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

Beatrice Giannetta[a], César Plaza[b], Costantino Vischetti[a], M. Francesca Cotrufo[c], Claudio Zaccone[*,d]

[a] *Department of Agricultural, Food and Environmental Sciences, Polytechnic University of Marche, via Brecce Bianche 10, 60131 Ancona, Italy*

[b] *Instituto de Ciencias Agrarias, Consejo Superior de Investigaciones Científicas, Serrano 115 bis, 28006 Madrid, Spain*

[c] *Department of Soil and Crop Sciences & Natural Resources Ecology Laboratory, Colorado State University, Fort Collins, Colorado, United States of America*

[d] *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

Abstract

~~Accrual of carbon (C) in soil is a significant and realizable management option to mitigate the relentless increase in atmospheric CO_2.~~ A clear understanding of the mechanisms controlling the persistence of carbon (C) and nitrogen (N) 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~~most 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~~. A~~lthough 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

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

Soil C stock and sink capacity were recently described as "ecosystem properties" (Schmidt et al.~~,~~ 2011), sharply affected by several factors including land use (*e.g.*, undisturbed *versus* agricultural soils subjected to aggressive human imprints) (Amundson et al.~~,~~ 2015). Besides climate, v~~V~~egetation and parent material are main drivers of soil formation (Jenny~~,~~ 1941), and their role in SOM stocks and persistence has been investigated in different ecosystems, spanning from spruce and deciduous forests to grasses and arable soils (Guggenberger et al.~~,~~ 1994, 1995; Leifeld and Kögel-Knabner~~,~~ 2005; Simpson et al.~~,~~ 2007; Clemente et al.~~,~~ 2011; Wie~~i~~smeier et al.~~,~~ 2012; Denef et al.~~,~~ 2013; Catoni et al.~~,~~ 2016). Despite all these research efforts, an accurate assessment of soil organic C and total N distribution across pools with different mechanisms of physical and chemical protection is still needed to improve our knowledge of the response of soil properties, including C stocks, to land management and/or changes in land use and cover (Guo and Gifford~~,~~ 2002; Wie~~i~~smeier et al.~~,~~ 2012; Wei et al.~~,~~ 2017). This would help better understand if SOM persistence, and therefore the distribution among fractions, is an ecosystem property rather than the main common denominator across different soils and ecosystems. In addition, technosols, *i.e.* man-made soils, are widely distributed throughout the world as they are found where human activity has led to the construction of artificial soils, sealing of natural soil, or extraction of material normally not affected by surface processes (*e.g.*, mines, dumps, oil spills, earth movements, coal fly ash deposits) (IUSS Working Group WRB~~,~~ 2006). Many technosols, in particular those in dumps, are originated covering man-made substrate with a layer of natural soil in order to permit revegetation. Although technosols have been recently included in soil classifications, often associated with other soil groups in a complex pattern, here they are considered as a "modern form" of soil in anthropogenic ecosystems. Only few attempts to understand SOM stabilization dynamics in technosols are reported in literature (Chaudhuri et al.~~,~~ 2013; Rodríguez-Vila et al.~~,~~ 2016; Moreno-Barriga et al.~~,~~ 2017), pointing to the need of including them when contrasting different ecosystems, for a generalizable understanding of the characteristic of SOM pools protected by different mechanisms of stabilization.

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

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-*~~*Ostrya 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~~. T~~hese 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 °C (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 auger and mixed to obtain a composite sample. The depth of sampling varied from 10 to 25 cm, and corresponded to the top soil horizon (Table 1).

Bulk soil characterization

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

Physical fractionation of SOM

The physical fractionation of SOM was carried out using the method described by Plaza et al. ~~(2012) with modifications detailed by Plaza et al.~~ (2013), with the aim to separate the heavy MAOM fraction from the light particulate~~r~~ organic matter (LF) fraction, and distinguish the latter into free LF and LF occluded in aggregates of different sizes. Specifically, we separated four fractions: (a) free LF, located between aggregates (unprotected C pool; LF_free); (b) LF occluded within macroaggregates (SOM pool weakly protected by physical mechanisms; LF_M); (c) LF occluded within microaggregates (SOM pool strongly protected by physical mechanisms; LF_m); and (d) a heavy SOM pool associated with the minerals (highly persistent mostly chemically-protected; MAOM). This method is based on the densimetric procedure by Golchin et al. (1994) and Sohi et al. (2001) for the fractionation of free and occluded LF, and on the method by Six et al. (2000, 2002) for the breakup of macroaggregates preserving microaggregates. First, 148 g of NaI at a density of 1.85 g mL^{-1} were~~was~~ added to 10 g of 2-mm sieved, air-dried soil in a 100-mL polycarbonate centrifuge tube. Free LF was separated from the heavy fraction by density flotation in the NaI solution and subsequent suction, filtration through a glass fiber filter and scrupulous washings with deionized water. Macroaggregates remaining in the heavy fraction were broken up, using the microaggregate isolator device designed by Six et al. (2000, 2002), into stable microaggregates, MAOM and LF_M, which was collected after a second density separation. Another density separation after an ultrasonic disruption at an energy input of 1500 J g^{-1} allowed the LF_m isolation from the MAOM. The recovered LF_free, LF_M, LF_m and MAOM fractions were oven-dried at 60ºC, weighed and ground with a ball mill for further analyses.

Organic C and total N analysis

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

Thermogravimetric analysis

Thermogravimetric analysis (TGA) was carried out on all SOM fractions using a Perkin Elmer Thermogravimetric Analyzer TGA 4000. An aliquot (*ca.* 20 mg) of each sample was placed in a ceramic crucible and heated from 30 to 850 °C at 20 °C min^{-1} under an oxidizing atmosphere of air at a flow rate of 30 mL min^{-1}. Two indexes of overall thermal stability were calculated: T_{50} (°C), representing the temperature at which 50% of the SOM mass is lost, and the thermal stability index (*H*), here defined as the ratio between the weight losses within 400-550 °C and 250-400 °C temperature ranges. These thermal indexes are reported on a moisture- and ash-free basis.

Data analysis

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

Results and discussion

Organic C and total N concentrations in soils

Organic C and total N contents of ~~the soils~~ examined soils span a broad range, as expected (Table 2). Grassland soils show the highest average values for both organic C (86 g kg^{-1}) 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}) (Table 2). In particular, both the organic C and total N average contents ~~follow the order~~rank as GL > CF > BF > TS > AG (Table 2), thus mirroring the link between organic C and total N dynamics. As a result, C/N across land uses vary in the order CF > BF ~ AG > GL ~ TS (Table 2).

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

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

Organic C and total N distribution

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

The second most abundant organic C pool is LF_free, with mean concentration values ranging from 1.8 g kg^{-1} in AG up to 26.2 g kg^{-1} in GL (Table 3). The LF occluded in macro- (LF_M) and micro-aggregates (LF_m) represent comparatively minor soil C pools, with no significant differences between them. The highest ~~proportion~~ percentage of C stabilized in LF_free is found in CF (38%), followed by GL (30%), BF (25%), TS (19%) and AG (16%) (Table 3). An opposite trend is observed for the LF_M and LF_m fractions, where the relatively highest C accumulation in LF_M and LF_m is found in AG (8.8% and 9.0%, respectively) and TS (8.2% and 11.5%, respectively) (Table 3).

Total N relative distribution among SOM fractions also follows the trend MAOM > LF_free ≥ LF_m ~ LF_M (Table 3). In particular, up to 5.3 and 2.1 g N kg^{-1} of soil are found in the MAOM and LF_free pools of GL, respectively, whereas contents detected in the LF_M and LF_m fractions are much lower (always ≤0.2 g kg^{-1}). As for organic C, no significant differences among ecosystem types are found for the percentage of N stabilized in the MAOM (58% in CF to 69% in AG) and LF_free pools (9% in AG to 23% in GL). On the opposite, significant differences among land uses are observed in the LF_M and LF_m fractions, with relatively highest N accumulation in LF_M and LF_m found in AG (6% and 7%, respectively) and TS (4% and 8%, respectively) (Table 3).

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

C/N ratio of SOM fractions

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

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

For all ecosystems, the C/N ratio of the MAOM pool is significantly lower than those of the other fractions (Table 4). A number of studies have reported a decrease in the C/N ratio from coarse to fine fractions suggesting both an increase of the decomposition degree of the SOM and a relative accumulation of microbial-derived materials (Kiem et al.~~,~~ 2002; Chen et al.~~,~~ 2014). Thus, the low C/N ratio of MAOM fractions is probably indicative of a depletion in lignin and plant-derived components and of an enrichment in materials of prevailing microbial origin (Paul and Clark~~,~~ 1989; Golchin et al.~~,~~ 1994; Guggenberger et al.~~,~~ 1995; Kögel-Knabner et al.~~,~~ 2008; Cotrufo et al.~~,~~ 2013; Courtier-Murias et al.~~,~~ 2013; Plaza et al.~~,~~ 2013, 2016). In a parallel work, Zaccone et al. (submitted) reported the MAOM fraction to be characterized by high total DNA contents and very low C/N ratios at the same time. According to the Microbial Efficiency Mineral Stabilization ~~(MEMS)~~ framework, high quality N litter may lead to greater accumulation of microbial products and concomitant formation of MAOM in soils with a high soil matrix capacity (Cotrufo et al.~~,~~ 2013). Moreno-Barriga et al. (2017), studying the evolution of C and N pools following the addition of different materials to mine tailings, concluded that easily-available organic compounds triggered microbial growth during the first days of incubation and enhanced the formation of aggregates. This phenomenon probably occurred in TS, mirroring the early stage of pedogenic evolution, and suggested that aggregate stability may increase following a decrease of labile C forms, being then either used as fuel by microorganisms or stabilized by association with mineral particles. The accumulation of organic C and total N in the MAOM fraction confirms that these pools 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 ^{15}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_free 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_{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).

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

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

Conclusions

We provide evidence that physical fractionation coupled with thermal analysis and C/N ratios allow obtaining new insights about SOM sequestration in mineral soils across different ecosystems. We found that MAOM, followed by the LF_free fraction, represents the major pool of both organic C (44-64% of the whole SOC stock) and total N (58-70% of the whole N stock) independently of ecosystem type. The lowest C/N ratios and the highest percentage of weight loss recorded between 150-250 °C, both characterizing the MAOM fraction, indicate that this pool is comparatively richer in labile compounds. At the same time, the very small variability observed for these proxies (*H*, T_{50} and C/N), independently of land use and SOM inputs, is indicative of new molecules resulting from a common microbial transformation process. Technosols store two-third of the whole organic C and TN in the MAOM fraction, and show the highest values of both *H* and T_{50} in all SOM fractions. Moreover, the significant correlation found between C/N ratio and both *H* and T_{50} exclusively in TS underlines how, in this young, man-made soils, mechanisms of SOM sequestration probably mirror processes taking place during the first phases of the pedogenetic processes. As a whole, different ecosystems may differ in terms of absolute SOM content among fractions; however, our results suggest a common mechanism of SOM dynamics, where plant input and their inherent recalcitrance control LF quality and distribution, while microbial by-products, largely independent of ecosystem type, govern SOM accumulation in the MAOM fraction. Further studies are needed to better understand not only the mechanisms of SOM stabilization at the molecular lever by state-of-the-art tools currently available, but also the implications of the molecular mechanisms at larger scales and the ecological functions of the different SOM pools in the environment.

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Figure caption

Figure 1. Thermogravimetric curves of free light fraction (LF_free), intra-macroaggregate light fraction (LF_M), intra-microaggregate light fraction (LF_m) and mineral-associated organic matter fraction (MAOM) of a representative sample for each soil group (coniferous forest soils, CF; broadleaved forest soils, BF; grassland soils, GL; technosols, TS; agricultural soils, AG).

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