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**Distribution and thermal stability of physically and chemically protected organic  
matter fractions in soils across different ecosystems**

Beatrice Giannetta<sup>a</sup>, César Plaza<sup>b</sup>, Costantino Vischetti<sup>a</sup>, M. Francesca Cotrufo<sup>c</sup>,  
Claudio Zaccone<sup>\*,d</sup>

<sup>a</sup> *Department of Agricultural, Food and Environmental Sciences, Polytechnic University  
of Marche, via Brecce Bianche 10, 60131 Ancona, Italy*

<sup>b</sup> *Instituto de Ciencias Agrarias, Consejo Superior de Investigaciones Científicas,  
Serrano 115 bis, 28006 Madrid, Spain*

<sup>c</sup> *Department of Soil and Crop Sciences & Natural Resources Ecology Laboratory,  
Colorado State University, Fort Collins, Colorado, United States of America*

<sup>d</sup> *Department of the Sciences of Agriculture, Food and Environment, University of  
Foggia, via Napoli 25, 71122 Foggia, Italy*

\* Corresponding author. E-mail address: claudio.zaccone@unifg.it

19   **Abstract**

20   ~~Accrual of carbon (C) in soil is a significant and realizable management option to mitigate~~  
21   ~~the relentless increase in atmospheric CO<sub>2</sub>.~~ A clear understanding of the mechanisms  
22   controlling the persistence of carbon (C) and nitrogen (N) in soil organic matter (SOM),  
23   and how they differ across different ecosystems, has never been more needed. ~~Soil~~  
24   ~~organic C accrual requires nitrogen (N); thus understanding of N distribution in soil is~~  
25   ~~equally important.~~ Here we investigated SOM distribution between physical and chemical  
26   stabilized fractions in soils from a variety of ecosystems (*i.e.*, coniferous and broadleaved  
27   forest soils, grassland soils, technosols and agricultural soils). Using elemental and  
28   thermal analyses, we examined changes in the quantity and quality of physically-  
29   fractionated SOM pools characterized by different mechanisms of protection from  
30   decomposition. Independently of ecosystem type, ~~the majority~~most of the organic C and  
31   total N were found in the mineral-associated SOM pool, known to be protected mainly  
32   by chemical mechanisms. Indexes of thermal stability and C/N ratio of this heavy SOM  
33   fraction were lower (especially in agricultural soils) compared to light SOM fractions  
34   found free or occluded in aggregates, and suggested a marked presence of inherently  
35   labile compounds. Our results confirm that the association of labile organic molecules  
36   with soil minerals is a major stabilization mechanism of SOM, and demonstrate that this  
37   is a generalizable finding occurring across different mineral soils and ecosystems.

38

39   Keywords: soil organic matter; soil N; physical fractionation; C sequestration; TGA;  
40   technosols

## 41    **Introduction**

42    Soils represent the largest terrestrial organic carbon (C) reservoir (Jobbágy and Jackson;  
43    2000) with the potential to accrue enough additional C to halt the ~~relentless~~ increase in  
44    atmospheric CO<sub>2</sub>, as proposed by the recently lounded 4 per mill initiative (Chabbi et al.;  
45    2017). Achieving this challenging objective requires a clear and generalizable  
46    understanding, across different soils and ecosystem types, of the mechanisms responsible  
47    for the buildup and persistence of soil organic matter (SOM).

48        The notion of recalcitrance as a key factor controlling SOM stability has been  
49    questioned during the last decades. More specifically, biochemical recalcitrance, an  
50    inherent molecular property of resistance to decomposition, has been usually associated  
51    with humified SOM or complex aromatic structures, such as lignin, cutin and pyrogenic  
52    organic matter (Stevenson; 1994; Sollins et al.; 1996; Krull et al.; 2003). However, recent  
53    works presented evidence against this concept, and stressed the importance of physical  
54    protection and mineral association as one of the most significant mechanisms responsible  
55    for SOM persistence on the long term in mineral soils (Kögel-Knabner et al.; 2008;  
56    Marschner et al.; 2008; Kleber; 2010; Schmidt et al.; 2011; Dungait et al.; 2012; Lehmann  
57    and Kleber; 2015). Thus, SOM in particulate form, such as the light fraction (LF), is  
58    generally prone to fast decomposition unless physically protected in soil aggregates.  
59    Aggregates are composite secondary SOM structures (Christensen; 2001) and therefore  
60    are not clearly distinguished by turnover times (Poeplau et al. submitted). Yet the organic  
61    matter protected within aggregates, and in particular within micro-aggregates (<250 µm),  
62    is considered among the most persistent form of SOM (Six et al.; 2000). ~~a-~~ Although the  
63    longest turnover times are assigned to heavy, fine organic matter associated with minerals  
64    (von Lutzow et al.; 2006; Poeplau et al.; submitted).

65        Microbial biomass is considered to be turned over much faster than plant residues

(Kästner 2000), thus suggesting that the molecular imprint of SOM by molecules and fragments derived from microbial biomass is probably much more important than previously considered (Miltner et al. 2012). When microorganisms degrade plant residues, they use low-molecular-weight compounds from the plant biomass to build their own biomass, and the majority of this material is then incorporated into non-living SOM (Kindler et al. 2006; Miltner et al. 2012, and ref. therein). Cotrufo et al. (2013) hypothesized that labile plant constituents are the main source of microbial by-products, and these in turn are the main precursors of SOM stabilized by the soil matrix, through the formation of physically or chemically realized organo-mineral associations (Vogel et al., 2014). For example, McGill and Paul (1976) and Turchenek and Oades (1979) observed that microbial cell wall materials were a major component of SOM accumulated in the coarse clay fraction, while Ladd et al. (1996, and ref. therein) reported that clay-size fractions were enriched of organic nitrogen (N) of microbial origin. Moreover, particle size fractionation after ultrasonic dispersion and subsequent dating showed young radiocarbon ages of the fine clay-associated organic C, indicating that this organic C was more active than that associated with the coarse clay (Anderson and Paul, 1984).

Several other studies have suggested that thermally labile, microbial derived materials and easily metabolizable compounds (*e.g.*, carbohydrates and amino acids) are dominant in the mineral-associated organic matter (MAOM) pool, whereas the LF free (LF\_free) or occluded within macro- (LF\_M) and especially micro-aggregates (LF\_m) are believed to be mainly of plant origin and characterized by an increasing degree of persistence (Kleber et al., 2011; Hatton et al., 2012; Keiluweit et al., 2012; Plaza et al., 2013, 2016). Given its chemical nature, the MAOM pool is also the richest in N among SOM pools, *i.e.*, with the lowest C/N (Christensen, 2001), pointing to the need of a better understanding of N distribution in the soil. In fact, despite the importance of N

91 sequestration to ecosystem functioning, to date there has not been a comprehensive  
 92 examination of the implications of SOM stabilization in ecologically meaningful fractions  
 93 for long-term N sequestration in different soils and ecosystems (Bingham and Cotrufo,  
 94 2016).

95 Soil C stock and sink capacity were recently described as “ecosystem properties”  
 96 (Schmidt et al., 2011), sharply affected by several factors including land use (*e.g.*,  
 97 undisturbed *versus* agricultural soils subjected to aggressive human imprints) (Amundson  
 98 et al., 2015). Besides climate, ~~v~~Vegetation and parent material are main drivers of soil  
 99 formation (Jenny, 1941), and their role in SOM stocks and persistence has been  
 100 investigated in different ecosystems, spanning from spruce and deciduous forests to  
 101 grasses and arable soils (Guggenberger et al., 1994, 1995; Leifeld and Kögel-Knabner,  
 102 2005; Simpson et al., 2007; Clemente et al., 2011; ~~W~~ieismeier et al., 2012; Denef et al.,  
 103 2013; Catoni et al., 2016). Despite all these research efforts, an accurate assessment of  
 104 soil organic C and total N distribution across pools with different mechanisms of physical  
 105 and chemical protection is still needed to improve our knowledge of the response of soil  
 106 properties, including C stocks, to land management and/or changes in land use and cover  
 107 (Guo and Gifford, 2002; ~~W~~ieismeier et al., 2012; Wei et al., 2017). This would help better  
 108 understand if SOM persistence, and therefore the distribution among fractions, is an  
 109 ecosystem property rather than the main common denominator across different soils and  
 110 ecosystems. In addition, technosols, *i.e.* man-made soils, are widely distributed  
 111 throughout the world as they are found where human activity has led to the construction  
 112 of artificial soils, sealing of natural soil, or extraction of material normally not affected  
 113 by surface processes (*e.g.*, mines, dumps, oil spills, earth movements, coal fly ash  
 114 deposits) (IUSS Working Group WRB, 2006). Many technosols, in particular those in  
 115 dumps, are originated covering man-made substrate with a layer of natural soil in order

116 to permit revegetation. Although technosols have been recently included in soil  
117 classifications, often associated with other soil groups in a complex pattern, here they are  
118 considered as a “modern form” of soil in anthropogenic ecosystems. Only few attempts  
119 to understand SOM stabilization dynamics in technosols are reported in literature  
120 (Chaudhuri et al., 2013; Rodríguez-Vila et al., 2016; Moreno-Barriga et al., 2017),  
121 pointing to the need of including them when contrasting different ecosystems, for a  
122 generalizable understanding of the characteristic of SOM pools protected by different  
123 mechanisms of stabilization.

124 In this study, we measured: (1) the organic C and total N contents of bulk soils under  
125 five different ecosystems, including coniferous and broadleaved forests, grasslands,  
126 technosols in anthropogenic systems and agroecosystems; and (2) their distribution in  
127 ecologically meaningful SOM fractions, namely: a light fraction ( $LF < 1.85 \text{ g cm}^{-3}$ ), found  
128 free or occluded in macro- (2000-250  $\mu\text{m}$ ), and micro-aggregates ( $>250 \mu\text{m}$ ), thus  
129 stabilized by either inherent recalcitrance and/or an increasing strength of physical  
130 protection, and the heavy ( $> 1.85 \text{ g cm}^{-3}$ ) MAOM fraction, mostly stabilized by chemical  
131 association to minerals. The main objectives of this work were to: (a) elucidate SOM  
132 distribution among primary fractions characterized by different mechanisms of  
133 protection, in a variety of soils from different ecosystems; and (b) investigate if the  
134 thermal fingerprint of SOM fractions (possibly mirroring their “inherent recalcitrance”)  
135 is an ecosystem property or can be generalized across different soils and ecosystems.  
136 Finally, including technosols in this study would improve our understanding of the kind  
137 and rate of stabilization mechanisms taking place during the initial phases of pedogenetic  
138 processes (Zaccone et al., 2014), being technosols young soils that develop in technogenic  
139 man-made parent materials.

140

141

## 142 **Materials and methods**

### 143 *Study sites and soil sampling*

144 Five ecosystem types, known to have contrasting climatic and edaphic conditions, were  
145 considered in this study. To achieve a broad range of soils, we collected samples from 4  
146 to 5 different sites for each of the five ecosystem types (~~Table 1~~). Five coniferous forests  
147 (CF), five broadleaved forests (BF), five grassland soils (GL) and five technosols (TS)  
148 were sampled between May and June 2016 in different sites of the Marche Region (Italy).  
149 The coniferous forests consisted mainly of *Pinus nigra* woodlands occurring between 350  
150 and 1400 m a.s.l., the broadleaved ~~forests-woodlands~~ in stands of different species (e.g.,  
151 *Fagus sylvatica*, ~~Orno~~-*Ostrya carpinifolia*etum associations with, *Quercus pubescens*)  
152 growing between 320 and 1300 m a.s.l., the grassland soils in meadows and pastures  
153 characterized by meso- to xerophilous species (e.g., *Bromus erectus*) and *Medicago sativa*  
154 and developing between 890 and 1700 m a.s.l.. The technosols included two open-pit  
155 mines, filled and then reclaimed with *Helianthus annuus* (since 2010) and with *Triticum*  
156 (since 2000), respectively, two dumps, reclaimed in 2000 and in 2011, respectively, and  
157 a site of disposal of compost and other non-hazardous waste materials (all TS sites <300  
158 m a.s.l.). Mean annual temperature of sampling sites ranged from 7.3 and 15.0 °C,  
159 whereas mean annual precipitation ranged between 810 and 1570 mm. Four agricultural  
160 soils (AG) cropped with barley and collected in Arganda del Rey (Madrid, Spain) were  
161 also included in this study: 1. These soils were located at 530 m a.s.l. and characterized  
162 by a mean annual precipitation of about 440 mm and a mean annual temperature of 14 °C  
163 (Plaza et al., 2016). More details about the sampling sites are reported in Table 1.

164 At each location, 3 to 10 soil samples were collected randomly from several points,  
165 depending on site surface and homogeneity separated 50 to 100 cm apart, using a soil

166 auger and mixed to obtain a composite sample. The depth of sampling varied from 10 to  
167 25 cm, and corresponded to the top soil horizon (Table 1).

168

#### 169 *Bulk soil characterization*

170 Prior to analysis, soil samples were gently crushed and passed through a 2-mm sieve. Soil  
171 reaction (pH units) was measured on suspensions of 1:2.5 sample:water and sample:KCl  
172 (1M) (Thomas 1996), whereas electrical conductivity (EC) was measured on water  
173 extracts obtained at a sample-to-water ratio of 1:5 (RoadesSparks, 1996). All soils were  
174 also analyzed for texture by the pipette method, whereas C and N elemental composition  
175 was determined as described in section below. Soil properties averaged by ecosystem type  
176 are reported in Table 2.

177

#### 178 *Physical fractionation of SOM*

179 The physical fractionation of SOM was carried out using the method described by Plaza  
180 et al. ~~(2012) with modifications detailed by Plaza et al.~~ (2013), with the aim to separate  
181 the heavy MAOM fraction from the light particulate~~r~~ organic matter (LF) fraction, and  
182 distinguish the latter into free LF and LF occluded in aggregates of different sizes.  
183 Specifically, we separated four fractions: (a) free LF, located between aggregates  
184 (unprotected C pool; LF\_free); (b) LF occluded within macroaggregates (SOM pool  
185 weakly protected by physical mechanisms; LF\_M); (c) LF occluded within  
186 microaggregates (SOM pool strongly protected by physical mechanisms; LF\_m); and (d)  
187 a heavy SOM pool associated with the minerals (highly persistent mostly chemically-  
188 protected; MAOM). This method is based on the densimetric procedure by Golchin et al.  
189 (1994) and Sohi et al. (2001) for the fractionation of free and occluded LF, and on the  
190 method by Six et al. (2000, 2002) for the breakup of macroaggregates preserving

191 microaggregates. First, 148 g of NaI at a density of 1.85 g mL<sup>-1</sup> ~~were~~<sup>was</sup> added to 10 g  
192 of 2-mm sieved, air-dried soil in a 100-mL polycarbonate centrifuge tube. Free LF was  
193 separated from the heavy fraction by density flotation in the NaI solution and subsequent  
194 suction, filtration through a glass fiber filter and scrupulous washings with deionized  
195 water. Macroaggregates remaining in the heavy fraction were broken up, using the  
196 microaggregate isolator device designed by Six et al. (2000, 2002), into stable  
197 microaggregates, MAOM and LF\_M, which was collected after a second density  
198 separation. Another density separation after an ultrasonic disruption at an energy input of  
199 1500 J g<sup>-1</sup> allowed the LF\_m isolation from the MAOM. The recovered LF\_free, LF\_M,  
200 LF\_m and MAOM fractions were oven-dried at 60°C, weighed and ground with a ball  
201 mill for further analyses.

202

### 203 *Organic C and total N analysis*

204 Organic C and total N contents of both bulk soil samples and SOM fractions were  
205 determined by dry combustion using a Thermo Flash 2000 NC Soil Analyzer. For organic  
206 C determination, the whole soil samples and mineral-associated SOM fractions were  
207 subject to acid fumigation before analysis to remove carbonates (Harris et al., 2001). All  
208 analyses were carried out in triplicate.

209

### 210 *Thermogravimetric analysis*

211 Thermogravimetric analysis (TGA) was carried out on all SOM fractions using a Perkin  
212 Elmer Thermogravimetric Analyzer TGA 4000. An aliquot (*ca.* 20 mg) of each sample  
213 was placed in a ceramic crucible and heated from 30 to 850 °C at 20 °C min<sup>-1</sup> under an  
214 oxidizing atmosphere of air at a flow rate of 30 mL min<sup>-1</sup>. Two indexes of overall thermal  
215 stability were calculated: T<sub>50</sub> (°C), representing the temperature at which 50% of the SOM

216 mass is lost, and the thermal stability index ( $H$ ), here defined as the ratio between the  
217 weight losses within 400-550 °C and 250-400 °C temperature ranges. These thermal  
218 indexes are reported on a moisture- and ash-free basis.

219

## 220 *Data analysis*

221 Data were ~~analyzed with the Kruskal-Wallis test~~ ~~subjected to one-way analysis of variance~~  
222 ~~(ANOVA)~~, with ecosystem type or SOM fraction as factors. ~~The Dunn's Tukey's honest~~  
223 ~~significant difference (HSD)~~ test at the 0.05 level was used for multiple mean comparison.  
224 All statistical analyses were performed using R, version 3.4.1 (R Core Team, 2017) ~~and~~  
225 ~~the R package agricolae, version 1.2.8 (De Mendiburu, 2017).~~

226

227

## 228 **Results and discussion**

### 229 *Organic C and total N concentrations in soils*

230 Organic C and total N contents of ~~the soils~~ examined soils span a broad range, as expected  
231 (Table 2). Grassland soils show the highest average values for both organic C (86 g kg<sup>-1</sup>)  
232 and total N (9 g kg<sup>-1</sup>), whereas the lowest are found in AG (11.8 g C kg<sup>-1</sup> and 1.1 g N kg<sup>-1</sup>)  
233 (Table 2). In particular, both the organic C and total N average contents ~~follow the~~  
234 ~~order rank as~~ GL > CF > BF > TS > AG (Table 2), thus mirroring the link between organic  
235 C and total N dynamics. As a result, C/N across land uses vary in the order CF > BF ~  
236 AG > GL ~ TS (Table 2).

237 The conversion of grassland or forest soils to agricultural soils is well-known to cause  
238 reductions of SOM contents, due to decreased inputs of plant-derived materials and  
239 increased decomposition rates through tillage (Matson et al., 1997; Post and Kwon, 2000).  
240 Wei et al. (2012) reported that grassland and forest ecosystems showed *ca.* 40%

241 higher SOC content compared to croplands depending on soil properties. Some studies  
242 have found that SOM ~~tends to increase~~ increases with as the clay content ~~increases~~ (e.g., Magdoff  
243 and Weil, 2004). However, in our work, neither organic C nor total N are significantly  
244 correlated with clay or clay + silt contents.

245

#### 246 *Organic C and total N distribution*

247 Independently of ecosystem, most soil organic C is accumulated in the MAOM fraction,  
248 with average concentrations ranging from 6.1 g kg<sup>-1</sup> in AG up to 49.3 g kg<sup>-1</sup> in GL (Table  
249 3). In particular, the ~~proportion percentage~~ of organic C accumulated in MAOM ranges  
250 between 44% for CF and 64% for TS, although these values are not significantly different  
251 from each other (Table 3). This finding suggests that different ecosystems, and thus SOM  
252 inputs, do not significantly affect this pool. Nonetheless, it is particularly noteworthy that  
253 TS exhibit the highest percentage of organic C in the MAOM fraction (Table 3), in spite  
254 of their low SOM contents (Table 2).

255 The second most abundant organic C pool is LF<sub>free</sub>, with mean concentration values  
256 ranging from 1.8 g kg<sup>-1</sup> in AG up to 26.2 g kg<sup>-1</sup> in GL (Table 3). The LF occluded in  
257 macro- (LF<sub>M</sub>) and micro-aggregates (LF<sub>m</sub>) represent comparatively minor soil C  
258 pools, with no significant differences between them. The highest ~~proportion percentage~~  
259 of C stabilized in LF<sub>free</sub> is found in CF (38%), followed by GL (30%), BF (25%), TS  
260 (19%) and AG (16%) (Table 3). An opposite trend is observed for the LF<sub>M</sub> and LF<sub>m</sub>  
261 fractions, where the relatively highest C accumulation in LF<sub>M</sub> and LF<sub>m</sub> is found in  
262 AG (8.8% and 9.0%, respectively) and TS (8.2% and 11.5%, respectively) (Table 3).

263 Total N relative distribution among SOM fractions also follows the trend MAOM >  
264 LF<sub>free</sub> ≥ LF<sub>m</sub> ~ LF<sub>M</sub> (Table 3). In particular, up to 5.3 and 2.1 g N kg<sup>-1</sup> of soil are  
265 found in the MAOM and LF<sub>free</sub> pools of GL, respectively, whereas contents detected

266 in the LF\_M and LF\_m fractions are much lower (always  $\leq 0.2 \text{ g kg}^{-1}$ ). As for organic C,  
267 no significant differences among ecosystem types are found for the percentage of N  
268 stabilized in the MAOM (58% in CF to 69% in AG) and LF\_free pools (9% in AG to  
269 23% in GL). On the opposite, significant differences among land uses are observed in the  
270 LF\_M and LF\_m fractions, with relatively highest N accumulation in LF\_M and LF\_m  
271 found in AG (6% and 7%, respectively) and TS (4% and 8%, respectively) (Table 3).

272 Plant litter decomposability is thought to strongly affect the accumulation of organic  
273 matter in soils within different ecosystems. According to Castellano et al. (2015),  
274 recalcitrant C compounds, characteristic of coniferous needles, may confer to litter a low  
275 decomposition rate and microbial use efficiency (Kooch et al., 2012; Cotrufo et al., 2013),  
276 leading to a relatively higher C accumulation in LF\_free. We thus hypothesized a  
277 different distribution of organic C and total N among SOM pools under forests and  
278 grasses as compared to the more heavily managed agricultural soils and technosols, due  
279 to different mineralization rates of surface litter and roots. In agricultural soils and  
280 especially in technosols, an increased aggregate disruption and aeration might have been  
281 expected to lead to SOM accumulation mainly in MAOM. As a whole, however, our  
282 results indicate that, although soils under different ecosystems exhibit markedly different  
283 total organic C and N contents, the relative distribution of organic C and total N among  
284 SOM pools is pretty consistent, in spite of their different vulnerability to anthropogenic  
285 management.

286

#### 287 *C/N ratio of SOM fractions*

288 Organic matter in bulk soils shows C/N ratios ranging from 9 in TS to 14 in CF (Table  
289 2). The C/N ratios of all the fractions are significantly higher for CF than for the other  
290 ecosystems (Table 4), consistently with the relatively lower decomposition degree of

291 SOM in CF compared to the other ecosystems (Castellano et al., 2015). On the opposite,  
292 AG soils generally show the lowest C/N ratios for all fractions, with the exception of  
293 LF\_free (Table 4).

294 For all ecosystems, the C/N ratio of the MAOM pool is significantly lower than those  
295 of the other fractions (Table 4). A number of studies have reported a decrease in the C/N  
296 ratio from coarse to fine fractions suggesting both an increase of the decomposition  
297 degree of the SOM and a relative accumulation of microbial-derived materials (Kiem et  
298 al., 2002; Chen et al., 2014). Thus, the low C/N ratio of MAOM fractions is probably  
299 indicative of a depletion in lignin and plant-derived components and of an enrichment in  
300 materials of prevailing microbial origin (Paul and Clark, 1989; Golchin et al., 1994;  
301 Guggenberger et al., 1995; Kögel-Knabner et al., 2008; Cotrufo et al., 2013; Courtier-  
302 Murias et al., 2013; Plaza et al., 2013, 2016). In a parallel work, Zaccone et al. (submitted)  
303 reported the MAOM fraction to be characterized by high total DNA contents and very low  
304 C/N ratios at the same time. According to the Microbial Efficiency Mineral Stabilization  
305 (~~MEMS~~) framework, high quality N litter may lead to greater accumulation of microbial  
306 products and concomitant formation of MAOM in soils with a high soil matrix capacity  
307 (Cotrufo et al., 2013). Moreno-Barriga et al. (2017), studying the evolution of C and N  
308 pools following the addition of different materials to mine tailings, concluded that easily-  
309 available organic compounds triggered microbial growth during the first days of  
310 incubation and enhanced the formation of aggregates. This phenomenon probably  
311 occurred in TS, mirroring the early stage of pedogenic evolution, and suggested that  
312 aggregate stability may increase following a decrease of labile C forms, being then either  
313 used as fuel by microorganisms or stabilized by association with mineral particles. The  
314 accumulation of organic C and total N in the MAOM fraction confirms that these pools  
315 are less susceptible to further mineralization and offer a longer-term storage (Courtier-

Murias et al., 2013).

The low C/N of MAOM recorded for all the land uses is also consistent with the adsorption of nitrogenous compounds (*e.g.*, amino acids, amino sugars, proteins, cell wall constituents), possibly of microbial origin (Stevenson 1994; Ladd et al. 1996; Knicker, 2004; Sollins et al., 2006; Nannipieri and Paul, 2009), ~~possibly of microbial origin~~, onto mineral surfaces.

In fact, clay strongly affects stabilization of organic N directly (Ladd et al. 1996; Nielsen et al. 2006; Sollins et al. 2006) and through the formation of aggregate-protected particulate and non-particulate organic matter (*e.g.*, Yoo and Wander 2008), although most of these studies have been carried out in vitro using purified clay particles and purified proteins (Nannipieri and Paul 2009). By means of a 14-month laboratory incubation of a sandy loam soil amended with <sup>15</sup>N-clover, DiCosty et al. (2003) showed that both in whole soil and particle-size fractions, the clover-derived N was always 85–90% amide. Simpson et al. (2007) estimated that 88%, 86% and 82% of organic N in the soil humic fractions from grasses, mixed-aspen dominated forests and pine forests respectively was of microbial origin, in the form of amide in peptides and proteins. On the opposite, according to Rasmussen et al. (2005), the high C/N ratio found in LF<sub>free</sub> and occluded LF fractions suggests a relatively high occurrence of slightly-to-partially decomposed plant materials in these fractions.

#### *Thermal stability of SOM pools*

Being the thermal stability of any material related to its chemistry and surface properties, the thermal stability of SOM may serve as a proxy for biogeochemical stability and provide insight about the energy of interaction between SOM and soil minerals (Plante et al. 2009). At the same time, thermal analysis does not provide information at molecular

level, and is affected by several operational parameters, including arrangement and type of sample holder, packing density and grain size of sample, heating rate, initial sample mass, crucible material, and furnace atmosphere (Plante et al. 2009 and ref. therein).

Thermogravimetric (TG) curves of all SOM fractions generally show two main steps of weight loss in the range of temperature 100-550 °C. The first, between 250 and 350 °C, is associated with more easily oxidizable compounds, including polysaccharides (*e.g.*, cellulosic material) and aliphatic structures (Plante et al. 2009). The second, between 350 and 550 °C, due to the thermal degradation of recalcitrant, aromatic structures including lignin and non-hydrolyzable compounds (Schnitzer and Hoffman 1966; Lopez-Capel et al. 2005) (Fig. 1). The ratio between these two weight losses, here defined as *H*, as well as the *T*<sub>50</sub> value, *i.e.* the temperature at which 50% of the SOM mass is lost, are thus generally used to describe the SOM stability and/or recalcitrance (*e.g.*, Rovira and Vallejo 2000). In fact, within each fraction, the *H* and *T*<sub>50</sub> values found are significantly correlated to each other ( $R=0.666-0.981$ ,  $p\leq 0.001$ ,  $n=24$ ), independently of the land use.

Differences in *H* and *T*<sub>50</sub> among pools in CF, BF and GL soils are not statistically significant, while TS and AG the LF\_free and MAOM fractions showed lower values of both thermal indexes compared to LF\_M and LF\_m (Table 4), being these latter fractions the result of residual plant material depleted in labile compounds. Technosols generally show the highest values of both *H* and *T*<sub>50</sub> in all SOM fractions (Table 4). Moreover, TS is the only soil group in which a significant correlation was found between C/N ratios and both *H* ( $R=0.463$ ,  $p=0.040$ ,  $n=20$ ) and *T*<sub>50</sub> ( $R=0.630$ ,  $p=0.003$ ,  $n=20$ ).

When the weight loss (%) between 150 and 250 °C is considered (Fig. 2), the occurrence of more labile molecules in the MAOM fraction becomes evident, in accordance with the low C/N ratios characterizing this pool. In fact, the percentage of weight loss in the MAOM fraction is always higher than in the other fractions (Fig. 2).

366 The interaction of labile molecules (*e.g.*, sugars, amino acids, proteins) with mineral  
367 surfaces has been reported to be a key mechanism for the long-term stabilization of  
368 organic C (Lynch and Cotnoir, 1956; Baldock and Skjemstad, 2000; Kiem and Kögel-  
369 Knabner, 2003), allowing low molecular weight metabolic compounds to be stored in  
370 relatively “passive” pools for up to millennia (Paustian et al., 1992). For example,  
371 Schulten and Leinweber (1999), analyzing the Ah horizon of a Gleysol, found pyrolysis-  
372 field ionization mass spectra and thermograms of the light SOM fraction ( $< 2.0 \text{ g cm}^{-3}$ )  
373 to be similar to those of primary plant materials while the corresponding heavy fraction  
374 ( $> 2.0 \text{ g cm}^{-3}$ ) characterized by larger abundances of carbohydrates, lignin decomposition  
375 products, N-containing compounds and peptides.

376 Finally, the comparatively small variation in both  $H$  and  $T_{50}$  values characterizing the  
377 MAOM fraction ( $<16\%$  and  $<3\%$ , respectively) regardless of the ecosystem from each it  
378 is derived (Table 4) are indicative of new molecules resulting from a common microbial  
379 transformation process rather than highly degraded litter inputs. This finding is in  
380 agreement with Kallenbach et al. (2016) who provided evidences about the influence of  
381 substrate–microbe interactions on the synthesis of novel SOM constituents that become  
382 stabilized by clay minerals.

383

384

## 385 **Conclusions**

386 We provide evidence that physical fractionation coupled with thermal analysis and C/N  
387 ratios allow obtaining new insights about SOM sequestration in mineral soils across  
388 different ecosystems. We found that MAOM, followed by the LF\_free fraction, represents  
389 the major pool of both organic C (44-64% of the whole SOC stock) and total N (58-70%  
390 of the whole N stock) independently of ecosystem type. The lowest C/N ratios and the

391 highest percentage of weight loss recorded between 150-250 °C, both characterizing the  
392 MAOM fraction, indicate that this pool is comparatively richer in labile compounds. At  
393 the same time, the very small variability observed for these proxies ( $H$ ,  $T_{50}$  and  $C/N$ ),  
394 independently of land use and SOM inputs, is indicative of new molecules resulting from  
395 a common microbial transformation process. Technosols store two-third of the whole  
396 organic C and TN in the MAOM fraction, and show the highest values of both  $H$  and  $T_{50}$   
397 in all SOM fractions. Moreover, the significant correlation found between  $C/N$  ratio and  
398 both  $H$  and  $T_{50}$  exclusively in TS underlines how, in this young, man-made soils,  
399 mechanisms of SOM sequestration probably mirror processes taking place during the first  
400 phases of the pedogenetic processes. As a whole, different ecosystems may differ in terms  
401 of absolute SOM content among fractions; however, our results suggest a common  
402 mechanism of SOM dynamics, where plant input and their inherent recalcitrance control  
403 LF quality and distribution, while microbial by-products, largely independent of  
404 ecosystem type, govern SOM accumulation in the MAOM fraction. Further studies are  
405 needed to better understand not only the mechanisms of SOM stabilization at the  
406 molecular level by state-of-the-art tools currently available, but also the implications of  
407 the molecular mechanisms at larger scales and the ecological functions of the different  
408 SOM pools in the environment.

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647

648 **Figure caption**

649

650 **Figure 1.** Thermogravimetric curves of free light fraction (LF\_free), intra-  
651 macroaggregate light fraction (LF\_M), intra-microaggregate light fraction (LF\_m) and  
652 mineral-associated organic matter fraction (MAOM) of a representative sample for each  
653 soil group (coniferous forest soils, CF; broadleaved forest soils, BF; grassland soils, GL;  
654 technosols, TS; agricultural soils, AG).

655

656 **Figure 2.** Percentage of weight loss (mean  $\pm$  st. dev.) occurring in the temperature range  
657 150-250 °C for each SOM fraction and within each ecosystem type (coniferous forest  
658 soils, CF; broadleaved forest soils, BF; grassland soils, GL; technosols, TS; agricultural  
659 soils, AG).

660