

1 Major comments

2 ***“Terminology you cited one reference for “microbial degradation index”, but I could not***
3 ***understand how the ratio described in the provided equation (Page 7, Line 10) may indicate the***
4 ***degree of microbial degradation. Furthermore, your interpretation of this ratio as an index of old C***
5 ***is also very confusing. As commented below, I would suggest you to articulate your rationale,***
6 ***supported by some data available from your or other studies”.***

7 The microbial degradation index is based on the De Clercq et al., 2015 paper. In this study, the
8 authors demonstrated that the organic matter in the occluded micro-aggregate and silt clay fractions
9 was less degraded than the organic matter in the free micro-aggregate and silt clay fractions. They
10 did it by combining a fractionation method similar to ours with a stable isotope approach first
11 developed by Conen et al (2008). Results from this study corroborates the aggregate formation
12 theory as proposed by Six et al. (2004) and Segoli et al., (2013), where the fresh residue is converted
13 to POM and serves as the core of newly formed macro-aggregates. Inside of these macro-aggregates,
14 the POM is further degraded and occluded micro-aggregates are formed. According to De Clercq et
15 al., 2015 interpretation, the younger and intermediate SOM is contained in the POM and occluded
16 fractions, while the older C is contained in the free OC fractions. Other authors (Jastrow et al., 1996;
17 Deneff et al., 2001) have also reported that the OC associated to free particles has a slower turnover
18 rate than that associated to macro-aggregates, highlighting that free particles are an important
19 factor contributing to OC sequestration and stabilization. However, being aware of the fact that this
20 interpretation can be conflicting, and given that distinguishing between “old” and “young” organic
21 carbon was not the main aim of our approach, we have now left out this conceptual interpretation
22 through the manuscript. Therefore, the “microbial degradation index” is now only used as an index
23 that shows the high or low degree of OC stability (in terms of OC decomposability), as it has been
24 now mentioned in page 8 lines 6, 8, 15. Please note that many other OC quality indicators that have
25 been used through the manuscript (e.g., C:N ratios, basal respiration, and OC resistant to oxidation)
26 are consistent/support this index.

27

28 ***Another term “protected OC” would also require some theoretical or empirical back-ups.***

29 According to literature, the OC protection mechanism can be chemical, that means "OC adhesion to
30 soil mineral particles", or physical, that means "particle soil aggregates that promotes the protection
31 of organic matter against decomposition and oxidation" (Jastrow et al., 2007; Six et al., 2002).
32 According to these concepts and taking into account that our results indicate a hierarchical order of
33 aggregation in which macroaggregates are the nucleus for microaggregate formation, and that inside
34 of these macro aggregates the POM is further degraded and occluded microaggregates are formed,
35 as it has been previously described by many authors (Six et al., 2004; Golchin et al., 1994, Segoli et al.,
36 2013;), we have used the “OC protection ratio” as an index to assess the different stabilization
37 degree of macro-aggregates present in soils and sediments at the different locations across the
38 catchment. This ratio is based in the “macroaggregate turnover index” by Six et al. (2000) and it is
39 interpreted as follows: the higher the ratio, the higher the OC is stabilized within the macro-
40 aggregates. Thus, we compare the two OC fractions occluded within macro-aggregates (i.e., micro-
41 aggregate and mineral associated OC) with the most active OC fraction (Mpom). This allows us to
42 have a relative estimation of how much of this active OC pool is being incorporated and stabilized
43 within the micro-aggregates.

44 Nevertheless, we have renamed this index in the revised version of the manuscript, according to the
45 comments made by the editor (in the text below), in order to avoid reader misunderstandings. We
46 have also changed: “OC protection ratio” to “macro-aggregate stabilization index” throughout the
47 manuscript. Moreover, we have now included the reference by Six et al (2000) in the methodology
48 section (page 7, lines 22-24) to clarify that the above.-mentioned index is based on this author
49 previous work.

50 ***“Interpretation of results on OC in deposited sediments. One of your main conclusions is***
51 ***stabilization of OC in deposited sediments. However, your measurements of basal respiration may***
52 ***indicate a higher lability of OC in your sediment samples compared with the source soils. Except***
53 ***some indirect indices (protected OC and microbial degradation index), you don’t have any other C***
54 ***quality data that can support your arguments for more stable and older C in sediments. That’s why***
55 ***you first need to provide robust backgrounds for the two ratios as well as a more in-depth***
56 ***discussion of the conflicting results on BR and OC compared between the source soils and***
57 ***sediments”.***

58 Please note that our statements are not contradictory at all. On the one hand, basal respiration is a
59 surrogate of microbial activity and therefore can be used as an index of the lability of the material
60 being degraded by the microorganisms. Microorganisms are activated by the presence of labile
61 (easily decomposable) organic matter and then respire more. But this labile organic matter is further
62 transformer into other bio-products which can be more or less recalcitrant, can be chemically
63 attached to other molecules and even physically protected by aggregates, as it has been pointed out
64 by many previous studies (Six et al., 2000; 2002; Cotrufo et al 2013; Denef et al 2004, etc).At the end,
65 organic matter can be protected (and preserved) by their inherent chemical composition, by physical
66 protection mechanisms (intra-POM), or both. See Schmidt et al 2011 for further details.

67 Nevertheless, higher basal respiration in sediments compared to the source soils only occurred at the
68 surface layer of the deposits (e.g., alluvial wedges and reservoir; please see Table 4), where at the
69 same time aggregate formation is occurring (as explained before). On the contrary, at the deep layers
70 of the alluvial wedges and the reservoir, where the carbon is being stored and stabilized, much lower
71 basal respiration rates than at the upper layers, and even than in the source soils are observed.

72 ***“... In addition, your incubation settings did not consider different in situ conditions of the three***
73 ***setups (source soils, stream sediments, and deposited sediments). For example, aerobic conditions***
74 ***can accelerate degradation of sediment OM otherwise limited by O₂? You did not provide any***
75 ***detail on this and other important environmental conditions such as initial moisture levels. Please***
76 ***discuss how the arbitrary lab conditions might deviate from the actual “field conditions”, affecting***
77 ***the measured rates of BR.”***

78 We agree with the comment made by the editor that soil and sediment respiration rates under field
79 conditions may differ from those under laboratory controlled conditions. Indeed different techniques
80 (soil chambers vs laboratory incubations) are used. It’s also true that aerobic conditions can
81 accelerate degradation of sediment OM otherwise limited by O₂. However, it was necessary to run
82 the soil and sediment incubations under standardized controlled conditions (28 °C and at 60% of its
83 water holding capacity) in order to be able to make comparisons between the large variety of soils
84 and sediments distributed within the catchment, and exposed to different local environmental
85 conditions. Please note that given the large number of sampling points, sometimes located some
86 kilometers apart from each other, it was not possible to perform *in situ* field soil respiration
87 measurements with soil respiration chambers within the same period (e.g., from 10 am to 13 pm) to

88 avoid variations in soil temperature and moisture (well-known as major environmental drivers of soil
89 respiration spatial and temporal variability; see Almagro et al., 2009 and references therein). It is
90 well-known that respiration is highly sensitive to moisture conditions, and such variations could have
91 biased our experiment outcomes. Moreover, this approach is not valid to estimate respiration rates
92 in deep soil or sediment layers. Because of all those drawbacks, we had to perform soil and sediment
93 incubations in the laboratory. Nevertheless, as our aim was to assess the potential microbial activity
94 (as a surrogate of basal respiration rates) as well as characterize the quality of the organic carbon
95 present in the different soils and sedimentary deposits (i.e., basal rates rates are also an indicator of
96 the lability or recalcitrance of OC) we had to perform the incubations under standardized controlled
97 conditions in order to avoid temperature and moisture variation among samples. In such a way, we
98 can state that the observed variability in basal respiration rates among soils and sediments are
99 explained by the different quality of their OC contents.

100 Regarding the initial moisture levels of the collected soils and sediments we estimated them before
101 setting the incubations and the variability among samples was huge. We used the initial value of each
102 sample to estimate the amount of water that had to be added to each specific soil or sediment
103 sample in order to achieve a 60% of its water holding capacity (likewise estimated for each soil and
104 sediment sample). Therefore, all samples were incubated under the same environmental conditions
105 to avoid bias associated with different soil moisture contents.

106 ***3."Editorial improvement Although the manuscript is generally well organized and written, I found
107 numerous typos and scattered short paragraphs that can be more coherently organized, as some
108 specific examples are indicated below. Please pay attention to details and revise the manuscript
109 thoroughly.***

110 Thanks very much for your suggestion. We have now revised the manuscript changing all those
111 paragraphs that were not clear and corrected all typographic errors (marked on the manuscript).

112 <Specific comments:

113 ***"L 24: Do you mean "representing suspended load and bedload in the main channel"?"***

114 Yes, we have changed it (page 1, Line 24).

115 ***"L 30: Please define (or elaborate the meaning of) "physical stabilization" and "chemical
116 protection".***

117 In our study , we have used two main mechanisms of SOC stabilization (from Six et al 2002): (1)
118 physical protection, which refers to the isolation of microbes and enzymes from carbon substrates by
119 the physical barriers of soil aggregates; and (2) chemical protection, which means the protection of
120 OC by binding to soil minerals.

121 We agree with the editor that it is necessary to clarify this terminology that can be sometimes
122 confusing through the manuscript because the physico-chemical protection mechanisms lead to the
123 organic carbon stabilization. In order to clarify the terminology and be consistent throughout the
124 manuscript we have now changed physical stabilization by "physical protection" and we have added
125 between brackets the meaning of physical and chemical protection: "OC within aggregates", and "OC
126 adhesion to soil mineral particles", respectively): page 1, lines 30-31.

127 ***P 2, L 1-2: Please be more specific in providing your major conclusion about the relative importance***
128 ***of “temporary and permanent deposits. Do you mean that both sources are equally important?”***

129 According to the results from our work we consider that both, temporary and permanent deposits
130 are very important from the point of view of the physico-chemical mechanisms of OC protection and
131 stabilization that are occurring across the catchment. In addition, it is not our purpose to determine
132 if one of them is more important than the other. We consider that both temporary and permanent
133 deposits should be preserved, although not beyond the natural fluvial dynamics specially for the
134 natural transitory deposits (short-term residence times), due to their high potential as C sinks.

135 ***“P 2, L 8: Please remove “but” and begin the following sentence with “However,”.***

136 It has been done

137 ***“P 3 L 10-28: Please combine these into one paragraph”. –***

138 It has been combined.

139 ***“P 3 L 24: Please fix this and other “numerous” super- and subscript typos throughout the***
140 ***manuscript” .***

141 Thanks and sorry for the errors. We have now revised and changed all of them. MPOM has been
142 unified and change by Mpom through the manuscript; Km² has been changed to Km² (marked on the
143 manuscript).

144 ***“P 3 L 26-28: Given the importance of aggregate structure for POC stabilization during transport***
145 ***and deposition, you need to provide a more detailed review of the previous works on this topic. I***
146 ***would suggest you to expand the short introduction misplaced at the end of the first paragraph (- P***
147 ***2 L 20-26) with these (Six et al,,,) and more recent citations in a separate paragraph”.***

148 Thanks very much. Your suggestion has been accepted. We have now moved the paragraph,
149 extended our arguments and new references have been added to support our statements in the
150 introduction: Hoffman et al., 2013 (page 2, line 20) Lal, 2005; Boix Fayos et al., 2015; Berhe et al.,
151 2013, 2018 ; Nie et al., 2018. See page 2 lines 26-31.

152 ***P 4 L 11: “more detailed” description? –***

153 Because there are many previous works in this study area (already cited in this section (Boix-Fayos et
154 al., 2007, 2015,)), Quiñonero-Rubio et al., (2016), and Pérez-Cutillas et al., (2018)), we consider that
155 extend the description could revert in repeating information already published.

156 ***P 4 L 27-28: Please provide more details on soil sampling: depth, sampling method, etc. –***

157 More details on soil sampling has been included

158 ***P 5 L 1: “after a flooding event”? As you know, the bulk of suspended load is transported “during”***
159 ***rainfall events, so sampling timing is a critical information. Please specify when and how long***
160 ***suspended sediment was collected. –***

161 We have specified that the sampling was done immediately after each flooding event (page 7, line 7).

162 ***P 5 L 26: What is “min”? –***

163 Sorry for the abbreviation. It means minutes. We have changed it (page 6, lines 13-14).

164 **P 6 L6: 40oC or 50oC? Please provide reason in case you used different temperatures.**

165 *Sorry, it was a mistake. 50°C is the temperature for both analyses (page 7, line 7).*

166 **P 6 L 25: “protected OC” is a misnomer, because this is actually a ratio of “protected OC to MPOM”.**

167 This has been already responded in “major comments” point 2.

168 **P 7 L 10: Why don’t you use simply “OC-M” as denominator? In addition, it is assumed here that OC**
169 **in free microaggregates and mineral fractions is older than OC in macroaggregates. Do you have**
170 **any data supporting this assumption? If not, you need to reformulate relevant sentences**
171 **throughout the manuscript.**

172 We agree with the editor that the total OC in M could have been used in the denominator (the sum
173 of each sub-fraction is equal to the total OC in macroaggregates). However, we feel that displaying
174 the different sub-fractions contained within the macroaggregates is a clearer way to present this
175 index.

176 The second question has been already answered in “major comments” point 1.

177 **P 7 L 17: 30 g soil “on a dry mass basis”?**

178 No, it was weighted on a fresh mass basis. It has been specified in the test (page 8, line 19).

179 **“P 7 L 14: There must have been significant reductions in soil moisture given the high incubation**
180 **temperature and 32 days of incubation. Please clarify this.**

181 As stated in the previous MS version (see lines 24-25 in page 8) the moisture content of the samples
182 was regularly checked for potential water losses by evaporation by weighting the bottles, but there
183 was not water losses and therefore it was not necessary to add any water". Please note that 32 days
184 of incubation is not a long period for water losses to occur.

185 **“P 8 L 1: Please describe why you opted for the nonparametric test. You might need to mention any**
186 **prior test for normal distribution”.**

187 We used a non-parametric test because our sampling design was not balanced. That is, we did not
188 have the same amount of representative samples (n° of replicates) across eroding, transport and
189 depositional areas (see Table 1). Please note that prior normality distribution tests are not required
190 when non-parametric tests are performed.

191 **Sections 3.2-3.3: These two short sections can be better combined into one section, in a more**
192 **coherent way to compare OC fractions among the three watershed components.**

193 Ok, in agreement to the editor we have integrated 3.2 and 3.3

194 **Sections 3.4 & 3.5: Please also consider to integrate these sections in the preceding one or in a**
195 **separate section on OC quality.**

196 Ok, in agreement to the editor we have integrated 3.4 and 3.5 sections.

197 ***“P 11 L 14: Please rephrase “macroaggregates are the nucleus for microaggregate formation”.***
198 ***How can larger macroaggregates function as the nucleus?”***

199 This sentence is based in Oades (1984) theory who postulated that “the roots and hyphae holding
200 together the macroaggregate form the nucleus for microaggregate formation in the center of the
201 macroaggregates. Other authors also explain this: “inside the macroaggregates, the presence of
202 decomposed organic matter, metabolites and biogenic products, polyvalent cations, and other
203 binding agents promoted the solid phase reaction between organic matter and clay and silt particles
204 leading to the formation of stable microaggregates (Edwards and Bremner, 1967; Golchin et al.,
205 1994). In addition in Six et al., 2004 there is a review of different models of soil aggregation where
206 this kind of hierarchical order of aggregation is included

207 ***P 12 L 20-25: This type of 1 to 1 comparison between sediment and source soils does not make***
208 ***sense, because three sources have different source capacity. Please take into consideration***
209 ***estimates of source capacity in evaluating C enrichment or depletion during OC transport.***

210 In contrast with the editor view, we feel that comparing sediment with each one of the potential
211 sources, specially, at coarser scales, is a key aspect to determine the potential sources of eroded OC
212 in the sedimentary deposits through the catchment and also to progress in the knowledge on the
213 stabilization mechanisms in the redistribution of eroded carbon by water erosion processes.
214 Furthermore, our own experience in previous works (Boix Fayos et al., 2015, 2017) where a mean
215 value of OC was assumed as representative of the whole catchment sources can lead to an
216 overestimation of the role of mineralization during the redistribution of sediments when a very
217 plausible reason is that sediments come from low-OC sources as bank and channel sediments.

218 ***P 13 L 4: Typo at the end of the sentence.***

219 It has been corrected

220 - ***P 13 L 6: “sediments”?*** –

221 We have rewritten the sentence (page 15, lines 1-2).

222 ***P 13 L 12: “soil forming” or “aggregate forming”?***

223 We have changed soil forming by aggregate forming besides both terms have the same meaning
224 (page 15, line 9).

225 ***“P 13 L 17-21: Please rewrite (better split) this long and vague sentence. This and the following***
226 ***sentences are logically conflicting, because you are arguing that OC in the deposited sediments is***
227 ***more stabilized than the source soils, even though more labile materials, as evidenced by higher***
228 ***BR rates, exist in the same sediments. Please clarify this”.***

229 Please note that our statements are not contradictory at all. On one hand, higher basal respiration is
230 a surrogate of microbial activity and therefore can be used as an index of the lability of the material
231 being degraded by the microorganisms. Microorganisms are activated by the presence of labile
232 (easily decomposable) organic matter and then respire more. But this labile organic matter is further
233 transformed in other bioproducts which can be more or less recalcitrant, can be chemically attached
234 to other molecules and even physically protected by aggregates, as it has been pointed out by many
235 previous studies (Six et al., 2000; 2002; Cotrufo et al 2013; 2015, Deneff et al 2014, etc). At the end

236 organic matter can be protected (and preserved) by their inherent chemical composition, by physical
237 protection mechanisms (intra-POM), or both. See Schmidt et al 2011 for further details.

238 ***P 13 L 21: typo "Thw"***

239 It has been changed (page 15, line 19).

240 ***P 13 L 22: Please specify what you meant by "microbial induced processes"***

241 We have changed it to clarify (page 15, lines 21-22).

242 ***"P 14 L 10: You did not measure "microbial activity".***

243 We have measured "Basal respiration" that is an estimate of the total microbial activity in soils
244 (Vanhala et al., 2005). Any way, we have change microbial activity by basal respiration rates to clarify
245 (page 16, line 8).

246 ***P 14 L 16-18: Again, this short paragraph can find a better place in the preceding one***

247 Ok, it has been done (page 15, lines 15-18)

248 ***P 15 L5: Again, you need to clarify how more stabilized OC in deposited sediments exhibited higher
249 rates of BR compared with those measured for the source soils. –***

250 It has been already clarified

251 ***Fig 3: Please clarify in the figure legend whether significant differences indicated by different
252 letters are among the compared fractions or soil/sediment samples.***

253 Thanks for the suggestion. We agree that the Figure legend was not clear enough. Thus we have
254 changed it specifying that the differences are among soils/sediment for each aggregate class. We
255 have changed it in Figure 3A, 3B, 4A and 4B (page 17, lines 16-23).

256

257 Elliott et al., 1991 has been now included in the reference list and Page 4, line 5 (suggesting by the
258 review 1)

259 We have also modified the contribution of each author (see Page 17, lines 16-23)

260 **References mentioned in this response:**

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