
223 practices and accurately predict their potential effect on soil C sequestration in a context of  
224 climate change.

225 This paper starts with a general bibliometric analysis. The aim was to identify knowledge  
226 gaps, that impede the implementation of a dynamic modelling of the soil aggregation  
227 process and its effect on C conservation in soils. The lack of consideration of the role of soil  
228 ecosystem engineers in general soil processes, is one such possible gap indicated by several  
229 authors (Lavelle, 2000; Bottinelli et al., 2015; Filser et al., 2016; Jouquet et al., 2016). We  
230 then propose a simple conceptual model where the conservation of C in soil depends on its  
231 protection into macro-aggregates.

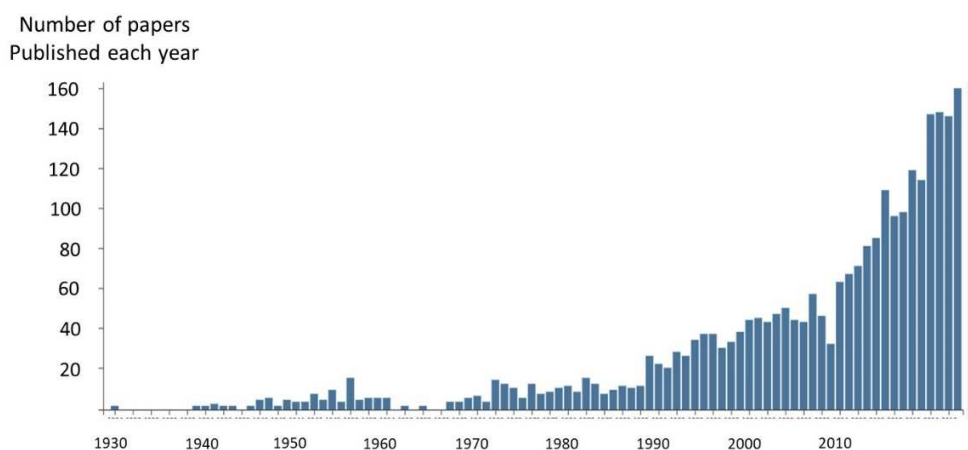
232 We finally discuss the coherence and feasibility of this approach in view of the assumptions  
233 made and identify research needs to improve it. Particular emphasis is set on providing  
234 concepts and methodologies accessible to non-specialized scientists, farmers and  
235 technicians.

236

## 237 2. Soil aggregation in the scientific literature

238

239 A search of the ISI Web of knowledge in November 2019, with the words 'soil 'and  
 240 'aggregat\*' in title provided 2,692 papers, with a clear exponential increase occurring in the  
 241 rates of publication on these and related topics over recent years. With the same keywords  
 242 in topic, we obtained 77,427 articles. The large number of citations (>77,000), further  
 243 illustrates the enormous interest in, and the importance of, this topic.



244  
 245 *Figure 1: Number of papers published each year with words "soil" and "aggregate\*" in title*  
 246 *(source ISI Web) from 1900 to present.*

247  
 248 The frequency of associated words in titles showed the major themes studied. **Organic**  
 249 **matter** or **Carbon** was the most important topic associated with soil aggregates, treated in  
 250 757 (28.1%) papers. This shows the strong association perceived between the aggregation  
 251 process and soil organic matter cycling. **Stability** was the second topic in importance (572  
 252 occurrences, 21.2%), a very important attribute of aggregates that measures their  
 253 resistance to breakdown by physical stress, especially by water (301; 11.1%). **Soil**  
 254 **management** (or till\* or crop\*) considered in 430 papers (16%) reflects the ongoing  
 255 concern for physical degradation in managed soils and interest to identify options for  
 256 reverting degradation. **Aggregate size** (418; 15.5%) is an important morphological attribute,  
 257 considered an indicator of aggregate stability. **Water and erosion** (358; 6.7%) and **texture**  
 258 (clay or text\* or sand) (198; 7.4%) are studied for their important role in aggregate  
 259 stabilization. In comparison, biological actors of aggregation comprised a low proportion of  
 260 the papers. **Microbial** relationships with aggregation (microb\* or microor\* or bact\* or

261 fung\* or mycor\*) were studied in only 307 (11.4%) papers, soil **macroinvertebrates**  
262 (earthworm\* or lumbric\* or termit\* or ant\* or formic\* or macroinvert\*) in 56 papers (2%),  
263 the same as **roots** (55; 2%). Finally, **turnover** of aggregates (7) or of organic matter in  
264 aggregates (9) have been very little considered in aggregate research.

265

### 266 3. An alternative approach to assess aggregate-associated C dynamics

267

268 In their seminal paper, Tisdall and Oades (1982) proposed a comprehensive conceptual  
269 framework to explain the origin, stabilization and dynamics of soil aggregation. They listed a  
270 number of key features that characterize the physical organization of aggregated structures  
271 and a diversity of mechanisms that provide stability against disruption. Such elements have  
272 been thoroughly described and discussed in later reviews (Elliott, 1986; Feller and Beare,  
273 1997; Six et al., 2002a, 2014; Tötsche et al., 2018) and different elements have been  
274 introduced in recent modelling attempts (De Gryze et al., 2001, Marquez et al., 2019).  
275 Although this model has inspired a large number of studies, measuring the different  
276 compartments is still difficult and often requires rather high levels of expertise and  
277 equipment. Modelling the ageing and disruption dynamics of aggregates is even more  
278 difficult, partly because of the initial diversity of aggregate composition and structures that  
279 has not been considered so far in monitoring and modelling attempts. Stabilization or de-  
280 stabilization dynamics further depend on chemical, physical and biological processes  
281 associated with local soil conditions and the quality and quantity of organic material  
282 present in the aggregate.

283

#### 284 3.1. General conceptual model

285

286 Our conceptual model is based on three simple assumptions:

- 287 1. Aggregate fractions should be separated according to their origin. This will prevent  
288 the mixing of very different aggregates into a single category based solely on their  
289 resistance to physical stress. We will recognize various pools of physicogenic or  
290 biogenic aggregates of root or invertebrate origin (Topoliantz et al., 2000; Velasquez  
291 et al., 2007a; Zanella et al., 2018). We expect each pool to have different dynamics

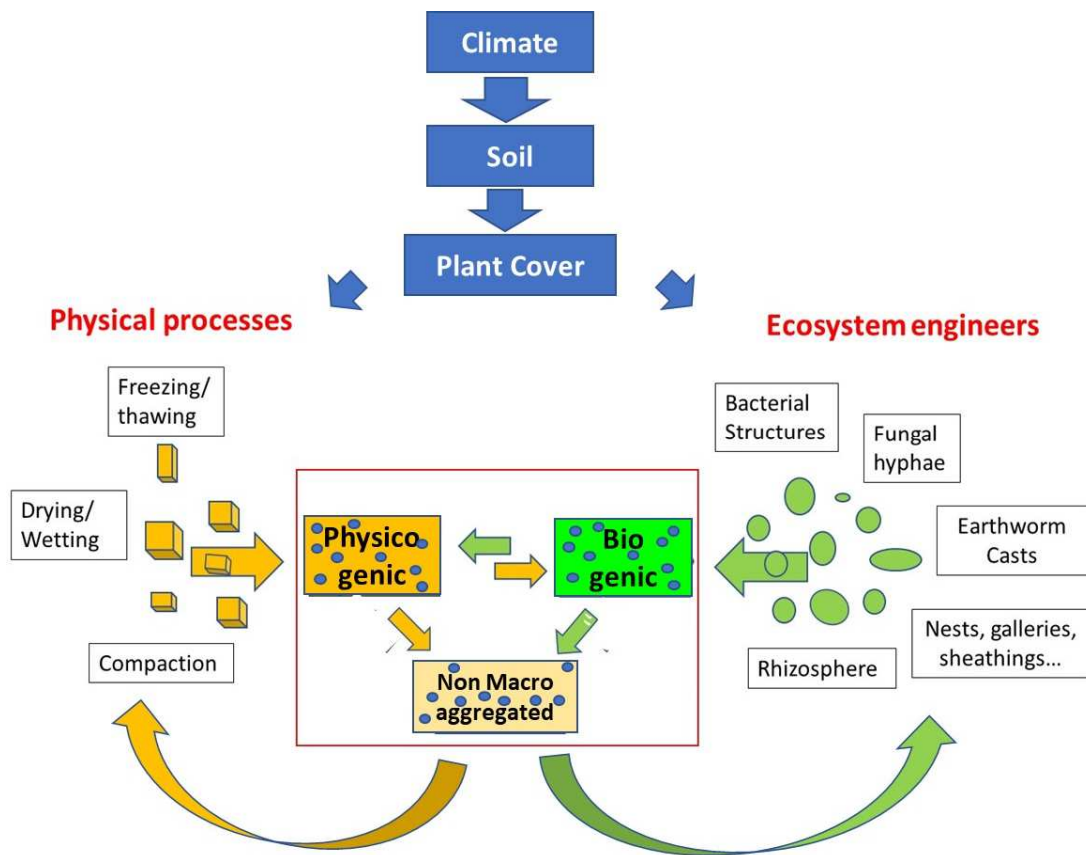
292 according to their initial diversity of compositions and structures (Hedde et al.,  
293 2005).

294 2. Microaggregates are mainly formed within macroaggregates (Six et al., 2004) and  
295 their dynamics are likely associated with the one of the larger structures that hosts  
296 them. We expect, however, that disaggregation of macro-aggregated structures will  
297 not go along with disaggregation of microaggregates and a pool of free  
298 microaggregates may be comprised in the non-macro-aggregated soil fraction  
299 (Tötsche et al., 2018).

300 3. Organic matter transformations are closely linked with aggregate dynamics. The  
301 incorporation of organic matter to the soil matrix is mostly a biological process  
302 associated with root growth, rhizodeposition, comminution, feeding and  
303 bioturbation processes. Flux of water-soluble organic matter that may transit  
304 through the porous space of the soil is expected to be negligible (Lavelle and Spain,  
305 2001; Jimenez et al., 2006). Organic matter is present in different forms and  
306 quantities in the different aggregated and non-macro-aggregated pools and  
307 associated to specific mineral components and porous spaces (Daniel et al., 1997;  
308 Capowiez et al., 2011; Arai et al., 2019). Mineralization rates are therefore likely  
309 determined by conditions that occur in aggregates.

310 In a given set of environmental - climate, soil and plant cover- conditions, macro-  
311 aggregates created by different processes and actors (see Fig. 2) accumulate in three  
312 compartments, which have different turnover times and mineralization rates and  
313 differently affect the overall C dynamics (Fig. 3).

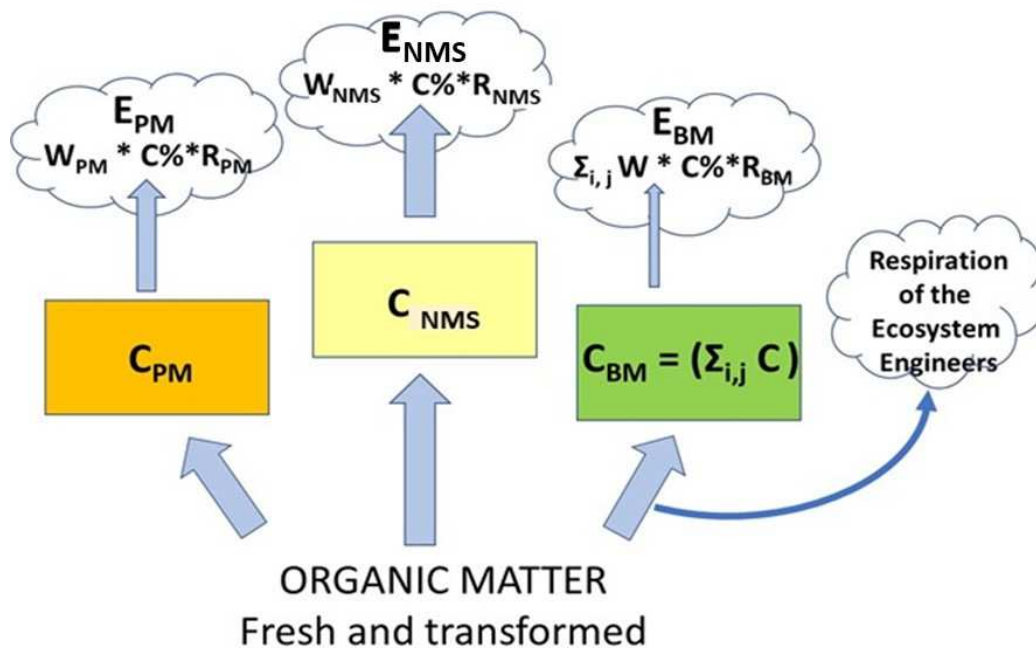
314



315

316 *Figure 2: Conceptual framework for the diagnosis of soil macro-aggregation status and*  
 317 *dynamics. Small circles in each category box symbolize microaggregates. Macroaggregates*  
 318 *are classified in 3 categories, each of them having a definite number of sub categories*  
 319 *indicated in boxes. Their dynamics involves creation from other aggregate pools, ageing and*  
 320 *disruption that transfers their material to the non-macroaggregated pool, or a direct*  
 321 *transformation into biogenic macroaggregates from the other 2 pools.*

322



323

324 Figure 3: Flow of organic matter and gas emissions through the aggregated and non-macro-  
 325 aggregated soil compartments.

326

327 *Carbon in physical macro-aggregates ( $C_{PM}$ ), non-macro-aggregated soil ( $C_{NMS}$ ) and biogenic*  
 328 *macro-aggregates ( $C_{BM}$ ).*

329  *$E_{PM}$ ,  $E_{NMS}$  and  $E_{CBM}$  are gases released by respiratory activities from the  $C_{PM}$ ,  $C_{NMS}$  and  $C_{BM}$*   
 330 *pools respectively.  $W$ : respective weights of the  $C_{PM}$ ,  $C_{NMS}$  and  $C_{BM}$  pools;  $C\%$ : % carbon*  
 331 *contained in the respective pools;  $R_{PM}$ ,  $R_{NMS}$  and  $R_{BM}$ : emission rate in %C of the different*  
 332 *pools. Note that respiration of soil ecosystem engineers when they produce biogenic*  
 333 *aggregates is an additional source of gas emissions.*

334

335 Three major steps are necessary to run this general model of soil aggregation and  
 336 associated C dynamics (Figure 2 and 3).

337

- Diagnosis of the aggregation status of the soil that considers the origins and relative amounts of the different types of aggregates (Fig.2);

338

339

- Assessing the production and turnover of aggregates by measuring the flows of materials among the different aggregated and non-aggregated fractions (Fig 2);

340

341

- C cycling and aggregate dynamics: an analysis of the quality and quantity of C pools contained within each macro-aggregate or non-macro-aggregated fraction, their mineralization rates in the respective pools and the C losses and gains associated with their production, stabilization, ageing and disruption cycles (Fig 3).

342

343

344

345

346 *3.2 Diagnosis of aggregation status*

347

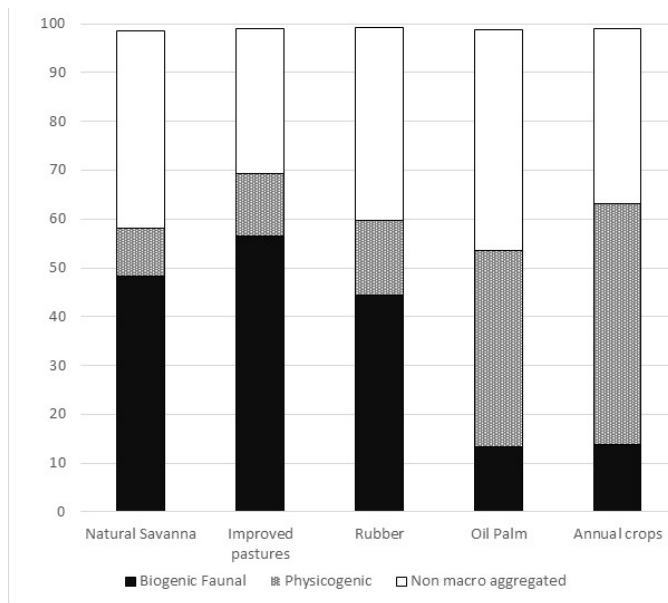
348 Separation and identification of the origins of aggregates is the first step in our approach.

349

350 Large macro-aggregated compartments

351 **Size limits.** There is a general agreement that a 250  $\mu\text{m}$  size separates macro- from  
352 microaggregates. Since it was first proposed by Tisdall and Oades (1982), this limit has  
353 dominated the research although sub categories have been proposed within each class.  
354 This paper focuses on the large macroaggregates > 2 mm sub category that can be easily  
355 separated and identified.

356 **Origin and identity.** Manual fractionation allows the separation of soil into three main  
357 fractions (non-macro-aggregated, physicogenic and biogenic macro-aggregates) based on  
358 their sizes and general morphologies (Topoliantz et al., 2000; Pulleman et al., 2005;  
359 Velasquez et al., 2007a). This method has been applied successfully to describe macro-  
360 aggregation in a wide range of soils, with sandy to clayey textures, in temperate and  
361 tropical areas. An example is provided in Figure 4. A great proportion of soils worldwide,  
362 protected by permanent vegetation and possessing suitable moisture contents, comprise  
363 large proportions of up to 60% macro-aggregates of both physical and biogenic types  
364 (Velasquez et al., 2019).



365

366 *Figure 4: Relative proportions of biogenic, physicogenic macro-aggregates and non-macro-*  
 367 *aggregated soil in different types of land use in the Eastern Plains of Colombia (Lavelle et*  
 368 *al., 2014).*

369

370 Physical aggregates are soil blocks produced by mechanical processes that create fissures in  
 371 a continuous soil matrix (Boersma and Kooistra, 1994; Jongmans et al., 2001; Pulleman et  
 372 al., 2005). They have angular blocky shapes with a dominance of sharp edges and plane  
 373 surfaces. Particles within blocks are held together in a continuous matrix by organic and  
 374 inorganic binding agents together with inter-particle binding due to Van der Waals forces  
 375 (Hu et al., 2015). There is evidence that the same alternation of drying and rewetting  
 376 events, that created these aggregates, may also activate the release of organic molecules,  
 377 by microorganisms, that act as glues and consolidate these structures (Degens and  
 378 Sparling, 1995; Cosentino et al., 2006). The addition of high molecular weight humic  
 379 compounds may have similar effects (Piccolo et al., 1997; Yamaguchi et al., 2004). This set  
 380 of aggregation processes when enhanced by physical compaction through use of tractors  
 381 or cattle trampling in managed systems, tends to create very large clods and hard pans  
 382 (Keen et al., 2013).

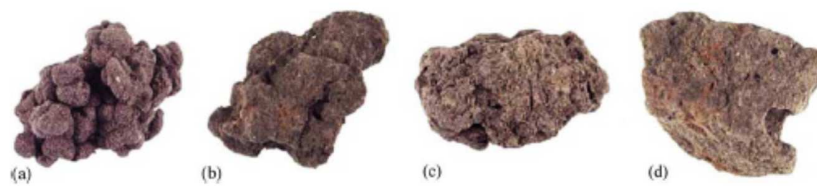
383

384 Biogenic aggregates are produced by organisms classified as ecosystem engineers for their  
 385 ability to modify soil conditions through their physical activities (Blouin et al., 2013; Lavelle



386 et al., 2016). Ecosystem engineers produce a wide diversity of macro-aggregated  
387 structures that differ significantly in their morphologies, chemical and biochemical  
388 compositions (Decaëns et al., 2001; Mora et al., 2003; Velasquez et al., 2007b) (Figure 5).  
389 Specific mineral compositions and organic matter contents and natures gives them rather  
390 specific spectral signatures when illuminated with Near Infrared light (Hedde et al., 2005;  
391 Dominguez-Haydar, 2018).

392



393

394 *Figure 5: Different kinds of soil macro-aggregates: a. biogenic (fresh earthworm cast); b.*  
395 *biogenic (welded cast); c. intermediate (rounded aggregate); d. physicogenic (angular*  
396 *blocky aggregate) (Photo: Pulleman et al., 2005).*

397

398

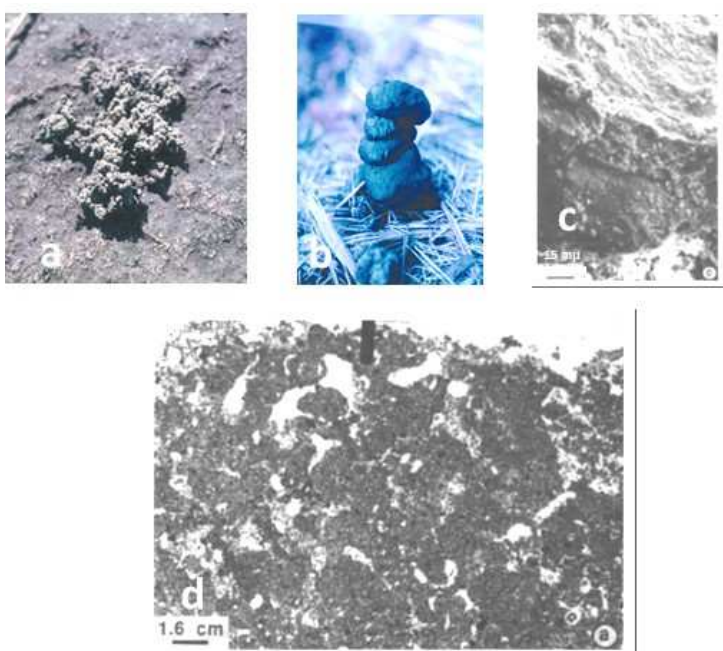
399 We recognize here four main categories which in turn comprise a large diversity of sub  
400 categories.

401 *Fungal macro-aggregates* are created when fungal hyphae entangle organic debris and  
402 mineral particles into stable aggregates that resist slaking and dispersion under the  
403 mechanical stresses applied in classical tests. They are usually not separated in soils with a  
404 permanent plant cover and are mostly seen in intensive cropping systems where  
405 communities of other ecosystem engineers are depleted.

406 Most of the macro-aggregation described in soils of cropped fields and laboratory tests is  
407 actually produced by this process (Tisdall and Oades, 1982; Plante and Mc Gill. 2002).  
408 Mycorrhizal fungi are considered major producers of this type of aggregation (Rillig and  
409 Mummey, 2006).

410 *Earthworms*\_when present are major agents of soil bioturbation with widely different  
411 effects depending on their ecological strategies (Bouché, 1977; Lavelle and Spain, 2001).  
412 Epigeic earthworms live in the litter layers and are active composting agents that transform  
413 litter into largely organic pellets. Anecic species live in deep, subvertical galleries

414 surrounded by zones of compacted soil that may behave as stable aggregated structures.  
415 Their casts are usually relatively loose structures stabilized by high contents of 20 to 40%  
416 on average (Judas, 1992) of little-decomposed particulate organic matter. Endogeic  
417 earthworms live within the mineral soil horizons and feed on soil, with different  
418 concentrations of organic matter depending on their specific – oligohumic, mesohumic or  
419 polyhumic- adaptive strategies (Lavelle and Spain, 2001). They are the major producers of  
420 biogenic macro-aggregates in many soils. They typically ingest from 1 to 10 and sometimes  
421 up to 30 times their own weight of soil daily and total values of up to 1150 Mg dry soil ha<sup>-1</sup>  
422 have been measured, equivalent to a 10 cm thick soil layer (Lavelle, 1978). Their casts are  
423 either highly compacted structures, with bulk densities as high as 1.8 to 2.0 Mg m<sup>-3</sup>  
424 (Blanchart et al., 1993), or loose structures, easily disaggregated, produced by the endogeic  
425 decomposing species (Blanchart et al., 1997).  
426



427  
428 *Figure 6: Loose (Chuniodrillus zieleae) (a) and compact (Reginaldia omodeoi) (b) earthworm*  
429 *casts in African savanna (Lamto, Ivory Coast). (Photos P. Lavelle). Right: (c) Fracture of a*  
430 *surface cast of R. omodeoi observed with SEM. Observe the cortex made of fine particles*  
431 *that gives the surface of the cast a smooth and close aspect (Blanchart et al., 1993).*  
432 *(d) Thin section in the 0-10 cm layer of a soil from a humid savanna (Lamto, Ivory Coast)*  
433 *showing accumulation of globular casts mainly produced by the earthworm Reginaldia*  
434 *omodeoi (Blanchart et al., 1997).*

435

436 The destruction and reorganization of aggregates may actually be a product of earthworm  
437 activity itself in some soils.

438

439 *Social insect structures.* Termites and ants may be active producers of soil macro-  
440 aggregates. Their bioturbation of several Mg ha<sup>-1</sup> year<sup>-1</sup> is the result of a mixture of digging,  
441 building and casting activities (Lobry de Bruyn & Conacher 1990; Folgarait, 1998; Jouquet et  
442 al., 2011). Aggregates formed that way vary from very loose deposits and faeces to very  
443 compact tunnel-shaped aggregates or complex nest structures that may last years to  
444 decades and even more (Rajagopal et al., 1982; Bonell et al., 1986; Mermut et al., 1994;  
445 Humphreys, 1994; Gorosito et al., 2005; Korb, 2011; Erens et al., 2015).

446 Maybe even more remarkable is the formation by humivorous termites of structures  
447 identified as pseudo-sand aggregates that end up comprising the entire volume of some  
448 tropical ferralsols (Wielemaker, 1984, Eschenbrenner, 1986; Balbino et al., 2002; Reatto et  
449 al., 2009; Millogo et al., 2011).

450

451 *Root aggregates* are structures that adhere to the roots. They are formed through five  
452 different processes: local compaction of soil, water regime alteration, rhizodeposition of  
453 gluing materials, decomposition of root material, and entanglement of soil particles by fine  
454 (0.2 to 1 mm in diameter) roots (Monroe and Kladvko, 1987; Miller and Jastrow, 1990;  
455 Morel et al., 1991; Materechera et al., 1992; Dorioz et al. 1993; Degens et al., 1994; Alami  
456 et al. 2000, Czarnes et al. 2000, Gale et al. 2000, Feeney et al. 2006; Demenois et al., 2018).  
457 An estimated 20% of plant photosynthesis is directly released at root tips with strong  
458 biological effects and induced aggregation (Miller and Jastrow, 1990; Lavelle and Spain,  
459 2001). Macro-aggregation by root activities may be a rather fast process. In a five-week  
460 laboratory experiment, *Trifolium pratense* plants produced an average of 217.1 g and  
461 *Plantago lanceolata*, 142.1 g of macro-aggregates in pots that contained 800 g of dry soil  
462 moistened to field capacity (Zangerlé et al., 2011).

463

464 Mixed structures. Although some spectacular structures are produced by well-identified  
465 single actors in nature or laboratory experiments, aggregation seems frequently to be a  
466 cooperative process that involves different kinds of organisms, especially microorganisms.  
467 There is also evidence that fine roots often colonize fresh earthworm casts and thus  
468 possibly add their aggregative effects to those of the earthworm (Decaëns et al., 1999;  
469 Zangerlé et al., 2011; Fonte et al. 2012). Fungal hyphae likely participate in the  
470 consolidation of all sorts of other macroaggregates built by large ecosystem engineers.

471

#### 472 Macro-aggregate specific identity and age

473 For a finer identification of physicogenic and biogenic macroinvertebrate or root macro-  
474 aggregates, Near Infrared Spectrometry (NIRS) has proven to be a very efficient and  
475 practical tool (Hedde et al., 2005). NIRS is a non-destructive method that reflects the  
476 texture and amount and quality of organic matter contained in a soil sample. For each  
477 sample, NIRS provides a spectrum that can be divided into separate ranges of wave length  
478 longitudes (Velasquez et al., 2007b). Manually separated biogenic, physicogenic and non-  
479 macro-aggregated aggregates exhibit significant differences (Dominguez-Haydar et al.,  
480 2018). The same method perfectly discriminated structures produced by different macro  
481 invertebrates (Hedde et al., 2005). Rather small samples of a few cg can be analysed  
482 separately. It is therefore possible to analyse individually all the macro-aggregates from a  
483 sample of a standard 10 x 10 x 10 cm size sample and group aggregates with similar  
484 spectral signatures into homogeneous categories. Using this method, Zangerlé et al. (2016)  
485 were able to separate eight categories of macro-aggregates from a forest soil in  
486 Luxemburg. They further identified the earthworm species that had produced the structure  
487 by comparing the spectral signatures with a bank of signatures established under  
488 laboratory conditions. In another experiment, the same authors observed changes in the  
489 spectral signature of casts as they aged (Zangerlé et al., 2014) showing potential of this  
490 method to identify different phases in the stabilization, ageing and disaggregation process  
491 of aggregates. Alternatively, enzymatic activities, microbial communities PLFA fingerprints  
492 or physical and chemical variables have been shown to discriminate among biogenic

493 structures of different origins (Decaëns et al., 2001; Mora et al., 2003; Hedde et al., 2005;  
494 Jouquet et al., 2013).

495

### 496 *3.3. Assessing the production and turnover of aggregates*

497

498 Our literature survey has confirmed that very few studies have been devoted to aggregate  
499 production and turnover in natural or managed systems.

500

#### 501 Field reaggregation or disaggregation tests

502 A simple way to approach the dynamics of aggregation can be obtained by applying an in-  
503 situ re-aggregation or disaggregation test as done by Blanchart (1992), Barros et al. (2001  
504 and Gorosito (2007()). Blocks of soil from 10 x 10 x 10 cm to 25 x 25 x 30 cm depending on  
505 local conditions are taken in the field and a diagnosis of macro-aggregation is performed  
506 using the methodology proposed in section 3.1. Soil is then crushed and passed through a  
507 sieve with a 1 mm mesh size and taken back to fill the hole from which it had been  
508 excavated and reaggregation is monitored. The unit is covered with some litter taken from  
509 the surroundings and/or protected with a mesh to prevent the direct impact of rainfall that  
510 would create physicogenic macro-aggregates. Different sizes of mesh may be used to allow  
511 invertebrates and roots of different sizes to enter the experimental unit. Under these  
512 conditions, ecosystem engineers from the surrounding soil and natural physical processes  
513 will progressively form aggregates from the non-macro-aggregated soil. Experimental units  
514 are excavated at regular time intervals to follow the restoration of the macro-aggregated  
515 structure. In the experiment conducted by Blanchart (1992), the average percentage of  
516 macro-aggregates > 2mm was 12.9% when macroinvertebrates were not allowed to enter  
517 the unit, 49.9% when the mesh size allowed them to recolonize and 60.6% when the units  
518 had been inoculated with endogeic earthworms. The dynamics of disaggregation can also  
519 be observed putting the intact soil block, after fauna has been eliminated by a temporary  
520 drowning, in a net that will not allow roots and invertebrates to come in (Blanchart et al.,  
521 1997).

522 In some sites, macro-aggregate production can be measured using direct methodologies.  
523 Production of surface and subterranean casts by earthworms, root macro-aggregates and  
524 aggregated soil in the nest structures of termites and ants has been measured by ecologists  
525 in a range of laboratory and field situations (Lavelle, 1978; Lobry de Bruyn et al., 1990;  
526 Jouquet et al., 2013; Lavelle and Spain, 2001; Zangerlé et al., 2011) or simulated with  
527 mechanistic models (Martin and Lavelle, 1992). Data are still scarce because these  
528 measurements that require highly specific biological expertise are generally difficult and  
529 time consuming, especially when measuring structures produced in the soil matrix.

530

#### 531 Spectral analysis of individual macro-aggregates

532 Macro-aggregates of biogenic root or invertebrate origin have a specific spectral signature  
533 that mostly reflect specific quality and concentrations of organic elements and texture  
534 (Hedde et al., 2005; Velasquez et al., 2007a; Zhang et al., 2009; Zangerlé et al., 2011).  
535 These specific signatures seem to progressively converge towards a common “bulk soil”  
536 signature as the structure ages. In a laboratory study conducted with the endogeic species  
537 *Aporrectodea caliginosa*, spectral signature of casts changed first rapidly, in the first 2 days  
538 after deposition and then slowly until day 45 to 60 when it was difficult to separate it from  
539 the bulk soil signature. This method allowed to separate casts aged less than 60 days and  
540 get an estimate of macro-aggregates of a given category during a 2-month period.  
541 (Zangerlé et al., 2016).

542

#### 543 Turnover time in steady state situations

544 In a steady state situation, the amount of new structures created is expected to be  
545 compensated by the destruction of an equal amount. Based on reaggregation experiments  
546 conducted in natural environment, Blanchart (1992) was able to evaluate at 20 months and  
547 28 months, in grass and shrub savannas respectively, the average residence time of casts of  
548 the earthworm *Reginaldia omodeoi* in a savanna of Ivory Coast. In this case,  
549 complementary studies have shown that macro-aggregated structures created by this

550 earthworm are eaten by another earthworm species from the Eudrilidae family that  
551 transform these highly compact casts into fragile erodible soil pellets (Blanchart et al.,  
552 1997). Very fast transformations of the soil structure have been observed with this method.  
553 In an experiment conducted in Amazonia, Barros et al, (2001) exchanged undisturbed soil  
554 blocks from a pasture and the natural forest to the other site. After one year, the heavily  
555 compacted soil of the pasture had recovered a macro-porosity close to that of the forest,  
556 while forest blocks inoculated in the pasture has almost attained the levels of compaction  
557 observed in this pasture. A similar experiment allowed Gorosito (200) to demonstrate that  
558 soil blocks from rice fields derived from a natural savanna were rapidly colonized, with  
559 similar abundances and diversities after one year and even higher values after 2 years.  
560 Similar approaches developed with plants and other producers of biogenic aggregates  
561 might provide some estimates on the life duration of these structures. Further studies will  
562 then be required to identify the predominant disruption process and the fate of  
563 microaggregates that they contained.

564

#### 565 *4 C cycling and soil aggregation*

566

567 Once recognized and measured the different aggregate pools in soil, the effect of soil  
568 aggregation dynamics on C cycling can be assessed using a simple conceptual framework  
569 (Figure 3). Assessing the effect of each recognized pool on soil C cycle first requires an  
570 evaluation of the energy cost (as mineralized C) of creating these macro-aggregates, if  
571 relevant. Then we need a precise knowledge of the amount and quality of the organic  
572 matter contained in this pool and an estimate of the persistence and general dynamics of  
573 the specific aggregate pool. In addition, one must have some ideas on the processes that  
574 lead to their disruption and transfer to the non-macro-aggregated pool or to another pool.

575

#### 576 Soil C in aggregates and aggregation dynamics

577 Analysis of C contents separated among particulate organic matter (POM) and MAOM  
578 fraction associated to the soil mineral fraction will be applied to samples from the different  
579 recognized aggregate pools. This will allow measuring stocks of C associated to the

580 different pools. A number of studies has shown strong effects of plant cover and soil  
581 management on these stocks and their temporal variations (Dexter et al., 1999; Conant et  
582 al., 2001; Post et al., 2000; Martens, 2000; Zhang et al., 2013; 2016).

583 We hypothesize that transformations of particulate and mineral-associated organic matter  
584 are linked to those of the aggregated or non-aggregated mineral pools that comprise them.  
585 Dead fine and coarse root material and root exudates are transferred to the root biogenic  
586 macro-aggregated structures (CBM roots) through rhizospheric activities. Organic debris of  
587 root origin occasionally ingested by invertebrates will be further included in their faecal  
588 pellets as earthworm casts or as organic macro-aggregates of rhizophagous coleoptera  
589 larvae in other fractions of the CBM pool. Organic matter deposited in the leaf litter layer,  
590 the other source of organic materials, is first transformed by comminution and natural  
591 composting processes and progressively incorporated to the soil by digestion and  
592 bioturbation processes associated with macroinvertebrate feeding activities and leaching of  
593 dissolved organic matter. The decomposition rate of this organic matter is influenced by  
594 the same processes that lead to its incorporation in the aggregates, gut transit or burial  
595 below earthworm casts and insect deposits at the soil surface.

596

#### 597 Energy cost of macro-aggregate production

598 As indicated before, the formation of certain biogenic macroaggregates has a significant  
599 cost in energy and C emission. This C loss may be evaluated by comparing C contents of the  
600 non-macroaggregated material originally used with the macroaggregates produced, or  
601 measuring the respiratory costs associated with the mechanical activities associated to  
602 their formation (Lavelle, 1978).

603

#### 604 Carbon emission from the aggregated and non-macro-aggregated pools

605 A large number of studies have compared the respiration activity of aggregates of different  
606 size fractions and in different conditions of soil management (Dexter et al., 1999; Ashman  
607 et al., 2003; Zhang et al., 2013; 2016). Carbon from each pool is mineralized at different



608 rates. For example, Martin (1991) showed that C mineralization proceeded at a much lower  
609 rate in casts of *Reginaldia omodeoi* than in the non-ingested soil sieved at 2mm. Digestion  
610 of the soil prior to cast release had decreased the C content of the soil by 9%, but  
611 mineralization inside the very compact cast had decreased microbial activity to such a low  
612 value that the non-digested soil and the cast had the same C contents after 100 days. With  
613 each day past this limit, the difference in C contents between macro-aggregated and non-  
614 macro-aggregated soil would increase. Where casts may persist from 20 to 28 months, the  
615 ability of this species and type of biogenic aggregate to conserve C may be very important.  
616 The expected 'protection' of C from mineralization however will likely depend on conditions  
617 created within the structure: aeration, temperature and moisture conditions,  
618 stoichiometric and other chemical environment conditions.

619 The major water-soluble organic sources, root exudates, earthworm intestinal mucus or  
620 termite saliva that represent inputs of several Mg C per ha per year are mixed with the  
621 mineral soil and soon become part of the macro-aggregated soil fraction, in the forms of  
622 earthworm casts, root macro-aggregates or termite constructions.

623 In the absence of invertebrate ecosystem engineers, leaf litter organic matter stays at the  
624 soil surface and the production of organo-mineral macro-aggregates is limited to root  
625 aggregate production. Water soluble organic matter leached from the litter layer may well  
626 circulate among macro-aggregated fractions as part of the soil drainage process and  
627 stimulate microbial decomposing activities creating priming effects (Fontaine et al., 2007).  
628 It should, however, not have a significant effect since it represents a rather limited flow as  
629 compared to other components of organic matter inputs (Lavelle and Spain, 2001; Jimenez  
630 and Lal, 2006). It is also expected to be rapidly flocculated in the presence of clay minerals  
631 (Toutain, 1981) or consumed upon entering the soil matrix and does not enter easily inside  
632 compact aggregates

633 Carbon in the non-macro-aggregated soil pool ( $C_{NMS}$ ) may have been released from  
634 disaggregated biogenic or physical aggregates or be generated by flocculation or  
635 condensation processes of water-soluble organic matter occurring in the interaggregate

636 space. Carbon of the physicogenic macro-aggregate pool ( $C_{PM}$ ) may have been contained in  
637 the non-aggregated pool before it was transformed into physical aggregates.

638

## 639 5 Discussion

640

641 Our literature review confirmed that biological processes are severely undermined in  
642 aggregate studies was confirmed: only 11.5% of the papers considered microbial  
643 components, and 4% roots and invertebrate activities. This is clearly the result of restricted  
644 disciplinary focus where a holistic view is necessary (Lavelle et al., 2016; Briones, 2018).  
645 Five other important gaps, addressed below, appeared that are susceptible to hinder a  
646 global and practical understanding of processes at hand and ways to enhance their benefits  
647 in terms of ecosystem services. The approach that we propose to attain this goal is based  
648 on a number of assumptions, conceptual simplifications and the choice of technical options  
649 that often coincide with these gaps.

650

651 Definition and classification of aggregates: The need for a comprehensive separation  
652 technique that combines viewing and isolating approaches.

653 Soil macroaggregates may be seen in very different ways by scientists depending on the  
654 discipline they belong to. For soil ecologists, technicians and farmers, they often are visible  
655 and easily recognizable structures created by roots and invertebrate ecosystem engineers  
656 (Lavelle et al., 2006; Blouin et al., 2013; Jouquet et al., 2016). A severe drawback of using  
657 stress resistance of aggregates as a way to isolate them is that part of the resistance is  
658 provided by chemical and biological binding effects associated to the soil type, and not to  
659 the macro-aggregation process itself. On the other hand, a direct relationship between  
660 stabilization and conservation of C in soil conditions has not been proven to our knowledge.  
661 The manual separation technique that we propose is a combination of viewing and  
662 isolation techniques. This technique has proven very efficient at showing scientists,  
663 students and farmers the importance and vulnerability of the macro aggregation in soils.  
664 Synthetic indicators made with data provided with this method are always linked with

665 other physical, chemical or biological attributes of soil fertility and ecosystem services  
666 provisioning (Velasquez et al., 2007b; Lavelle et al., 2014; Grimaldi et al., 2014; Velasquez  
667 ad Lavelle, 2019). When the objective is to show the importance of the macro aggregation  
668 process to students or farmers and differential effects of soil management options, no  
669 specific training is required since trainees will separate structures the same way, whatever  
670 it is, in different soils and perceive differences. Efforts should be done however, to better  
671 standardize this technique (Jouquet et al., 2009). Clear recommendations should be made  
672 on how to properly disrupt the soil matrix according to natural breaking surfaces, in  
673 different conditions of soil texture and plant root densities. Separation of root and other  
674 biogenic aggregates may require specific actions depending on their sizes and structures.  
675 Soil texture - especially when clayey- or effects of mismanagement -when soils are highly  
676 compacted- may complicate this technique in some situations. An important issue is the  
677 size of the elements that have been identified as macro-aggregates. When felt important,  
678 smaller sieves could be used to isolate fractions down to 1, or even 0.25 mm. In this case,  
679 the observation of subsamples with a stereo microscope may provide the accuracy  
680 required by specific scientific studies (Topoliantz et al., 2000).

681

682 Macro vs. microaggregates. Can microaggregates be independent of macro-aggregates?  
683 And to what extent?

684 The idea that microaggregates are formed inside macro-aggregates (Six et al. 2000) offers a  
685 welcome simplification in our approach. It is also in accordance with the self-organized soil  
686 model that proposes a hierarchical organization of soil, with successive functional units  
687 nested in each other as their size increases (Lavelle et al., 2016). While this view of soil  
688 organization is widely acknowledged and demonstrated by an important number of studies  
689 (Six et al., 2000; Bossuyt et al., 2004; Fonte et al., 2012), a few questions remain. First  
690 microaggregates are not a homogeneous category (Tötsche et al, 2018) although, there is  
691 some expectation that microaggregates have compositions derived from that of  
692 macroaggregates, although with generally less C and lower C:N ratio (Blanchart et al.,  
693 2000). Maybe more important is knowing what happens to microaggregates when

694 macroaggregates get disrupted. In the model of aggregate dynamics proposed by Marquez  
695 et al. (2018), microaggregates may be either disrupted when the macro-aggregate that  
696 comprise them is disrupted or be part of a transient fractions that is further reincorporated  
697 into macro-aggregates.

698

699 Identify aggregate origins to understand biological or other processes that determine their  
700 creation and further dynamics.

701 The very low proportion of articles that consider root or invertebrate generation of macro-  
702 aggregates points at a very important gap. Specificities of composition, structure and  
703 microbial communities at species level of these macro-aggregates are very important  
704 determinants of their further stability and temporal dynamics (Hedde et al., 2005; Mora et  
705 al., 2003; Jouquet et al., 2013). NIRS has been showed to offer a cheap and efficient way to  
706 solve the problem. However, NIR spectral signatures have also proved to be unstable in  
707 ageing earthworm casts (Zangerlé et al., 2014). Changes in spectral signature probably  
708 reflect the rapid changes of microbial biomass and mineral N contents observed in freshly  
709 deposited earthworm casts (Lavelle et al., 1992). The Intriguing observation that casts aged  
710 >45 days have a signature similar to the bulk soil should be further investigated. Does this  
711 mean that the surrounding soil was actually mostly comprised of aged earthworm casts of  
712 the species considered? Or can we think of an alternative hypothesis? Fresh macro-  
713 aggregates are hotspots of microbial activity where some microbial species are enhanced,  
714 not the whole soil community. Would the specific signature observed in freshly created  
715 structures reflect important transient biomass of these components? And signature  
716 observed later on an indication that these populations have decreased to their initial  
717 abundance in the bulk soil? Comparable observations made by Blackwood and Paul (2003)  
718 show the importance of this question.

719

720

721

722

723 Interactions among physicogenic and biogenic agents in soil macro-aggregation dynamics

724 In a finite soil volume, formation of new macro-aggregates in a steady state formation has  
725 to be done at expenses of other pools of aggregated or non-aggregated soil. Stable macro-  
726 aggregates produced by a given set of invertebrates or roots can be destroyed by other  
727 invertebrates that may use them as food, or roots and invertebrates that just need to make  
728 their way through the soil to grow or develop their porous domains. Organisms may  
729 cooperate in forming macro-aggregates. This is the case for example for earthworms and  
730 roots, although cooperation may well be specific (Zangerlé, et al., 2011). The equilibrium  
731 among ecosystem engineers with opposite compacting or decompacting effects may be a  
732 critical element when considering soil management options (Blanchart et al., 1997; Chauvel  
733 et al., 1999). Research on this topic is virtually absent.

734

735 C loss and conservation associated to aggregation

736 While soil aggregation is largely associated with C accumulation in soils, detailed underlying  
737 mechanisms are far from being completely understood. The option of our model,  
738 considering C cycling as a discrete process split among different aggregate pools of  
739 different origins with different histories probably deserves improved theoretical and  
740 experimental foundations. In surface soil layers where biological activity is concentrated,  
741 aggregation seems to be the best option for limiting C mineralization. A better  
742 understanding and classification of biological aggregation processes would probably help.  
743 The overall effects of these aggregation processes on C cycling might well differ along a  
744 continuum from short term fungal hyphae particle entanglement observed in poorly  
745 aggregated agricultural soils to potentially long-lived earthworm casts stabilized by a drying  
746 event or aggregates associated with social insect nests. In both extreme cases, respiration  
747 of the organisms responsible for the creation of aggregates have very different energy  
748 costs that should be incorporated in models. One can hypothesize that in steady state  
749 conditions, the cost of creating aggregates is compensated by their protection effect on soil  
750 organic matter (Lavelle and Spain, 2001; Lubbers et al., 2017). Disaggregation of soils

751 associated to intensive cropping practices may be explained by increased microbial  
752 mineralization in conditions where macro-aggregation by ecosystem engineers is deficient.  
753 In other cases, excessive activity of invasive ecosystem engineers, like European earthworm  
754 species in northern America forests, may well increase soil aggregation while soil C stocks  
755 decrease. Bioturbation activities then exceed the effect of physical protection in  
756 aggregates (Bohlen et al., 2004).

757 With soil depth, oxygen concentration naturally decreases and the mere rarefaction of  
758 biological activity is thought to favor C conservation. Mechanisms and conditions that allow  
759 migration of C in deep layers also require better understanding.

760

## 761 6 Conclusion

762

763 Synthesis of knowledges generated in different disciplinary fields led us to propose an  
764 alternative approach to evaluate the process of aggregate formation and its contribution to  
765 the conservation and accumulation of organic matter in soils. The methodology proposed is  
766 based on relatively simple and low-cost techniques that should be accessible to a large  
767 number of scientists, technicians and farmers. This condition is important to develop multi-  
768 functional agricultural practices that contribute to restore soil- based ecosystem services  
769 and especially soil critical contribution to the mitigation of global warming.

770 Explicitly considering biological mechanisms that determine soil aggregation will allow  
771 harnessing this process to ecosystem restoration practices and sustainable management  
772 options. In soils considered as self-organized systems, aggregates are constructed  
773 structures that have positive feedbacks on the communities that produced them (Lavelle et  
774 al., 2016; Bedano et al., 2019). Aggregation is part of the “potential” of the ecosystem as  
775 defined by Holling (2000), a capital that varies along adaptive cycles and that determines  
776 ecosystem evolution. In situ soil reaggregation tests, that indirectly evaluate the ability of  
777 organisms to collectively organize and improve their habitat, might well appear as a simple  
778 way to measure such an important attribute. This evaluation of potential combined with an  
779 assessment of the connectance, mostly linked to local diversity and abundance of plant and

780 invertebrate ecosystem engineer communities, should allow progressing in the base line  
781 evaluation of ecosystems before initiating restoration practices.

782

783

## 784 7 References

785

786 Abiven, S., S. Menasseri and C. Chenu. 2009. The effects of organic inputs over time on soil  
787 aggregate stability - A literature analysis. *Soil Biology & Biochemistry* 41. 1: 1-12.

788

789 Alami, Y., Achouak, W; Marol, C. and Heulin, T. 2000. Rhizosphere soil aggregation and  
790 plant growth promotion of sunflowers by an exopolysaccharide-producing *Rhizobium sp.*  
791 strain isolated from sunflower roots. *Appl. Env. Microb.*, 66, 3393-3398.

792 Altieri, M. A., C. I. Nicholls, A. Henao and M. A. Lana. 2015. Agroecology and the design of  
793 climate change-resilient farming systems. *Agronomy for Sustainable Development* 35. 3:  
794 869-890.

795

796 Amezketa, E. 1999. Soil aggregate stability: a review. *Journal of Sustainable Agriculture* 14.  
797 83-151.

798

799 Ashman, M. R., P. D. Hallett and P. C. Brookes. 2003. Are the links between soil aggregate  
800 size class, soil organic matter and respiration rate artefacts of the fractionation  
801 procedure? *Soil Biology & Biochemistry* 35. 3: 435-444.

802

803 Bach, E. M., Hofmockel, K. S. 2016. A time for every season: soil aggregate turnover  
804 stimulates decomposition and reduces carbon loss in grasslands managed for bioenergy.  
805 *Glob. Ch. Biol. Bioener.* 8, 3 :588-99.

806 Balbino, L.C., Bruand, A., Brossard, M., Grimaldi, M., Hajnos, M., Guimaraes, M.F., 2002.  
807 Changes in porosity and micro aggregation in clayey Ferralsols of the Brazilian Cerrado on  
808 clearing for pasture. *Eur. J. Soil Sci.*, 53, 219-230.

809 Barois, I., Villemin, G., Lavelle, P., Toutain, F. 1993. Transformation of the soil structure  
810 through *Pontoscolex corethrurus* (Oligochaeta) intestinal tract. *Geoderma*, 56, 57-66

811 Barros, E., P. Curmi, V. Hallaire, A. Chauvel and P. Lavelle. 2001. The role of macrofauna in  
812 the transformation and reversibility of soil structure of an oxisol in the process of forest to  
813 pasture conversion. *Geoderma* 100, 1-2: 193-213.

814 Bedano, J.C., Vaquero, F., Dominguez, A., Rodriguez, M.P., Wall, L., Lavelle, P. 2019.  
815 Earthworms contribute to ecosystem process in no-till systems with high crop rotation  
816 intensity in Argentina. *Acta Oecologica* 98. 14–24

817

- 818 Blackwood, C. B. and E. A. Paul. 2003. Eubacterial community structure and population size  
819 within the soil light fraction, rhizosphere and heavy fraction of several agricultural  
820 systems. *Soil Biol. Biochem.* 35, 1245-1255.
- 821 Blanchart, E., 1992. Restoration by earthworms (Megascolecidae) of the macro-aggregate  
822 structure of a destructured savanna soil under field conditions. *Soil Biol. Biochem.*, 24,  
823 12,1587-94.
- 824 Blanchart, E., Bruand, A., Lavelle. P. 1993. The physical structure of casts of *Millsonia*  
825 *anomala* (Oligochaeta, Megascolecidae) in shrub savanna soils (Côte d'Ivoire). *Geoderma*,  
826 56 (1-4), 119-132.
- 827 Blanchart, E., P. Lavelle, E. Braudeau, Y. Le Bissonnais and C. Valentin. 1997. Regulation of  
828 soil structure by geophagous earthworm activities in humid savannas of Cote d'Ivoire. *Soil*  
829 *Biol. & Biochem.* 29. 3-4: 431-439.
- 830 Blanchart, E., Achouak, W., Albrecht, A., Barakat, M., Bellier, G., Cabidoche, Y.M., Hartmann,  
831 C., Heulin, T., Larré-Larrouy, C., Laurent, J. Y., Mahieu, M., Thomas, F., Villemin, G., Watteau,  
832 F. 2000 Déterminants biologiques de l'agrégation des vertisols des Petites Antilles.  
833 Conséquences sur l'érodibilité des sols. *Ét. Gest. Sols*, 7: 309-328.
- 834 Blouin, M., Hodson, M. E., Delgado, E. A., Baker, G., Brussaard, L., Butt, K. R., Dai, J.,  
835 Dendooven, L., Peres, G., Tondoh, J., Cluzeau, D., Brun, J.J. 2013. A review of earthworm  
836 impact on soil function and ecosystem services. *Eur. J. Soil Sci.*, 64(2), 161-82.
- 837 Boersma, O. H., Kooistra, M. J., 1994. Differences in soil structure of silt loam Typic  
838 Fluvaquents under various agricultural management practices. *Agric. Ecosyst. Environ.*, 51,  
839 21-42.
- 840 Bohlen, P. J., S. Scheu, C. M. Hale, M. A. McLean, S. Migge, P. M. Groffman and D.  
841 Parkinson. 2004. Non-native invasive earthworms as agents of change in northern  
842 temperate forests. *Front. Ecol. & Envir.* 2, 8: 427-435.
- 843 Bonell, M., Coventry, R. J., & J. A. Holt. 1986. Erosion of termite mounds under natural  
844 rainfall in semiarid tropical north-eastern Australia. *Catena*, 13, 11-28.
- 845 Bossuyt, H., Six, J., & P.F. Hendrix. 2004. Rapid incorporation of carbon from fresh residues  
846 into newly formed stable microaggregates within earthworm casts. *European Journal of*  
847 *Soil Science*, 55, 393-399.
- 848 Bossuyt, H., Six, J., & P.F. Hendrix. 2005. Protection of soil carbon by microaggregates  
849 within earthworm casts. *Soil Biology and Biochemistry*, 37, 251-258.
- 850 Bottinelli, N., Jouquet, P., Capowiez, Y., Podwojewski, P., Grimaldi, M., Peng, X., 2015. Why  
851 is the influence of soil macrofauna on soil structure only considered by soil ecologists? *Soil*  
852 *Till. Res.* 146, 118-24.



- 853 Bouché, M. B. 1977. Stratégies lombriciennes. Soil Organism as Components of  
854 Ecosystems. *Ecol. Bull. (Stockholm)*, 25, 122-32.
- 855 Briones, M. J. I. 2018. The Serendipitous Value of Soil Fauna in Ecosystem Functioning: The  
856 Unexplained Explained. *Front. Envir. Sci.* 6.
- 857 Bronick, C. J., Lal, R. 2005. Soil structure and management: a review. *Geoderma*. 124(1-  
858 2):3-22.
- 859 Cambardella, C. A., Elliot, E. T. 1993. Methods for physical separation and characterization  
860 of soil organic matter fractions. *Geoderma* 56. 449-457.
- 861 Carvalhais, N., Forkel, M., Khomik, M., Bellarby, J., Jung, M., Migliavacca, M., Mu, M.,  
862 Saatchi, S., Santoro, M., Thurner, M., Weber, U., Ahrens, B., Beer, C., Cescatti, A.,  
863 Randerson, J.T., Reichstein, M., 2014. Global covariation of carbon turnover times with  
864 climate in terrestrial ecosystems. *Nature*, 514, 7521, 213-217.
- 865 Castorena, E. V. G., M. D. Gutierrez-Castorena, T. G. Vargas, L. C. Bontemps, J. D. Martinez,  
866 E. S. Mendez and C. A. O. Solorio. 2016. Micro mapping of microbial hotspots and biofilms  
867 from different crops using digital image mosaics of soil thin sections. *Geoderma* 279, 11-21.
- 868 Chauvel, A., M. Grimaldi, E. Barros, E. Blanchart, T. Desjardins, M. Sarrazin and P. Lavelle.  
869 1999. Pasture damage by an Amazonian earthworm. *Nature* 398, 6722: 32-33.
- 870 Chenu, C., 1995. Extracellular polysaccharides: An interface between microorganisms and  
871 soil constituents. *Environmental impact of soil component interactions; natural and*  
872 *anthropogenic organics*. 1, 217-33.
- 873 Chenu, C., and Stotzky, G., 2002. Interactions between microorganisms and soil particles: an  
874 overview. *Interactions between soil particles and microorganisms. Impact on the terrestrial*  
875 *ecosystems*. P. M. Huang, J. M. Bollag and J. M. Senesi. Chichester, UK, John Willey and sons:  
876 3-40.
- 877 Conant, R. T., K. Paustian and E. T. Elliott. 2001. Grassland management and conversion into  
878 grassland: Effects on soil carbon. *Ecol. Appl.* 11. 2: 343-355.
- 879 Cosentino, D., Chenu, C., Le Bissonnais, Y. 2006. Aggregate stability and microbial  
880 community dynamics under drying-wetting cycles in a silt loam soil. *Soil Biol. Biochem.*  
881 38(8), 2053-62.
- 882 Cotrufo, M.F., Ranalli, M.G., Haddix, M.L. *et al.* Soil carbon storage informed by particulate  
883 and mineral-associated organic matter. *Nat. Geosci.* 12, 989–994 (2019)  
884 doi:10.1038/s41561-019-0484-6
- 885  
886 Czarnes, S., Hallett, P.D., Bengough, A.G., Young, I.M., 2000. Root- and microbial-derived  
887 mucilages affect soil structure and water transport. *Eur. J. Soil Sci.*, 51, 435-443.

- 888 Decaëns, T., Mariani, L., Lavelle, P., 1999. Soil surface macrofaunal communities associated  
889 with earthworm casts in grasslands of the Eastern Plains of Colombia. *App. Soil. Ecol.*,13(1),  
890 87-100.
- 891 Decaëns, T., 2000. Degradation dynamics of surface earthworm casts in grasslands of the  
892 eastern plains of Colombia. *Biol. Fertil. Soils*, 32:149-156.
- 893 Decaëns T, Galvis JH, Amezcua E., 2001. Properties of the structures created by ecological  
894 engineers at the soil surface of a Colombian savanna. *C.R.A. Sciences, III, Life Sci.* 324(5),  
895 465-478.
- 896 Degens, B.P., Sparling, G.P., Abbott, L.K., 1994. The contribution from hyphae, roots and  
897 organic carbon constituents to the aggregation of a sandy loam under long-term clover  
898 based and grass pastures. *Eur. J. Soil Sci.*, 45, 459-468.
- 899 Degens, B. P., Sparling, G.P. 1995. Repeated wet-dry cycles do not accelerate the  
900 mineralization of organic C involved in the macro aggregation of a sandy-loam soil. *Plant &*  
901 *Soil*,175, 2:197-203.
- 902 De Gryze, S., Six, J., Brits, C., Merckx, R. 2005. A quantification of short-term macro-  
903 aggregate dynamics: influences of wheat residue input and texture. *Soil Biol. Biochem.*, 37,  
904 55-66.
- 905 Demenois, J., F. Carriconde, P. Bonaventure, J. L. Maeght, A. Stoke and F. Rey. 2018. Impact  
906 of plant root functional traits and associated mycorrhizas on the aggregate stability of a  
907 tropical Ferralsol. *Geoderma* 312, 6-16.
- 908 Dexter, A. R., J. Arvidsson, E. A. Czyz, A. Trautner and B. Stenberg. 1999. Respiration rates of  
909 soil aggregates in relation to tillage and straw-management practices in the field. *Act. Agric.*  
910 *Scand. Section B-Soil and Plant Science* 49. 4: 193-200.
- 911 Dominguez-Haydar, Y., Castañeda, C., Rodriguez-Ochoa, R., Jimenez, J.J. 2018. Assessment  
912 of soil fauna footprints at a rehabilitated coal mine using micromorphology and near  
913 infrared spectroscopy (NIRS). *Geoderma*. 313, 135-145.
- 914 Dorioz, J.M., Robert, M., Chenu, C. 1993. The role of roots, fungi and bacteria on clay  
915 particle organization. An experimental approach. *Geoderma*, 56, 179-194.
- 916 Elliott, E.T., 1986. Aggregate structure and Carbon, Nitrogen, Phosphorus in native and  
917 cultivated soils. *Soil Sci. Soc. Am. J.*, 627-633.
- 918 Elliott, E. T., and Cambardella, C. A., 1991. Physical separation of soil organic matter. *Agr*  
919 *Ecosyst & Envir.*34: 407-19.
- 920 Erens, H., Boudin, M., Mees, F., Mujinya, B. B., Baert, G., Van Strydonck, M., Boeckx, P., &  
921 Van Ranst, E. 2015. The age of large termite mounds-radiocarbon dating of *Macrotermes*

- 922 *falciger* mounds of the Miombo woodland of Katanga, DR Congo. *Palaeoclim. Palaeoeco.*  
923 435, 265-271.
- 924 Eschenbrenner, V. 1986. Contribution des termites à la micro-agrégation des sols  
925 tropicaux. *Cah. ORSTOM, sér. Pédol.* 22(4),397-408.
- 926 Feeney, D.S., Crawford, J.W., Daniell, T., Hallett, P.D., Nunan, N., Ritz, K., Rivers, M., Young,  
927 I.M. 2006. Three-dimensional micro organization of the soil-root-microbe system. *Microb.*  
928 *Ecol.*, 52, 151-158.
- 929 Feller, C., Beare, M. H., 1997. Physical control of soil organic matter dynamics in the tropics.  
930 *Geoderma*, 79(1-4), 69-116.
- 931 Filser, J., Faber, J.H., Tiunov, A.V., Brussaard, L., Frouz, J., De Deyn, G., Uvarov, A.V., Berg,  
932 M.P., Lavelle, P., Loreau, M., Wall, D.H., Querner, P., Eijsackers, H., Jiménez, J.J. 2016. Soil  
933 fauna: key to new carbon models. *SOIL*, 2(4), 565-582.
- 934 Folgarait, P. J., 1998. Ant biodiversity and its relationship to ecosystem functioning: a review.  
935 *Biodiv. and Conserv.*, 7(9): 1221-1244.
- 936 Fonte, S.J., Quintero, C.D., Velasquez, E., Lavelle, P., 2012. Interactive effects of plants and  
937 earthworms on the physical stabilization of soil organic matter in aggregates. *Plant &*  
938 *Soil*.359, 1-2
- 939 Gale, W.J., Cambardella, C.A., Bailey, T.B. 2000. Root-derived carbon and the formation and  
940 stabilization of aggregates. *Soil Sc. Soc. Am. J.*, 64, 201-207.
- 941 Gorosito, N., Curmi, P., Hallaire, V., Folgarait, P., Lavelle, P. 2005. Morphological changes in  
942 *Camponotus punctulatus* (Mayr) anthills of different ages. *Geoderma*, 132 (3-4), 249-260.
- 943 Gorosito, M. 2007. *Camponotus punctulatus*: su rol en la comunidad de hormigas,  
944 colonización de nuevas áreas y causas de su explosión demográfica frente a un disturbio  
945 agrícola. Universidad Quilmes, Bernal, Argentina.  
946
- 947 Grimaldi, M., Oszwald, J., Doledec, S., Hurtado M. P., Miranda, I. Arnaud de Sartre, X., Assis,  
948 W. S., Castaneda, E., Desjardins, T., Dubs, F., Guevara, E., Gond, V., Lima,T., Marichal, R.,  
949 Michelotti, F., Mitja, D. N., Noronha C., Oliveira, M. N., Ramirez, B., Rodriguez, G., Sarrazin,  
950 M., da Silva, M. L., Silva Costa, L. G., de Souza, S. L., Veiga I., Velasquez, E., P. Lavelle. 2014.  
951 Ecosystem services of regulation and support in Amazonian pioneer fronts: searching for  
952 landscape drivers. *Landsc. Ecol.*, 29, 2: 311-328.
- 953 Gumus, I. and C. Seker. 2015. Influence of humic acid applications on modulus of rupture,  
954 aggregate stability, electrical conductivity, carbon and nitrogen content of a crusting  
955 problem soil. *Solid Earth* 6, 4: 1231-1236.  
956

- 957 Gutierrez-Castorena, M. C., E. V. Gutierrez-Castorena, T. Gonzalez-Vargas, C. A. Ortiz-  
958 Solorio, E. Suastegui-Mendez, L. Cajuste-Bontemps and M. N. Rodriguez-Mendoza. 2018.  
959 Thematic micro-maps of soil components using high-resolution spatially referenced  
960 mosaics from whole soil thin sections and image analysis. *Eur. J. Soil Sci.* 69, 2 : 217-231.  
961
- 962 Hedde, M., Lavelle, P., Joffre, R., Jimenez, J. J., Decaëns, T. 2005. Specific functional  
963 signature in soil macro-invertebrate biostructures. *Funct. Ecol.*, 19(5), 785-93.
- 964 Holling, C. S. 2001. Understanding the complexity of economic, ecological, and social  
965 systems. *Ecosystems* 4, 5: 390-405.
- 966 Humphreys, G.S., 1994. Bioturbation, biofabrics and the biomantle: an example from the  
967 Sydney Basin. *Micromorphology in Soil: Studies in management and Genesis* (eds A.J.  
968 Ringrose-Voase and G. Humphreys), Amsterdam, pp. 787-798.
- 969 Hu, F.N., Xu, C.Y., Li, H., Li, S., Yu, Z.H., Li, Y., et al. 2015. Particles interaction forces and  
970 their effects on soil aggregates breakdown. *Soil. Til. Res.* 147:1-9.
- 971 Jimenez, J. J. and R. Lal. 2006. Mechanisms of C sequestration in soils of Latin America. *Crit.*  
972 *Rev. Plant Sci.*, 25. 4: 337-365.
- 973 Jongmans, A. G., Pulleman, M. M., Marinissen, J. C. Y. 2001. Soil structure and earthworm  
974 activity in a marine silt loam under pasture versus arable land. *Biol. Fertil. Soils.* 33, 279-  
975 285.
- 976 Jouquet, P., Podwojewski, P., Bottinelli, N., Mathieu, J., Ricoy, M-, Orange, D., Tran, T.D.,  
977 Valentin, C. 2008. Above-ground earthworm casts affect water runoff and soil erosion in  
978 Northern Vietnam. *Catena*, 74, 1 :13-21.
- 979 Jouquet, P., A. Zangerlé, C. Rumpel, D. Brunet, N. Bottinelli and T. T. Duc. 2009. Relevance  
980 and limitations of biogenic and physicogenic classification : a comparison of approaches for  
981 differentiating the origin of soil aggregates. *Eur. J. Soil Sci.* 60, 6: 1117-1125.  
982
- 983 Jouquet, P., Traore, S., Choosai, C., Hartmann, C., Bignell, D. 2011. Influence of termites on  
984 ecosystem functioning. *Ecosystem services provided by termites.* *Eur. J. Soil Biol.*, 47(4):  
985 215-22.
- 986 Jouquet, P., P.-A. Maron, V. Nowak and D. Toan Tran. 2013. Utilization of microbial  
987 abundance and diversity as indicators of the origin of soil aggregates produced by  
988 earthworms. *Soil Biol. & Biochem.* 57, 950-952.
- 989 Jouquet, P., Guilleux, N., Caner, L., Chintakunta, S., Ameline, M., Shanbhag, R. R. 2016.  
990 Influence of soil pedological properties on termite mound stability. *Geoderma*, 262, 45-51.
- 991 Judas, M. 1992. Gut content analysis of earthworms (Lumbricidae) in a Beechwood. *Soil*  
992 *Biol. Biochem.*, 24, 12: 1413-17.

- 993 Keen, A., Hall, N., Soni, P., Gholkar, M. D., Cooper, S., Ferdous, J. 2013. A review of the  
994 tractive performance of wheeled tractors and soil management in lowland intensive rice  
995 production. *J. Terramec.* 50, 1:45-62.
- 996 Keiluweit, M., Nico, P. S., Kleber, M., Fendorf, S. 2016. Are oxygen limitations under  
997 recognized regulators of organic carbon turnover in upland soils? *Biogeochem.*,127(2-  
998 3):157-71.
- 999 Keyes, S. D., Gillard, F., Soper, N., Mavrogordato, M. N., Sinclair, I. Roose, T. 2016. Mapping  
1000 soil deformation around plant roots using in vivo 4D X-ray Computed Tomography and  
1001 Digital Volume Correlation. *J. Biomech.* 49, 9: 1802-1811.
- 1002 Koesters, R., A. C. Preger, C. C. Du Preez and W. Amelung. 2013. Re-aggregation dynamics  
1003 of degraded cropland soils with prolonged secondary pasture management in the South  
1004 African Highveld. *Geoderma* 192, 173-181.  
1005
- 1006 Korb, J. 2011 Termite mound architecture, from function to construction. *Biology of*  
1007 *Termites, a Modern Synthesis.* Y. Roisin, D.E. Bignell, N. Lo. Dordrecht, Springer: 349-373.
- 1008 Kravchenko, A., W. Otten, P. Garnier, V. Pot and P. C. Baveye. 2019. Soil aggregates as  
1009 biogeochemical reactors: Not a way forward in the research on soil-atmosphere exchange  
1010 of greenhouse gases. *Glob. Ch. Biol*, 25, 7: 2205-2208  
1011
- 1012 Kuzyakov, Y., Blagodatskaya, E., 2015. Microbial hotspots and hot moments in soil: Concept  
1013 & review. *Soil Biol. Biochem.*, 83 :184-99.
- 1014 Lavelle, P., 1978. Les Vers de Terre de la savane de Lamto (Cote d'Ivoire). Peuplements,  
1015 populations et fonctions dans l'écosystème. *Publ. Lab. Zool., ENS, 18, 301pp*
- 1016 Lavelle, P. & Meyer, J. A. 1983. Allez-les-Vers, a simulation model of dynamics and effect on  
1017 soil of populations of *Millsonia anomala* (Oligochæta, Megascolecidae). *New Trends in Soil*  
1018 *Biology.* P. Lebrun, A. M. André, A. de Medts, C. Gregoire-Wibo and G. Wauthy. Louvain-la-  
1019 Neuve, Dieu-Brichard : 503-519.
- 1020 Lavelle, P., G. Melendez, B. Pashanasi and R. Schaefer. 1992. Nitrogen mineralization and  
1021 reorganization in casts of the geophagous tropical earthworm *Pontoscolex corethurus*  
1022 (*Glossoscolecidae*). *Biol. Fert. Soil*14. 49-53.
- 1023 Lavelle, P., Bignell, D., Lepage, M., Wolters, V., Roger, P., Ineson, P., Heal, O. W., Dhillion, S.  
1024 1997. Soil function in a changing world: the role of invertebrate ecosystem engineers. *Eur. J.*  
1025 *Soil Biol.* 33, 4:159-93.
- 1026 Lavelle, P., Spain, A., 2001. *Soil Ecology.* Kluwer Pub., 654pp.
- 1027 Lavelle, P., 2000. Ecological challenges for soil science. *Soil Sci.*,165(1):73-86.

- 1028 Lavelle, P. 2002. Functional domains in soils. *Ecol. Res.*17, 4: 441-450.
- 1029 Lavelle, P., Rodriguez, N., Arguello, O., Bernal, J., Botero, C., Chaparro, P., Gomez, Y.,  
1030 Gutierrez, A., Hurtado, M.P., Loaiza, S., Pullido, S.X., Rodriguez, E., Sanabria, C., Velasquez,  
1031 E., Fonte, S.J., 2014. Soil ecosystem services and land use in the rapidly changing Orinoco  
1032 River Basin of Colombia. *Agr. Ecos. & Envir.*,185:106-117.
- 1033 Lavelle, P., Spain, A., Blouin, M., Brown, G., Decaëns, T., Grimaldi, M., Jimenez, J.J., McKey,  
1034 D., Mathieu, J., Velasquez, E., Zangerlé, A. 2016. Ecosystem Engineers in a Self-organized  
1035 Soil: A Review of Concepts and Future Research Questions. *Soil Sci.*, 181, 91-109.
- 1036 Le Bayon, R. C., Binet, F. 2001. Earthworm surface casts affect soil erosion by runoff water  
1037 and phosphorus transfer in a temperate maize crop. *Pedobiologia*, 45, 5:430-442.
- 1038 Le Bissonnais, Y. 1996. Aggregate stability and measurement of soil crustability and  
1039 erodibility: I. Theory and methodology. *Eur. J. Soil Sci.*47. 425-437.  
1040
- 1041 Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. *Nature.*;528  
1042 (7580):60-68.
- 1043 Le Quéré, C., Andrew, R. M., Friedlingstein, P., Sitch, S., Pongratz, J., Manning, A. C.,  
1044 Korsbakken, J. I., Peters, G. P., Canadell, J. G., Jackson, R. B., Boden, T. A., Tans, P. P.,  
1045 Andrews, O. D., Arora, V. K., Bakker, D. C. E., Barbero, L., Becker, M., Betts, R. A., Bopp, L.,  
1046 Chevallier, F., Chini, L. P., Ciais, P., Cosca, C. E., Cross, J., Currie, K., Gasser, T., Harris, I.,  
1047 Hauck, J., Haverd, V., Houghton, R. A., Hunt, C. W., Hurtt, G., Ilyina, T., Jain, A. K., Kato, E.,  
1048 Kautz, M., Keeling, R. F., Klein Goldewijk, K., Körtzinger, A., Landschützer, P., Lefèvre, N.,  
1049 Lenton, A., Lienert, S., Lima, I., Lombardozzi, D., Metz, N., Millero, F., Monteiro, P. M. S.,  
1050 Munro, D. R., Nabel, J. E. M. S., Nakaoka, S.-I., Nojiri, Y., Padin, X. A., Peregon, A., Pfeil, B.,  
1051 Pierrot, D., Poulter, B., Rehder, G., Reimer, J., Rödenbeck, C., Schwinger, J., Séférian, R.,  
1052 Skjelvan, I., Stocker, B. D., Tian, H., Tilbrook, B., Tubiello, F. N., van der Laan-Luijkx, I. T., van  
1053 der Werf, G. R., van Heuven, S., Viovy, N., Vuichard, N., Walker, A. P., Watson, A. J.,  
1054 Wiltshire, A. J., Zaehle, S., Zhu, D. 2018. Global Carbon Budget 2017, *Earth Syst. Sci. Data*,  
1055 10, 405-448, <https://doi.org/10.5194/essd-10-405-2018>.
- 1056 Lobe, I., A. Sandhage-Hofmann, S. Brodowski, C. C. du Preez and W. Amelung. 2011.  
1057 Aggregate dynamics and associated soil organic matter contents as influenced by  
1058 prolonged arable cropping in the South African Highveld. *Geoderma* 162. 3-4: 251-259.
- 1059 Lobry de Bruyn, L. A. & Conacher, A. J. 1990. The role of termites and ants in soil  
1060 modification: A review. *Aust. J. Soil Res.* 28: 55-93.
- 1061 Lubbers, I., Puleman, M., Van Groenigen, J. W. 2017. Can earthworms simultaneously  
1062 enhance decomposition and stabilization of plant residue carbon? *Soil Biol. Biochem.*  
1063 105:12-24.

- 1064 Marquez, C. O., V. J. Garcia, R. C. Schultz and T. M. Isenhardt. 2019. A conceptual framework  
1065 to study soil aggregate dynamics. Eur. J. Soil Sci. 70. 3: 466-479.
- 1066 Martin A. 1991. Short- and long-term effects of the endogeic earthworm *Millsonia anomala*  
1067 (Omodeo) (Megascolecidae, Oligochaeta) of tropical savannas, on soil organic matter. Biol.  
1068 Fertil. Soils, 11. 234-238.
- 1069 Martin, S., and Lavelle, P. ,1992. A simulation model of vertical movements of an earthworm  
1070 population (*Millsonia anomala* Omodeo, Megascolecidae) in an African savanna (Lamto,  
1071 Ivory Coast). Soil Biol. Biochem., 24, 1419-1424.
- 1072 Materechera, S.A., Dexter, A.R., Alston, A.M., 1992. Formation of aggregates by plant roots  
1073 in homogenized soils. Plant & Soil. 142, 1, 69-79.
- 1074 Mermut, A R, Arshad, M A., & Arnaud, R J. 1984. Micro pedological study of termite  
1075 mounds of three species of *Macrotermes* in Kenya. Soil Sci. Soc. Amer- J. 48. 613-620.
- 1076 Miller, R.M., & Jastrow, J.D. 1990. Hierarchy of root and mycorrhizal fungal interactions  
1077 with soil aggregation. Soil Biol. Biochem., 22, 5, 579-584.
- 1078 Millogo, Y., Hajjaji, M. & Morel, J. C. 2011. Physical properties, microstructure and  
1079 mineralogy of termite mound material considered as construction materials. App. Clay Sci.  
1080 52. 1-2: 160-164.
- 1081 Minasny, B., Malone, B. P., Mc Bratney, A. B., Angers, D. A, Arrouays, D., Chambers, A., et  
1082 al., 2017. Soil carbon 4 per mille. Geoderma, 292, 59-86.
- 1083 Monroe, C.D., Kladvko, E.J., 1987. Aggregate stability of a silt loam soil as affected by roots  
1084 of corn, soybeans and wheat. Communications in Soil Science and Plant Analysis 18, 10:  
1085 1077-1087.
- 1086 Mora P, Seuge C, Chotte JL, Rouland C. Physico-chemical typology of the biogenic  
1087 structures of termites and earthworms: a comparative analysis. 2003. Biol. Fert. Soil., 37(4),  
1088 245-249.
- 1089 Morel, J.L., Habib, L., Plantureux, S., Guckert, A. 1991. Influence of maize root mucilage on  
1090 soil aggregate stability. Plant & Soil, 136, 1, 111-119.
- 1091 Negassa, W. C, Guber, A. K, Kravchenko, A. N, Marsh, T. L, Hildebrandt, B., Rivers, M. L.  
1092 2015. Properties of Soil Pore Space Regulate Pathways of Plant Residue Decomposition and  
1093 Community Structure of Associated Bacteria. Plos One. 10(4).
- 1094 Nunan, N., Wu, K. J., Young, I. M., Crawford, J. W., Ritz, K., 2003. Spatial distribution of  
1095 bacterial communities and their relationships with the micro-architecture of soil. FEMS  
1096 Microb. Ecol. 44, 2, 203-15.

- 1097 Paul, E. A. 2016. The nature and dynamics of soil organic matter: Plant inputs, microbial  
1098 transformations, and organic matter stabilization. *Soil Biol. Biochem.* 98:109-26.
- 1099 Paustian K., Lehmann, J., Ogle, S., Reay, D., Robertson, G. P., Smith, P. 2016. Climate-smart  
1100 soils. *Nature.*, 532(7597), 49-57.
- 1101 Peth, S., Nellesen, J., Fischer G., Horn R. 2010. Non-invasive 3D analysis of local soil  
1102 deformation under mechanical and hydraulic stresses by  $\mu$  CT and digital image  
1103 correlation. *Soil & Till. Res.* 111, 1: 3-18.
- 1104 Piccolo, A., Pietramellara, G., Mbagwu, J. S. C., 1997. Use of humic substances as soil  
1105 conditioners to increase aggregate stability. *Geoderma*, 75 (3-4), 267-77.
- 1106 Plante, A. F., Mc Gill, W. B. 2002. Soil aggregate dynamics and the retention of organic  
1107 matter in laboratory-incubated soil with differing simulated tillage frequencies. *Soil. Till.*  
1108 *Res.* 66, 79-92.
- 1109 Poeplau, C. and A. Don. 2015. Carbon sequestration in agricultural soils via cultivation of  
1110 cover crops - A meta-analysis. *Agr. Ecos. & Envir.* 200, 33-41.  
1111
- 1112 Post, W. M. and K. C. Kwon. 2000. Soil carbon sequestration and land-use change:  
1113 processes and potential. *Glob. Ch. Biol.* 6, 3: 317-327.  
1114
- 1115 Powlson, D. S., C. M. Stirling, C. Thierfelder, R. P. White and M. L. Jat. 2016. Does  
1116 conservation agriculture deliver climate change mitigation through soil carbon  
1117 sequestration in tropical agro-ecosystems? *Agr. Ecos. & Envir.* 220, 164-174.
- 1118 Puget, P., Angers, D.A., Chenu, C., 1999. Nature of carbohydrates associated with water-  
1119 stable aggregates of two cultivated soils. *Soil Biol. Biochem.*, 31(1), 55-63.
- 1120 Pulleman, M. M. and J. C. Y. Marinissen. 2004. Physical protection of mineralizable C in  
1121 aggregates from long-term pasture and arable soil. *Geoderma* 120, 3-4: 273-282.  
1122
- 1123 Pulleman, M. M., J. Six, A. Uyl, J. C. Y. Marinissen and A. G. Jongmans. 2005. Earthworms  
1124 and management affect organic matter incorporation and microaggregate formation in  
1125 agricultural soils. *Appl. Soil. Ecol.* 29, 1: 1-15.
- 1126 Rajagopal, D., Sathyanarayana, T., & Veeresh, G. K. 1982. Physical and chemical properties of  
1127 termite mound and surrounding soils of Karnataka. *J. Biol. Ecol. Soil.* 2, 18-31.
- 1128 Reatto, A., Bruand, A., Martins, E. D., Muller, F., da Silva E. M., de Carvalho, O. A. , Brossard,  
1129 M. , Richard, G. 2009. Development and origin of the microgranular structure in latosols of  
1130 the Brazilian Central Plateau: Significance of texture, mineralogy, and biological activity.  
1131 *Catena* 76. 2: 122-134.



- 1132 Reid, J.B., Goss, M.J., 1981. Effect of living roots of different plant species on the aggregate  
1133 stability of two arable soils. *J. Soil Sci.*, 32, 4, 521-541.
- 1134 Rieu, M., & Sposito, G., 1991. Fractal fragmentation, soil porosity, and soil-water properties  
1135 .1. Theory. *Soil Sci. Soc. Amer. J.*, 55, 5, 1231-1238.
- 1136 Rillig, M. C., Mummey, D. L. 2006. Mycorrhizas and soil structure. *New Phytol.* 171(1), 41-  
1137 53.
- 1138 Roose, T., Keyes, ., S. D., Daly, K. R. , Carminati, A., Otten, W. , Vetterlein D. , Peth, S. 2016.  
1139 Challenges in imaging and predictive modeling of rhizosphere processes. *Plant & Soil* 407.  
1140 1-2: 9-38.
- 1141 Scarciglia, F. and D. Barca. 2017. A powerful tool for assessing distribution and fate of  
1142 potentially toxic metals (PTMs) in soils: integration of laser ablation spectrometry (LA-ICP-  
1143 MS) on thin sections with soil micromorphology and geochemistry. *Environmental Science*  
1144 *and Pollution Research* 24. 10: 9776-9790.
- 1145  
1146 Segoli, M., De Gryze, S., Dou, F., Lee, J., Post, W. M., Denef, K., Six, J. 2013. AggModel: A soil  
1147 organic matter model with measurable pools for use in incubation studies. *Ecol. Model.*  
1148 263: 1-9.
- 1149 Shipitalo, M. J., & R. Pots. 1988. Factors influencing the dispersibility of clay in worm casts.  
1150 *Soil Science Society of America Journal*, 52, 764-769.
- 1151 Six, J., Elliott, E.T., Paustian, K. 2000a. Soil macro-aggregate turnover and microaggregate  
1152 formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biol.*  
1153 *Biochem.*, 32, 14, 2099-2103.
- 1154 Six, J., Paustian, K., Elliott, E.T., Combrink, C. 2000b. Soil structure and organic matter: I.  
1155 Distribution of aggregate-size classes and aggregate-associated carbon. *Soil Sci. Soc. Am. J.*,  
1156 64, 2, 681-689.
- 1157 Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002a. Stabilization mechanisms of soil organic  
1158 matter: Implications for C-saturation of soils. *Plant & Soil*, 241, 155-176.
- 1159 Six, J., Feller, C., Denef, K., Ogle, S.M., Sa, J.C.D., Albrecht, A. 2002b. Soil organic matter,  
1160 biota and aggregation in temperate and tropical soils - Effects of no-tillage. *Agronomie*, 22,  
1161 7-8, 755-775.
- 1162 Six, J., H. Bossuyt, S. De Gryze and K. Denef. 2004. A history of research on the link between  
1163 (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil & Tillage Research* 79.  
1164 1: 7-31.
- 1165 Six, J. and Paustian, K. 2014. Aggregate-associated soil organic matter as an ecosystem  
1166 property and a measurement tool. *Soil Biol. & Biochem.* 68: A4-A9.

- 1167 Smith, P., House, J. I., Bustamante, M., Sobocka, J., Harper, R., Pan, G. X., et al. 2016. Global  
1168 change pressures on soils from land use and management. *Glob. Ch. Biol.* 22(3):1008-28.
- 1169 Sollins, P., P. Homann and B. A. Caldwell. 1996. Stabilization and destabilization of soil  
1170 organic matter: Mechanisms and controls. *Geoderma* 74, 1-2: 65-105.
- 1171 Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. *J. Soil*  
1172 *Sci.*, 33, 2, 141-163.
- 1173 Topoliantz, S., Ponge, J.F., Viaux, P. 2000. Earthworm and enchytraeid activity under  
1174 different arable farming systems, as exemplified by biogenic structures. *Plant & Soil*, 225, 1-  
1175 2, 39-51.
- 1176 Tötsche, K. U., Amelung, W., Gerzabek, M. H., Guggenberger, G., Klump, E., Knief, C.,  
1177 Lehndorff, E., Mikutta, R., Peth, S. Pechtel, A. Ray, N. Kogel-Knabner, I., 2018. Micro-  
1178 aggregates in soils. *J. Plant Nutr. Soil Sci.*, 181(1):104-36.
- 1179 Toutain F. 1981. Les humus forestiers : structure et mode de fonctionnement. R.F.F. XXXIII.  
1180 449-477.
- 1181 Tracy, S. R., K. R. Daly, C. J. Sturrock, N. M. J. Crout, S. J. Mooney and T. Roose. 2015. Three-  
1182 dimensional quantification of soil hydraulic properties using X-ray Computed Tomography  
1183 and image-based modeling. *Water Resources Research* 51. 2: 1006-1022.
- 1184 Velasquez, E., Lavelle, P., Andrade, M., 2007b. GISQ, a multifunctional indicator of soil  
1185 quality. *Soil Biol. Biochem.*, 39, 12, 3066-3080.
- 1186 Velasquez, E., Pelosi, C., Brunet, D., Grimaldi, M., Martins, M., Rendeiro, A. Barrios, E, & P.  
1187 Lavelle. 2007a This ped is my ped: visual separation and near infrared spectra allow  
1188 determination of the origins of soil macro-aggregates. *Pedobiologia*, 51, 75-87.
- 1189 Velasquez, E. and P. Lavelle. 2019. Soil macrofauna as an indicator for evaluating soil-based  
1190 ecosystem services in agricultural landscapes. *Acta Oecologica-International J. of Ecol.*, 100.
- 1191 von Lutzow, M., I. Kogel-Knabner, K. Ekschmitt, E. Matzner, G. Guggenberger, B. Marschner  
1192 and H. Flessa. 2006. Stabilization of organic matter in temperate soils: mechanisms and  
1193 their relevance under different soil conditions - a review. *Eur. J. Soil Sci.* 57. 4: 426-445.
- 1194 Wang, J. Y., Z. Q. Xiong and Y. Kuzyakov. 2016. Biochar stability in soil: meta-analysis of  
1195 decomposition and priming effects. *Glob. Ch. Biol. Bioenergy* 8, 3: 512-523.
- 1196 Wielemaker, W. G. 1984. Soil formation by termites. PhD thesis. Wageningen University.
- 1197 Wollenberg, E., Richards, M., Smith, P., Havlik, P., Obersteiner, M., Tubiello, F. Herold N, M.,  
1198 Gerber, P. Carter S., Reisinger A., van Vuuren D. P., Dickie A., Neufeldt, H. Sander B. O.,  
1199 Wassmann, R. Sommer R., Amonette, J. E. Falcucci A., Herrero. M, Opio, C. Roman-Cuesta,  
1200 R. M. Stehfest, E. Westhoek H. Ortiz-Monasterio, I, Sapkota, T. Rufino, M. C. Thornton P. K.,

- 1201 Verchot, L. West, P. C. Soussana, J. F. Baedeker, T. Sadler, M. Vermeulen S. , Campbell B. M.  
1202 2016. Reducing emissions from agriculture to meet the 2 degrees C target. *Glob. Ch. Biol.*  
1203 22, 12:3859-64.
- 1204 Yamaguchi, T., Takei, T., Yazawa, Y., Wong, M. T. F., Gilkes, R. J. , Swift, R. S. 2004. Effect of  
1205 humic acid, sodium, and calcium additions on the formation of water-stable aggregates in  
1206 Western Australian wheatbelt soils. *Aust. J. Soil Res.* 2004;42(4):435-9.
- 1207 Young, I. M., Crawford, J. W. 2004. Interactions and self-organisation in the soil-microbe  
1208 complex. *Science.* 304, 1634-1637.
- 1209 Yudina, A. and Y. Kuzyakov. 2019. Saving the face of soil aggregates. *Glob. Ch. Biol*25. 11:  
1210 3574-3577.
- 1211 Zangerlé, A., Pando, A., & P. Lavelle, 2011. Do earthworms and roots cooperate to build soil  
1212 macro-aggregates? A microcosm experiment. *Geoderma*, 167, 303-309.
- 1213 Zangerlé, A., Hissler, C., Blouin, M and Lavelle, P. 2014. Near Infrared spectroscopy (NIRS)  
1214 to estimate earthworm cast age. *Soil Biol. & Biochem.*, 70, 47-53.
- 1215 Zangerlé A., Hissler, C. McKey, D. & P. Lavelle. 2016. Using near infrared reflectance  
1216 spectroscopy (NIRS) to identify the contribution of earthworms to soil macro-aggregation in  
1217 field conditions. *Applied Soil Ecology*, 104, 138-147.
- 1218 Zhao, D., M. X. Xu, G. B. Liu, X. Yao, D. F. Tuo, R. R. Zhang, T. Q. Xiao and G. Y. Peng. 2017.  
1219 Quantification of soil aggregate microstructure on abandoned cropland during vegetative  
1220 succession using synchrotron radiation-based micro-computed tomography. *Soil & Til. Res.*  
1221 165, 239-246.
- 1222 Zhang, S., Q. Li, Y. Lu, X. Zhang and W. Liang. 2013. Contributions of soil biota to C  
1223 sequestration varied with aggregate fractions under different tillage systems. *Soil Biol. &*  
1224 *Biochem.* 62, 147-156.
- 1225 Zhang, S., J. Li, X. Yang and B. Sun. 2016. Long-term effects of soil management regimes on  
1226 carbon contents and respiration rates of aggregate size fractions. *Soil Use & Manag.* 32, 4:  
1227 525-534.
- 1228 Zhu, G. Y., Z. P. Shangguan and L. Deng. 2017. Soil aggregate stability and aggregate-  
1229 associated carbon and nitrogen in natural restoration grassland and Chinese red pine  
1230 plantation on the Loess Plateau. *Catena* 149, 253-260.
- 1231