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

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1 **Distribution and thermal stability of physically and chemically protected organic**

2 **matter fractions in soils across different ecosystems**

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Abstract

~~Accrual of carbon (C) in soil is a significant and realizable management option to mitigate the relentless increase in atmospheric CO₂.~~ A clear understanding of the mechanisms controlling the persistence of <u>carbon (C) and nitrogen (N)</u> in soil organic matter (SOM), and how they differ across different ecosystems, has never been more needed. ~~Soil organic C accrual requires nitrogen (N); thus understanding of N distribution in soil is equally important~~. Here we investigated SOM distribution between physical and chemical stabilized fractions in soils from a variety of ecosystems (*i.e.*, coniferous and broadleaved forest soils, grassland soils, technosols and agricultural soils). Using elemental and thermal analyses, we examined changes in the quantity and quality of physically-fractionated SOM pools characterized by different mechanisms of protection from decomposition. Independently of ecosystem type, ~~the majority~~<u>most</u> of the organic C and total N were found in the mineral-associated SOM pool, known to be protected mainly by chemical mechanisms. Indexes of thermal stability and C/N ratio of this heavy SOM fraction were lower (especially in agricultural soils) compared to light SOM fractions found free or occluded in aggregates, and suggested a marked presence of inherently labile compounds. Our results confirm that the association of labile organic molecules with soil minerals is a major stabilization mechanism of SOM, and demonstrate that this is a generalizable finding occurring across different mineral soils and ecosystems.

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

Introduction

Soils represent the largest terrestrial organic carbon (C) reservoir (Jobbágy and Jackson, 2000) with the potential to accrue enough additional C to halt the ~~relentless~~ increase in atmospheric CO_2, as proposed by the recently lounged 4 per mill initiative (Chabbi et al., 2017). Achieving this challenging objective requires a clear and generalizable understanding, across different soils and ecosystem types, of the mechanisms responsible for the buildup and persistence of soil organic matter (SOM).

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

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

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66 (Kästner 2000), thus suggesting that the molecular imprint of SOM by molecules and

67 fragments derived from microbial biomass is probably much more important than

68 previously considered (Miltner et al. 2012). When microorganisms degrade plant

69 residues, they use low-molecular-weight compounds from the plant biomass to build their

70 own biomass, and the majority of this material is then incorporated into non-living SOM

71 (Kindler et al. 2006; Miltner et al. 2012, and ref. therein). Cotrufo et al. (2013)

72 hypothesized that labile plant constituents are the main source of microbial by-products,

73 and these in turn are the main precursors of SOM stabilized by the soil matrix, through

74 the formation of physically or chemically realized organo-mineral associations (Vogel et

75 al., 2014). For example, McGill and Paul (1976) and Turchenek and Oades (1979)

76 observed that microbial cell wall materials were a major component of SOM accumulated

77 in the coarse clay fraction, while Ladd et al. (1996, and ref. therein) reported that clay-

78 size fractions were enriched of organic nitrogen (N) of microbial origin. Moreover,

79 particle size fractionation after ultrasonic dispersion and subsequent dating showed young

80 radiocarbon ages of the fine clay-associated organic C, indicating that this organic C was

81 more active than that associated with the coarse clay (Anderson and Paul, 1984).

82 Several other studies have suggested that thermally labile, microbial derived

83 materials and easily metabolizable compounds (*e.g.*, carbohydrates and amino acids) are

84 dominant in the mineral-associated organic matter (MAOM) pool, whereas the LF free

85 (LF_free) or occluded within macro- (LF_M) and especially micro-aggregates (LF_m)

86 are believed to be mainly of plant origin and characterized by an increasing degree of

87 persistence (Kleber et al., 2011; Hatton et al., 2012; Keiluweit et al., 2012; Plaza et al.,

88 2013, 2016). Given its chemical nature, the MAOM pool is also the richest in N among

89 SOM pools, *i.e.*, with the lowest C/N (Christensen, 2001), pointing to the need of a better

90 understanding of N distribution in the soil. In fact, despite the importance of N

4

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, 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; Wiesmeier 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; Wiesmeier 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 g cm^{-3}), found

128 free or occluded in macro- (2000-250 μm), and micro-aggregates (>250 μm), thus

129 stabilized by either inherent recalcitrance and/or an increasing strength of physical

130 protection, and the heavy (> 1.85 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

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Materials and methods

Study sites and soil sampling

Five ecosystem types, known to have contrasting climatic and edaphic conditions, were

considered in this study. To achieve a broad range of soils, we collected samples from 4

to 5 different sites for each of the five ecosystem types (Table 1). Five coniferous forests

(CF), five broadleaved forests (BF), five grassland soils (GL) and five technosols (TS)

were sampled between May and June 2016 in different sites of the Marche Region (Italy).

The coniferous forests consisted mainly of *Pinus nigra* woodlands occurring between 350

and 1400 m a.s.l., the broadleaved forests woodlands in stands of different species (*e.g.,*

*Fagus sylvatica, Orno Ostry*a *carpinifolia*etum associations with, *Quercus pubescens*)

growing between 320 and 1300 m a.s.l., the grassland soils in meadows and pastures

characterized by meso- to xerophilous species (*e.g.*, *Bromus erectus*) and *Medicago sativa*

and developing between 890 and 1700 m a.s.l.. The technosols included two open-pit

mines, filled and then reclaimed with *Helianthus annuus* (since 2010) and with *Triticum*

(since 2000), respectively, two dumps, reclaimed in 2000 and in 2011, respectively, and

a site of disposal of compost and other non-hazardous waste materials (all TS sites <300

m a.s.l.). Mean annual temperature of sampling sites ranged from 7.3 and 15.0 °C,

whereas mean annual precipitation ranged between 810 and 1570 mm. Four agricultural

soils (AG) cropped with barley and collected in Arganda del Rey (Madrid, Spain) were

also included in this study; t. These soils were located at 530 m a.s.l. and characterized

by a mean annual precipitation of about 440 mm and a mean annual temperature of 14 ºC

(Plaza et al., 2016). More details about the sampling sites are reported in Table 1.

At each location, 3 to 10 soil samples were collected randomly from several points,

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   (1*M*) (Thomas 1996), whereas electrical conductivity (EC) was measured on water

173   extracts obtained at a sample-to-water ratio of 1:5 (Roades~~Sparks,~~ 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 particula~~te~~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^{-1} werewas 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^{-1} allowed the LF_m isolation from the MAOM. The recovered LF_free, LF_M,

200 LF_m and MAOM fractions were oven-dried at 60ºC, 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^{-1} under an

214 oxidizing atmosphere of air at a flow rate of 30 mL min^{-1}. Two indexes of overall thermal

215 stability were calculated: T_{50} (°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^{-1})

232 and total N (9 g kg^{-1}), whereas the lowest are found in AG (11.8 g C kg^{-1} and 1.1 g N kg^{-1}

233 $^{-1}$) (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 Wieismeier 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 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^{-1} in AG up to 49.3 g kg^{-1} 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 particular noteworthy that

253 TS exhibit the highest percentage of organic C in the MAOM fraction (Tables 3), in spite

254 of their low SOM contents (Table 2).

255   The second most abundant organic C pool is LF_free, with mean concentration values

256 ranging from 1.8 g kg^{-1} in AG up to 26.2 g kg^{-1} in GL (Table 3). The LF occluded in

257 macro- (LF_M) and micro-aggregates (LF_m) represent comparatively minor soil C

258 pools, with no significant differences between them. The highest proportion percentage

259 of C stabilized in LF_free 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_M and LF_m

261 fractions, where the relatively highest C accumulation in LF_M and LF_m 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_free ≥ LF_m ~ LF_M (Table 3). In particular, up to 5.3 and 2.1 g N kg^{-1} of soil are

265 found in the MAOM and LF_free pools of GL, respectively, whereas contents detected

266　in the LF_M and LF_m fractions are much lower (always ≤0.2 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 that 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-

13

316   Murias et al., 2013).

317   The low C/N of MAOM recorded for all the land uses is also consistent with the

318   adsorption of nitrogenous compounds (*e.g.*, amino acids, amino sugars, proteins, cell wall

319   constituents), possibly of microbial origin (Stevenson 1994; Ladd et al. 1996; Knicker,

320   2004; Sollins et al., 2006; Nannipieri and Paul, 2009), possibly of microbial origin, onto

321   mineral surfaces.

322   In fact, clay strongly affects stabilization of organic N directly (Ladd et al. 1996;

323   Nielsen et al. 2006; Sollins et al. 2006) and through the formation of aggregate-protected

324   particulate and non-particulate organic matter (*e.g.*, Yoo and Wander 2008), although

325   most of these studies have been carried out in vitro using purified clay particles and

326   purified proteins (Nannipieri and Paul 2009). By means of a 14-month laboratory

327   incubation of a sandy loam soil amended with ^{15}N-clover, DiCosty et al. (2003) showed

328   that both in whole soil and particle-size fractions, the clover-derived N was always 85–

329   90% amide. Simpson et al. (2007) estimated that 88%, 86% and 82% of organic N in the

330   soil humic fractions from grasses, mixed-aspen dominated forests and pine forests

331   respectively was of microbial origin, in the form of amide in peptides and proteins. On

332   the opposite, according to Rasmussen et al. (2005), the high C/N ratio found in LF_free

333   and occluded LF fractions suggests a relatively high occurrence of slightly-to-partially

334   decomposed plant materials in these fractions.

335

336   *Thermal stability of SOM pools*

337   Being the thermal stability of any material related to its chemistry and surface properties,

338   the thermal stability of SOM may serve as a proxy for biogeochemical stability and

339   provide insight about the energy of interaction between SOM and soil minerals (Plante et

340   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_{50} 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_{50} 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_{50} 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_{50} 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_{50} (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 g cm^{-3})

373   to be similar to those of primary plant materials while the corresponding heavy fraction

374   (> 2.0 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 lever 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.

409

410

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416

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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 ± 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