

## Organo-mineral associations largely contribute to the stabilization of century-old pyrogenic organic matter in cropland soils

Victor Burgeon, Julien Fouché, Jens Leifeld, Claire Chenu, Jean-Thomas Cornelis

## ▶ To cite this version:

Victor Burgeon, Julien Fouché, Jens Leifeld, Claire Chenu, Jean-Thomas Cornelis. Organo-mineral associations largely contribute to the stabilization of century-old pyrogenic organic matter in cropland soils. Geoderma, 2021, 388, 388, pp.114841. 10.1016/j.geoderma.2020.114841. hal-03048810

## HAL Id: hal-03048810 https://hal.science/hal-03048810

Submitted on 9 Dec 2020

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

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



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

# <sup>1</sup> Organo-mineral associations largely contribute to

<sup>2</sup> the stabilization of century-old pyrogenic organic

## <sup>3</sup> matter in cropland soils.

- 4 Victor Burgeon<sup>a</sup>, Julien Fouché<sup>a,b</sup>, Jens Leifeld<sup>c</sup>, Claire Chenu<sup>d</sup>, Jean-Thomas Cornélis<sup>a</sup>
- <sup>a</sup> TERRA, Teaching and Research Centre, Gembloux Agro-Bio Tech, University of Liège, Gembloux 5030,
  Belgium
- 7 <sup>b</sup> LISAH, Univ Montpellier, INRAE, IRD, Institut Agro, Montpellier, France
- 8 <sup>c</sup> Climate and Agriculture Group, Agroscope, Reckenholzstrasse 191, 8046 Zurich, Switzerland
- <sup>d</sup> UMR Ecosys, Université Paris-Saclay, INRAE, AgroParisTech, 78850 Thiverval-Grignon, France
- 10 Burgeon, victor.burgeon@uliege.be
- 11 Fouché, julien.fouche@supagro.fr
- 12 Leifeld, jens.leifeld@agroscope.admin.ch
- 13 Chenu, claire.chenu@inra.fr
- 14 Cornelis, jtcornelis@uliege.be

## 15 1. Abstract

- 16 Understanding the processes underlying carbon (C) stability in soils is of utmost importance in the
- 17 context of climate change. In this setting, biochar is often studied for its persistence in soils and
- 18 reported to have positive impacts on soil fertility. Whilst recent research has mostly focused on the
- 19 short-term effects of biochar amendments to soil, a better understanding of its long-term persistence in
- 20 soils is needed.
- 21 Our study focuses on agricultural soils enriched in charcoal residues, produced in preindustrial kiln sites
- *ca.* 220 years ago, as a proxy for aged biochar. Our aim is to better understand the processes governing
- 23 the long-term persistence of pyrogenic organic matter (PyOM) in cultivated soils. To achieve this, we
- focus on i) the effect of PyOM on soil aggregation, ii) its distribution amongst soil fractions and iii) its
- chemical and thermal properties. For this purpose, we combined a soil particle size-density fractionation
- 26 with elemental and thermal analyses on topsoil samples collected in charcoal enriched (CHAR) and
- 27 adjacent reference (REF) soils in a conventionally cropped field in Wallonia (Belgium).
- 28 The presence of charcoal in soils resulted in a 91 ± 34 % higher C content in CHAR soils than REF soils, of
- which 84 to 94 % was PyOM and 6 to 16 % was additional non-PyOM. In CHAR soils, macroaggregation
- 30 was promoted at the expense of microaggregation (CHAR<sub>MACRO</sub> =  $48.9 \pm 12.8$ ; CHAR<sub>MICRO</sub> =  $25.5 \pm 8.3$  g
- 31 100 g<sup>-1</sup> soil) whereas these were similar in REF soils (REF<sub>MACRO</sub> = 41.3  $\pm$  9.5; REF<sub>MICRO</sub> = 36.0  $\pm$  8.7 g 100 g<sup>-1</sup>
- 32 soil). Elemental and thermal analyses revealed that PyOM did not only occur as free light fractions but
- 33 was also occluded in aggregates or sorbed onto mineral phases (56.4 ± 22.9 % of total PyOM) suggesting
- 34 PyOM may be further stabilized through organo-mineral associations. Century-old PyOM showed
- 35 increased H:C and O:C atomic ratios compared to recently pyrolyzed OM, particularly in occluded as
- 36 opposed to free light fractions, vouching for the functionalization of its surfaces. Furthermore,
- 37 regardless of high C contents in CHAR soils, similar C:N ratios between studied soils in mineral
- 38 dominated fractions suggested increased amounts of N-rich non-PyOM in charcoal rich soils. Our results
- demonstrate that, over centuries in cultivated soils, PyOM plays an active role in aggregation patterns.
- 40 We conclude that century old PyOM is broken down from coarse to fine particles, and through
- 41 functionalization of its surface contributes to organo-mineral associations. Beyond its intrinsic chemical
- 42 recalcitrance, the long-term persistence of biochar is enhanced by occlusion and sorption processes.

#### 43 Keywords:

44 Black carbon, size-density fractionation, thermal analysis, soil aggregation, aged biochar

## 45 2. Introduction

- 46 Globally, human land use and agriculture in particular have resulted in a substantial loss of soil organic C
- 47 (SOC) (Sanderman et al., 2017). As soil organic matter (OM) plays a crucial role in both the global C cycle
- 48 and in ensuring food security (Le Quéré et al., 2009; Chenu et al., 2019) implementing practices that
- 49 allow for SOC restoration and sequestration in cultivated soils offers a great potential to contribute to
- 50 the mitigation of anthropogenic greenhouse gas emissions, whilst improving soil agronomic and
- 51 ecological functions (Lal, 2004; Minasny et al., 2017; Chenu et al., 2019).
- 52 Amongst the various strategies allowing for SOC sequestration (i.e., a net removal of atmospheric CO<sub>2</sub>),
- 53 the study of C forms displaying long residence times in soils such as biochar has received much
- 54 attention. Biochar is a form of pyrogenic organic matter (PyOM) whose purpose is to be amended to
- 55 soils for its conjoint potential in terms of long-term C storage (centennial or longer)(Forbes et al., 2006)
- as well as soil fertility improvement (Lehmann et al., 2006). Meta-analyses of short-term (annual to
- 57 decadal) biochar impacts in agro-ecosystems report an overall increased plant productivity as explained
- 58 by the increase in soil water holding capacity, plant nutrient availability and liming effects (Jeffery et al.,
- 59 2011; Biederman and Harpole, 2013). Such changes are particularly marked in highly weathered, coarse
- 60 textured, tropical soils (Crane-Droesch et al., 2013). Biochar properties and their resulting agronomic
- 61 benefits depend on its organic precursor and the pyrolysis conditions (e.g., heating rates and maximum
- 62 temperatures) (Zimmerman et al., 2011; B. P. Singh et al., 2012; Baveye, 2014; Fang et al., 2015). Beyond
- 63 reported positive impacts on soil fertility, biochar is studied for its potential as a C sequestration tool
- and related implications on the global C balance (Schmidt and Noack, 2000). The stability of biochar and
- 65 PyOM in general is attributed to its aromatic moieties which confer it recalcitrance against biotic or
- abiotic oxidation (Hammes et al., 2008; Kuzyakov et al., 2014). The distribution and organization of these
- aromatic moieties in charcoal fragments are characterized by the aromaticity and degree of aromatic
- 68 condensation of biochar (Wiedemeier et al., 2015) which will in turn determine its persistence in
- 69 ecosystems (Hammes et al., 2008; Kuzyakov et al., 2014). Despite this persistence in soils, biochar
- properties evolve in soils over time through breakdown and functionalization (i.e., ageing) (Cheng et al.,
- 2006; Nguyen et al., 2008; Cheng and Lehmann, 2009; Hardy et al., 2017b). Recent studies have shown
- that PyOM may decompose faster than initially predicted (Hamer et al., 2004; Hammes et al., 2008) and
- that the persistence of PyOM in soils may have been overestimated particularly in arable settings (Singh
- et al., 2012; Lehndorff et al., 2014; Lutfalla et al., 2017). The persistence of PyOM, as for other OM
- 75 forms, in soils is therefore strongly dependent on ecosystem properties rather than solely inherited
- 76 chemical recalcitrance (Schmidt et al., 2011; Lehmann and Kleber, 2015; Lehmann et al., 2020).
- 77 Therefore, spatial inaccessibility for decomposer organisms (i.e., occlusion in aggregates) and
- 78 interactions with mineral surfaces (i.e., adsorption) or metals (von Lützow et al., 2006; Kögel-Knabner et
- al., 2008) need to be carefully investigated as stabilization processes for PyOM in soils particularly as
- 80 ageing renders it an active soil constituent.
- 81 While short-term biochar-induced changes in agro-ecosystems have been extensively characterized
- 82 (Jeffery et al., 2011; Biederman and Harpole, 2013; Crane-Droesch et al., 2013), insights into its long-
- 83 term effects are needed to verify its potential in climate-smart agriculture (Baveye, 2020). Worldwide
- 84 various field models have been addressed to better understand the long-term fate of biochar in soils.

85 Amongst these are the fertile Amazonian Dark Earth (Glaser and Birk, 2012), soils containing wildfire

86 derived PyOM (Schmidt and Noack, 2000; Bird et al., 2015), historical industrial sites (Cheng et al.,

87 2008b), crop residues burning or relic charcoal hearths sites (Borchard et al., 2014; Lehndorff et al.,

88 2014; Hernandez-Soriano et al., 2016b; Kerré et al., 2016; Hardy et al., 2017a; Hirsch et al., 2017;

89 Schneider et al., 2019). All these studies address aged PyOM however varying production environments

90 may result in a final product differing from that obtained through oxygen regulated pyrolysis as is done

91 today to produce biochar (Bird et al., 2015).

92 In southern Belgium, pre-industrial charcoal production kiln sites are easily spotted in agricultural fields

as "black patches" (Hardy and Dufey, 2015). The charcoal still found in these sites stands as left-overs

94 from the production kiln that has been incorporated in agro-ecosystems since their conversion from

woodlands *circa* 150-200 years ago (Hardy et al., 2017a). Due to similar oxygen-limited conditions in
kilns as those obtained in modern biochar production units, this setting is ideal for the study of century-

97 old biochar in temperate conventional cropping systems (Hardy et al., 2017a). Recent studies

98 investigating similar kiln sites in the region showed that black patches stored 60 to 80% more C than

adjacent reference soils in cropped systems, of which from 25% to 40% was non-pyrogenic organic

100 matter (non-PyOM)(Hernandez-Soriano et al., 2016a; Kerré et al., 2016; Hardy et al., 2017a). The total N

101 content as well as the C:N and C:P ratios are higher in kiln soils than reference soils (Hardy et al., 2017a).

102 In addition, kiln soils display greater cation exchange capacity (CEC), which was associated with larger

103 content of exchangeable Ca<sup>2+</sup> and Mg<sup>2+</sup> (Hardy et al., 2017a). Long-term ageing and continuous

104 cultivation have resulted in the increase in H:C and O:C ratios of charcoal particles in comparison to

105 freshly pyrolyzed OM (Hardy et al., 2017b). These modifications are associated with the oxidation of

106 charcoal particles and the creation of functional groups (mostly carboxyl, phenol or carbonyl) at the

edge of aromatic rings (Lehmann et al., 2005; Cheng et al., 2008a) which in turn promotes the

108 precipitation of 2:1 phyllosilicates and CaCO<sub>3</sub> on the surfaces of PyOM (Hardy et al., 2017b).

109 In the present study we aim to investigate the various associations between century-old biochar and soil 110 minerals in order to assess their contribution to PyOM persistence in soils. For this purpose, we aim to 111 separate SOC into pools displaying similar stabilization mechanisms (i.e., chemical recalcitrance, spatial 112 inaccessibility, interactions with mineral surfaces) (von Lützow et al., 2006, 2007). Numerous physical 113 (size, density) and chemical (oxidation, extraction) fractionation protocols have been developed to 114 isolate SOC pools of comparable stabilization mechanisms and turnover rates (von Lützow et al., 2007; 115 Poeplau et al., 2018). In soils, the most labile SOC pool, displaying turnover rates < 10 years, are 116 represented by free microbial biomass and the coarse light fraction (LF - density < 1.6-2 g cm<sup>-3</sup>) whereas 117 the SOC pools with the highest turnover rates comprise the oxidation-resistant SOC pool and the heavy 118 fraction after macroaggregate dispersion (HF - density > 2.8 g cm<sup>-3</sup>)(von Lützow et al., 2007; Virto et al., 119 2010; Poeplau et al., 2018). In cultivated soils macroaggregates display rapid turnover as revealed by 120 their composition of a mix of young and older SOC originating from both labile organic matter, (i.e., 121 plant roots, mucilage, exudates, fungal hyphae, microbial biomass)(Rillig, 2004; Rasse et al., 2005) and 122 more persistent physically protected and adsorbed organic compounds (Moni et al., 2010; Poeplau et 123 al., 2018). Their dispersion is therefore required to isolate SOC groups with similar turnover rates (Virto 124 et al., 2010; Poeplau et al., 2018). The few fractionation studies performed on soils enriched in aged 125 biochar showed that the century-old biochar particles were mostly present as free particulate organic

- 126 matter (i.e., non-associated with mineral particles)(Glaser et al., 2000; Herath et al., 2014; Kerré et al.,
- 127 2016; Grunwald et al., 2017). However PyOM has also been reported to increase aggregate formation
- 128 (Awad et al., 2013) and stability (Pituello et al., 2018) as well as to increase the abundance of non-PyOM
- in the aggregate-protected silt and clay fraction (Liang et al., 2010; Hernandez-Soriano et al., 2016a;
- 130 Kerré et al., 2017) thus showing interactions with mineral phases.
- 131 Although the effects of PyOM in soils have been widely addressed, there is a lack of consensus
- 132 concerning the processes involved in its long-term persistence. Nonetheless it is known that ageing in
- soils causes an oxidation of the charcoals surfaces and results in the formation of phenolic and
- 134 carboxylic functional groups along with a charging of the structures surface (Cheng et al., 2008a; Nguyen
- et al., 2008; Cheng and Lehmann, 2009). As a result, physical protection through aggregation (Brodowski
- et al., 2006) as well as an increased association with the mineral phases (Nguyen et al., 2008; Rodionov
- et al., 2010; Hardy et al., 2017b) could play an important role in PyOM stabilization (Lehmann and
- 138 Kleber, 2015) beyond its chemical recalcitrance to oxidation.
- 139 In this study, we investigated the long-term persistence of PyOM in soils. To achieve this, soil samples
- 140 from five preindustrial charcoal kiln sites were compared to five adjacent reference soils in a
- 141 conventionally farmed Luvisol (Isnes, Belgium). These charcoal kilns are visible as black spots on bare
- soils and procure an ideal setting for the study of aged biochar in conventionally cropped systems. This
- site hence serves as a proxy to study the long-term effect of biochar in soils if it was to become a
- 144 common soil amendment in temperate soils of the region.
- 145 Our aim is to understand how and where PyOM persists in the soil matrix, as either free standing
- 146 particulate OM (POM) or protected through aggregate occlusion or mineral sorption. In particular, this
- study aimed i) to quantify the effects of long-term PyOM accumulation on soil aggregation through a
- size and density fractionation protocol, ii) to investigate the distribution of PyOM amongst the fractions
- and iii) to determine the physico-chemical and thermal characteristics of organic matter in soil fractions.
- 150 Numerous analytical techniques exist to identify, characterize and quantify PyOM; physical density
- 151 separations, chemical oxidation, thermal analyses, optical or molecular marking techniques (Schmidt
- and Noack, 2000; Brodowski et al., 2005; Hammes et al., 2007; Leifeld, 2007; Plante et al., 2009; Bird et
- al., 2015; Kerré et al., 2016). Among these, thermal analyses and differential scanning calorimetry (DSC)
- in particular, has been reported to be an efficient way to quantify and characterize number of samples
- rapidly within the whole OM continuum, and was hence used to characterize PyOM forms (Leifeld,
- 156 2007; Plante et al., 2009).
- 157 We postulate that charcoal is found in all, light and heavy, soil fractions as a consequence of particle
- 158 physical breakdown and functionalization of its surfaces. Furthermore, we postulate that the elemental
- composition of OM controls its thermal stability within the different fractions. Finally, we postulate that
- 160 pyrogenic C forms may be further stabilized as a result of organo-mineral associations which increases
- 161 its potential as a C sequestration tool.

## 162 3. Materials and Methods

#### **163** 3.1. <u>Study site</u>

164 In cropped agroecosystems of the province of Namur (Belgium), century-old charcoal kiln sites are easily spotted visually in the field or by aerial imagery as darker ellipses on the soil surface (Figure 1). The site 165 selected for this study is located in Isnes (50°31'N 4°44'E) and was converted from forest to cropland at 166 167 least 170 years ago as revealed by land occupation maps of Vandermaelen (1846-1854). Charcoal was 168 produced in kiln sites during the pre-industrial era to meet energy demands thus providing an estimated 169 age for the charcoal of interest of ca. 250 years (Hardy et al., 2017a). Hardy et al. (2017b) thoroughly 170 characterized kiln sites of the region and reported that charcoal mixtures are generally dominated by 171 deciduous hardwood such as oak (Quercus sp.), hornbeam (C. betulus), beech (F. sylvatica), hazel 172 (Corylus avellana L.) and birch (Betula sp.).

- 173 The soil of the studied site has a homogeneous silt loam texture throughout and is slightly sloped (Table
- 174 1). It is characterized as a Haplic Luvisol (WRB, 2014) developed from decarbonated quaternary loess.
- 175 The average annual precipitation is of 840 mm of rain and the mean annual temperature is 9.8°C. The
- 176 field follows a four-year crop rotation established approximately in 1985 and alternates between
- 177 chicory, winter wheat, beet root, potatoes and cover crops (mustard and phacelia).

178

#### # Figure 1: Aerial view of the study site.



179

Figure 1 - Aerial view of the study site. Kiln sites are visible as black patches when the soil is bare. The image comes from the
 "Service Public de Wallonie: Orthophotos 2009/2010", arrows indicate the approximate location of the five soil sampling sites
 per studied soil.

#### 183 3.2. Soil sampling and characterization

- 184 In this study, five field replicates of two soils, charcoal enriched (CHAR) and reference soils (REF) at least
- 185 40 m away from one another, were compared to study the effect of charcoal. Every soil sample collected
- 186 for each of the five replicates of CHAR and REF sites was bulked from five cores sampled in the topsoil
- 187 layer (0-30 cm) within a radius of 1 m. These samples were dried at 40 °C for 48h before further analysis.

- 188 A subsample of each soil was ground and sieved to 2 mm for baseline physico-chemical characterization.
- 189 Briefly, pH was determined in distilled water and KCl (1N) (ratio soil: solution 1:5, ISO 10390). The soil
- 190 particle-size distribution was determined by gravity sedimentation (NF X 31-107), C and N content were
- 191 quantified by near infra-red spectrometry after flash dry combustion (respectively ISO 10694; ISO
- 192 13878). We measured the cation exchange capacity (CEC) by percolation of 1 M ammonium acetate (pH
- 7) on soil columns according to the Metson procedure (Metson, 1956). The bioavailable major elements,
  Ca, Mg, P and K were extracted using ammonium acetate-EDTA 1M (pH = 4.65; (Lakanen and Erviö,
- Ca, Mg, P and K were extracted using ammonium acetate-EDTA 1M (pH = 4.65; (Lakanen and Ervi
   1971)) and quantified by atomic absorption spectroscopy (Ca, Mg, K) or spectrophotometry (P).

#### **196** 3.3. <u>Fractionation protocol</u>

- Soil samples from both studied soils followed a four step fractionation protocol (Figure 2), which was
  adapted from the method described by *Chenu and Plante*, (2006), *Gulde et al.* (2008) and *Virto et al.*
- 199 (2010). In the first step, wet sieving fractionated the soil into four fractions according to size (>2000  $\mu$ m,
- 200 coarse sand size: 2000-250  $\mu$ m, fine sand size: 250-50  $\mu$ m, silt and clay size (S&C) <50  $\mu$ m). The
- 201 particulate material bigger than 2 mm was discarded. The second step used density to differentiate light
- fractions (free LF, d < 1.85 g cm<sup>-3</sup>) from heavy fractions (HF: d > 1.85 g cm<sup>-3</sup>): Macroaggregate,
- 203 microaggregate, free silt and clay size heavy fractions (free S&C HF). Step 3 focused on breaking down
- the macroaggregates (2000-250  $\mu$ m) to isolate its components into three finer size class fractions
- 205 (coarse sand, macroaggregate protected fine sand, macroaggregate protected-S&C). Finally, step 4,
- through a sequential size and density fractionation and dispersion of microaggregates, isolated non-
- 207 occluded and occluded S&C LF from S&C-size HF within each size class sizes from step 1.
- We used the wording *free* for LF and HF after wet sieving, and *occluded* for LF obtained after ultrasonic
  dispersion of aggregates. This fractionation protocol was applied on both studied soils, CHAR or REF, and
  for all five replicates.
- 211 i. Step 1 Size separation by wet sieving
- 212 We applied the wet sieving size separation protocol presented by Six et al. (1998). A soil sample of 80 g, 213 previously air-dried at 40°C for 48 h was submerged in deionized water for 5 min prior to deposition in a 214 water bath on a 2 mm sieve. The sieve was then moved up and down and tilted in a rotational 215 movement for 50 rotations in two minutes at room temperature. The sieve was then removed from the 216 water bath and its inner and outer walls were rinsed with deionized water. The reject (> 2 mm) was 217 collected by back-washing in a glass tray and air dried at 60°C. This wet sieving operation was repeated 218 firstly using a 250 µm sieve on the soil that went through the 2 mm sieve, then repeated a second time 219 on a 50 µm sieve to separate the two smallest fractions. All sieve rejects were back-washed in glass trays 220 after rinsing the sieve and dried at 60°C. This way four fractions per sample were differentiated as 221 follows: particulate (>2000  $\mu$ m) > coarse sand size (2000-250  $\mu$ m) > fine sand size (250-50  $\mu$ m) > Silt &
- 222 Clay size materials (<50 μm) (Figure 2-step1).



#### 224

Figure 2 - Flow-chart of the four step fractionation protocol. S&C silt and clay; LF light fraction; HF heavy fraction; Mp
 macroaggregate protected; mp microaggregate protected; occ. occluded; non-occ non-occluded. Dashed squares highlight the
 difference between the three size classes

228 ii. Step 2 - Density fractionation

229 In this step we divided a 10 g subsample of the three size fractions smaller than 2 mm (coarse sand size 230 > fine sand size > S&C-size materials ) obtained from step 1 into a light fraction (LF) and a heavy fraction 231 (HF) as described by Six et al. (1998). For this density separation subsamples were submerged in 30 mL of sodium polytungstate (SPT,  $Na_6(H_2W_{12}O_{40}, -1.85 \text{ g cm}^{-3})$  in centrifuge tubes. These were then closed 232 and gently stroked 10 times to remove bubbles and without breaking aggregates. The centrifuge tubes 233 234 were then reopened and with an extra 20 ml of SPT its inner walls were rinsed to gather all the sample 235 in the solution. The samples were placed in a vacuum (-140 kPa) for 10 min, left to rest for 20 min and 236 centrifuged at 4700 g for 10 min. The supernatant was transferred on a filtering flask where a gentle 237 vacuum was applied (glass fiber filter Mechery-Nagel Germany,  $\Phi = 0.45 \,\mu$ m). After having repeated the 238 centrifuge and the filtering thrice, the LF on the filter was thoroughly rinsed to remove the remaining 239 SPT under a light vacuum. The LF was then recovered in a glass tray by gently back washing the filter 240 with deionized water and then oven dried (60°C).

To recover the HF, 50 ml of deionized water was added to the pellet in a centrifuge tube and the tube

242 gently stroked until the pellet detached. An additional 20 ml of deionized water was used to clean the

tubes inner walls before centrifuging for 10 min at 4700 g. This rinsing was repeated thrice to rid the HF

of SPT before recovering the sample in a glass tray and oven drying at 60 °C for 24h. This step produces a

LF and a HF per size class obtained after step 1. These LF are referred to as free LF (macroaggregate,
microaggregate or S&C-size) and the HF as macroaggregates, microaggregates or free S&C-size (Figure 2step 2).

248 iii. Step 3: Macroaggregate breakdown

249 This step focused on the HF collected after density fractionation (step 2) of the macroaggregate soil 250 fraction separated from coarse sand size (2000-250 μm) material. The purpose of this step was to 251 separate this HF into: Coarse sands, macroaggregate protected fine sands and macroaggregate 252 protected S&C-size material. To achieve this, the sample was placed in a 250 µm sieve in contact with 50 253 glass beads in a water bath previously installed on a shaker plate. Deionized water was set to run 254 continuously on the sample while maintaining a 2 cm water front on top. The sample running with the 255 water through the first sieve was collected by means of a funnel and deposited onto a 50 µm sieve. This 256 breakdown procedure was set to last for 5 min on the shaker plate. After this step, deionized water was 257 used to thoroughly rinse the funnel into the 50 µm sieve. The 250 µm sieve was back-washed and oven 258 dried (60°C for 24 h) in a glass tray. Then the wet sieving procedure was repeated for the fraction 259 collected in the 50 µm sieve whereby the sieve was tilted and lifted up and down 50 times within two 260 minutes. Again after rinsing the sieve, the reject was back-washed and collected in a glass tray. Both 261 fractions (250-50  $\mu$ m and <50  $\mu$ m) were collected in glass trays and oven dried (24 h at 60°C).

262 iv. Step 4: Isolating occluded LF and HF

263 The first purpose of this step was to isolate the non-occluded (non-occ) S&C-size fraction (<50 μm) 264 contained in macroaggregates from the S&C-size HF (i.e., S&C aggregates or free minerals). For this step 265 an adapted density fractionation was undertaken. The sample was submerged in 30 ml SPT (1.85 g cm<sup>-3</sup>) 266 and gently shaken into suspension. Using an extra 20 ml of SPT the inner walls of the centrifuge tube 267 were rinsed before being centrifuged at 4700 g for 60 min (all material bigger than 0.7 μm sediments). Using a micropipette, the topmost 15 ml were sampled and deposited on a glass fiber filter (0.4  $\mu$ m) 268 269 without vacuum application (composes the non-occ LF). After each filtration the filter was rinsed 270 thoroughly to rid it of the SPT and then back-washed in a glass tray to collect the LF in order to avoid 271 filter clogging, this step was repeated thrice. After the third centrifuge, 40 ml of the supernatant were 272 collected and poured in the filter, rinsed thoroughly with distilled water through the filter. Finally, this 273 filter was back-washed to collect the LF in a glass tray.

Next, using SPT, the pellet of the centrifuge tube was shaken into suspension and then dispersed by
 ultrasonication at 440 J ml<sup>-1</sup> for 2 minutes (Soniprep 150 MSE) (Amelung and Zech, 1999) in order to

- 276 break down remaining S&C-sized aggregates thus freeing the occluded LF. This step was undertaken for,
- 277 macroaggregate protected S&C-size material, microaggregates HF and free S&C-sized material.
- 278 Following this dispersion, the LF was separated from the HF through a density fractionation (1.85 g cm<sup>-3</sup>)
- 279 to obtain three new fraction: occluded LF in macroaggregates (occ Mp-LF), occluded LF in
- 280 microaggregates (occ mp-LF) and occluded LF (occ LF).

- 281 Finally, to conclude step 4, a size separation through wet sieving on the HF obtained following the
- 282 ultrasonication of microaggregate HF only is undertaken as described previously to separate fine sands
- 283 from S&C size HF.

284 The density of SPT was controlled before each density fractionation step. Previous studies reported

- solubilization of organic compounds with SPT use leading to a loss of C and a decrease the C recoveryafter density fractionation (Chenu and Plante, 2006).
- 287 The elemental composition (C, H, N) was determined via dry combustion for all fractions (LF and HF).

288 The O concentration was determined on LF only through sample pyrolysis at 1000 °C. The C, H, N, O

concentrations were quantified using a Euro EA elemental analyzer (Hekatech, Germany). Prior to the

analysis, HF samples were acidified with HCl to remove residual inorganic C.

#### 291 3.4. Differential Scanning Calorimetry (DSC)

We used thermal analysis to compare soil fractions isolated by the fractionation protocol to highlight the
 presence of PyOM in fractions as well as its position in the OM continuum by comparing CHAR and REF
 fractions. For this thermal analysis, three out of the five field replicates were selected for each studied

- soil. For these repetitions, all fractions isolated by the fractionation protocol were analyzed. In addition,
- three products were characterized as reference materials; hand-picked pyrolyzed OM (hand-picked
- 297 PyOM, n=3), hand-picked non pyrolyzed coarse OM (hand-picked non-PyOM, n=3) and freshly pyrolyzed
- 298 beech (F. Sylvatica fresh PyOM, n=3). Hand-picked non-PyOM are crop residues hand-picked from REF
- soils and hand-picked PyOM are charcoal fragments hand-picked from CHAR soils. The freshly pyrolyzed
- 300 beech was made in an industrial pyrolyzer (Greenpoch SA, Belgium). Finally, three bulk soils were also

301 used as comparison materials (n=3).

302 Samples were analyzed using a differential scanning calorimeter (Netzsch STA 449, Selb, Germany),

Agroscope, Zürich, Switzerland). Before sample analysis the sensor was calibrated for temperature and
 sensitivity (i.e. reaction enthalpy) with six different standards (indium, tin, bismuth, zinc, aluminium and
 silver).

- Prior to scanning, samples were ground to powder. For HF between 15 and 25 mg of samples were
- 307 weighed and placed in open Al<sub>2</sub>O<sub>3</sub> pans for analysis. For LF only 2 mg of samples were mixed to
- 308 approximately 18 mg Al<sub>2</sub>O<sub>3</sub> powder, precise weights were recorded to enable a correction by the
- dilution factor. Samples were analyzed under a constant flow of 50 ml min<sup>-1</sup> synthetic air from 50 °C to
- 310 700 °C at a heating rate of 20 °C min<sup>-1</sup>. During data treatment a baseline correction was applied on
- 311 thermograms to account for increased heat capacity of samples. Resulting thermograms were drawn
- between 200-600 °C to highlight the temperature range of interest as data below 200°C or above 600°C
- 313 were never used.
- To characterize the material obtained we used values of peak height as the heat flow at a given
- 315 temperature (W g<sup>-1</sup>), peak temperatures as the temperature at which main exotherms occur (°C), the
- area under the curve as the total heat of reaction of a sample (J g<sup>-1</sup>) delimited by the baseline, and the
- 317 thermogram 50% burn-off temperatures (°C) as the temperature where 50% of the total heat flow has
- 318 occurred. Finally ratios between areas or peak heights considered as thermally labile or stable were

- used (Leifeld, 2007). In accordance with previous studies, a tipping temperature of ~380-390 °C was
- used to differentiate the thermally labile or stable C forms (Dell'Abate et al., 2000; Lopez-Capel et al.,
- 321 2005).
- 322 Amongst various indicators available for the characterization of C forms using DSC, the sum of peak
- heights of the exotherms ( $R^2=0.78 p$ -value < 0.0001) and the total heat release ( $R^2=0.91 p$ -value < 0.0001) displayed strong linear correlation with the C content of fractions.

#### 325 3.5. <u>PyOM and non-PyOM quantification in charcoal rich soils</u>

- In CHAR soils, the PyOM was quantified as the additional heat flow occurring in the thermally stable
  region (>390 °C) as opposed to the heat flow in adjacent REF soils. This tipping temperature was
  established based on our results and in line with previous findings (Dell'Abate et al., 2000; Lopez-Capel
  et al., 2005). This additional heat flow over the sum of punctual heat flows (peak heights) of CHAR
  samples multiplied by the samples C content corresponds to the PyOM content of CHAR soils (eq 1).
- 331 eq 1.

332 
$$PyOM = \frac{\sum CHAR_{st.peaks} - \sum REF_{st.peaks}}{\sum CHAR_{peaks}} * C_{CHAR}$$

333 Where **PyOM**; the PyOM content (g kg<sup>-1</sup>), **st. peaks**; the heat flow of exotherms occurring above 390 °C 334 and  $C_{CHAR}$  the C content of CHAR samples.

- 335 Changes in non-PyOM contents,  $\delta$ (non-PyOM), were calculated as the ratio of differences in CHAR to 336 REF labile peaks height (< 390 °C) to the difference in sum of peaks between studied soils, the whole 337 multiplied by the difference of C content between CHAR and REF samples (eq 2.).
- 338 eq 2.
- 339

$$\delta(non PyOM) = \frac{CHAR_{lab.peak} - REF_{lab.peak}}{\sum CHAR_{peaks} - \sum REF_{peaks}} * (C_{CHAR} - C_{REF})$$

Where δ(non-PyOM); the change in non-PyOM content (g kg<sup>-1</sup>), lab.peak; the heat flow of
 exotherms occurring below 390 °C, C<sub>CHAR</sub> and C<sub>REF</sub> the C content of CHAR and REF samples (g kg<sup>-1</sup>).

#### 342 3.6. Data analysis and signal treatment

All data analysis, DSC signal treatment and graph plotting were performed using R studio v. 3.4.2 (R Core

Team, 2017). Results of all parameters are presented as the means ± standard deviation (SD).

345 Comparison between fractions of similar density and size class were undertaken by analysis of variances

346 (ANOVA) or by paired t-tests for soil baseline characteristics of bulk soils. Mean classification was

- 347 pursued through a least significant differences test (LSD-test) and indicated by different letters following
- 348 mean ± SD. For the application of this test the normality of the distribution and homoscedasticity of

349 variances was presumed given the low number of repetitions (n<10).

- 350 To ensure the validity of our soil fractionation results, the mass recovery and C content (mass by
- 351 concentration) were calculated at each fractionation step. These were obtained as the sum of fraction
- 352 masses and C content divided by that of sample used as input for the fractionation step.

### 353 **4. Results**

354 4.1. <u>Soil characteristics</u>

355 Beyond the presence of century-old charcoal kiln sites, the study site was selected for its homogeneity

- 356 (REF soil) in terms of texture (sand = 7.5 ± 0.4%, silt = 76.9 ± 0.8 %, clay = 15.5 ± 0.7 %), C concentration
- 357 (1.4 ± 0.0 %), CEC (12.0 ± 0.6 cmol<sub>c</sub> kg<sup>-1</sup>) and pH (H2O 7.8 ± 0.2). On average, C concentrations in CHAR
- soils ([1.9 − 3.3] %) were 91 ± 34 % higher than in REF soils ([1.3 − 1.4] %) (p-val. < 0.01). Similarly,
- organic N concentrations were  $66 \pm 11 \%$  higher (p-val. < 0.001) in CHAR ([2.1 2.6] g kg<sup>-1</sup>) than in REF
- soils ([1.2 1.5] %). C, N concentrations as well as C:N ratios were more variable among replicates in the
- 361 CHAR soils than in the REF soils. The two studied soils were of identical texture (silty loam) and had
   362 similar pH values. No significant differences in CEC were reported. Bioavailable Ca<sup>2+</sup> concentrations were
- similar pH values. No significant differences in CEC were reported. Bioavailable Ca<sup>2+</sup> concent
   on average 26 ± 15 % higher in CHAR soils (p-val. < 0.05) (Table 1).</li>
- Table 1 Main bulk soil physico-chemical parameters. Means ± SD (n=5). When followed by \* results are significantly different
   (p.val in text) according to a student paired T-test.

	CHAR		REF
Sand (%)	7,7 ± 0,3		7,5 ± 0,4
Silt (%)	77,3 ± 1,1		76,9 ± 0,8
Clay (%)	15,0 ± 1,0		15,5 ± 0,7
C (%)	$2,6 \pm 0,5$	*	1,4 ± 0,0
Norg ( <sup>Z</sup> )	2,3 ± 0,2	*	1,4 ± 0,1
C/N (-)	11,3 ± 1,5		9,9 ± 0,6
CEC (cmolc kg <sup>-1</sup> )	14,0 ± 1,8		12,0 ± 0,6
pH (H2O)	7,7 ± 0,2		7,8 ± 0,2
pH (KCI)	7,0 ± 0,3		7,1 ± 0,2
P (mg 100g <sup></sup> 1)	7,3 ± 0,9		8,0 ± 0,5
K (mg 100g <sup></sup> ')	21,4 ± 2,9		18,2 ± 1,1
Mg (mg 100g <sup></sup> 1)	9,9 ± 1,0		8,6 ± 0,7
Ca (mg 100g <sup>-1</sup> )	314,4 ± 39,1	* :	251,6 ± 31,7

- 366
- 367

#### # Table 1: Main bulk soil physico-chemical parameters.

- 368 4.2. Weight and Carbon distribution amongst fractions
- 369 In CHAR soils macroaggregates contributed significantly more (p-val. < 0.01) to total soil weight than
- 370 microaggregates or free S&C fractions (CHAR<sub>MACRO</sub> = 48.9 ± 12.8, CHAR<sub>MICRO</sub> = 25.5 ± 8.3, CHAR<sub>Free S&C</sub>=
- 371 25.6 ± 4.6 g 100 g<sup>-1</sup> soil). In reference soils, macroaggregate and microaggregate fractions were equal
- and accounted significantly more to total soil weight (p-val. < 0.01) than the free S&C fraction (REF<sub>MACRO</sub>=
- 373 41.3 ± 9.5, REF<sub>MICRO</sub>= 36.0 ± 8.7, REF<sub>Free S&C</sub>= 22.7 ± 2.6 g 100 g<sup>-1</sup> soil)(Figure 3).
- The weight recovery following the wet sieving procedure was of 99.4 ± 0.3 % and 99.6 ± 0.1 % for CHAR
- and REF respectively. For step 4 of our fractionation protocol, 93.6 ± 4.8 % and 89.0 ± 7.5 % of initial
- mass were recovered (CHAR and REF respectively). For this step the C content recovery was of 87.9 ±

377 52.1 % (CHAR) and 100.6 ± 20.1 % (REF).

Macroaggregate-size free LF was the most abundant free LF in CHAR soils (0.7 ± 0.2 %) and was also
 significantly more abundant (p-val. < 0.05) in CHAR soils than in REF soils (Table 2). Micro and S&C-size</li>

- 380 free LF displayed similar contribution to the soil mass between the two studied soils (Table 2).
- 381 Furthermore, free LF displayed systematically higher C contents (i.e., fraction C concentration multiplied
- by fraction mass) in CHAR soils than in REF soils (Table 2) due mainly to higher C concentrations (i.e.,
- 383 mass of C per mass of the fraction). Macroaggregate size free LF of charcoal rich soils contributed the
- 384 most to the C content compared to all other free LF studied soils for both treatments.
- 385 While no differences between studied soils were observed, occluded LF contributed more to the total
- soil weight than free LF (Table 2). In both studied soils, microaggregate-size fractions accounted the
- 387 most to the total soil C content for both occluded LF and free LF. Microaggregate size occluded LF stored
- 388 twice the amount of carbon than microaggregate-size free LF (Table 2).
- 389

# Figure 3: Size class distribution.



390

Figure 3 – Size class distribution. Weight distribution amongst fractions of different sizes following wet sieving of the studied
 soils. Points represent the 5 field replicates. Symbols and box shadings highlight different fraction sizes. Weight recoveries (mean ± SD) are: CHAR= 99.4 ± 0.3 %, REF= 99.6 ± 0.1 %.

The macroaggregate protected HF was significantly more abundant than microaggregate protected and free HF in charcoal rich soils. In reference soils, macroaggregate and microaggregate protected HF,

- 396 which displayed the same contribution to the total soil mass, were more abundant than free HF (Table
- 2). The C concentrations of HF ranged between 0.7 ± 0.2 to 0.8 ± 0.2 % in reference soils and from 0.9 ±
- 398 0.1 to 1.0 ± 0.2 % in CHAR soils. In charcoal rich soils, macroaggregate protected HF (3.5 ± 1.2 g kg<sup>-1</sup> soil)
- 399 contributed more to the total soil C content than microaggregate protected HF and free HF ( $2.2 \pm 0.6$ ,
- 400 2.2 ± 0.3 g kg<sup>-1</sup> soil). In REF soils, macroaggregate and microaggregate protected HF accounted similarly

401 to the total soil C content, which was a greater contribution than free HF (Table 2).

Summing all fractions regardless of size into either LF or HF showed that although HF accounted for
 most of the soil mass (CHAR=89.0 ± 2.2, REF= 86.5 ± 1.0 %), it merely accounted for 39.0 ± 25.0 % of the

- 404 recovered soil C in CHAR soils (CHAR<sub>HF</sub> =  $7.9 \pm 1.4 \text{ g C kg}^{-1}$ ) and  $48.0 \pm 23.0 \%$  in reference soils (REF<sub>HF</sub> =
- 405  $6.8 \pm 1.2$  g C kg<sup>-1</sup>). In charcoal rich soils, LF (free and occluded combined) comprised  $12.2 \pm 3.1$  g C kg<sup>-1</sup>
- soil of which  $6.8 \pm 3.0$  g C kg<sup>-1</sup> soil occurred in occluded LF rather than free LF ( $5.4 \pm 0.7$  g C kg<sup>-1</sup> soil). In
- 407 reference soils, LF combined comprised 7.5  $\pm$  1.5 g C kg<sup>-1</sup> soil, mainly as occluded LF (REF<sub>occ.LF</sub> = 4.7  $\pm$  1.3 g
- 408 C kg<sup>-1</sup> soil) rather than as free LF (REF<sub>freeLF</sub> =  $2.8 \pm 0.7$  g C kg<sup>-1</sup> soil). C concentrations of free LF increased
- with aggregate size and ranged between  $25.6 \pm na$  and  $41.9 \pm 7.4$  % for charcoal rich soils and between
- 410  $10.0 \pm 2.9$  and  $36.7 \pm 10.1$  % in reference. Microaggregate occluded LF had higher C concentrations for
- both treatments (CHAR=  $31.1 \pm 5.4$ , REF =  $30.3 \pm 4.2$  %) than macroaggregate occluded LF (CHAR = 26.1
- 412 ± 2.4, REF= 17.7 ± 3.4 %) or S&C-size occluded LF (CHAR= 24.4 ± 4.2, REF= 21.3 ± 2.4 %)(Table 2).
- 413 Table 2 Elemental composition of fractions. Mass, C & N concentration, C & N content, C:N ratio and PyOM content (mean ±
- 414 SD) distribution amongst fractions as a function of studied soils (CHAR, REF), size and density (LF, HF). Free LF, occluded (occ.) LF
- 415 and HF were isolated from the three major aggregate size classes. Different letters indicate significant differences ( $p \le 0.05$ )
- 416 between studied soils as a function of fraction. "na" is indicated when not enough fraction mass was recovered for a complete
- 417 set of analysis. Weight recovery (mean ± SD) CHAR= 93.6 ± 4.8 %, REF= 89.0 ± 7.5 %. C content recovery CHAR = 87.9 ± 52.1 %,
- 418

REF = 100.6 ± 20.1 %.

		il size (μm)	mass		с		Ntot			с	N			C:N		Py	OM
frac.	SOIL		(g kg <sup></sup> 1 soil)		(%)		(‰)		(g kg <sup></sup> 1 soil)		(mg kg <sup></sup> 1 soil)			(-)		(g kg <sup>-1</sup> soil)	
		2000-250	6,7 ± 2,0	А	41,9 ± 7,4	А	15,3 ± 2,8	А	2,7 ±	0,4 A	98,4 ±	17,7	А	27,5 ± 3,0	Α	1,1 ±	0,7
		250-50	4,2 ± 1,5	В	36,5 ± 4,5	А	14,6 ± 1,4	AB	1,7 ±	0,5 B	67,2 ±	18,4	В	25,0 ± 1,5	A	0,3 ±	0,1
<i>.</i>	CHAR	< 50	3,8 ± 3,3	В	25,6 ± n	а	11,8 ± 2,8	В	1,0 ±	na	49,5 ±	0,0	BCD	21,5 ±	na	0,1 ±	na
free LF		2000-250	$39 \pm 0.7$	в	36.7 + 10.1	А	148 + 12	AB	1.5 +	0.6 BC	58.2 +	10.3	BC	25.0 + 7.6	А		
	REE	250-50	$26 \pm 0.08$	B	349 + 39	AB	$155 \pm 29$	A	1,0 +	0.3 CD	42.6 +	16.0	CD	$22.8 \pm 2.5$	A		
	INC.	< 50	$3,6 \pm 1,8$	В	10,0 ± 2,9	C	6,5 ± 2,2	С	0,3 ±	0,2 D	22,3 ±	12,0	D	15,7 ± 1,8	В		
		2000-250	78 + 64	FG	261 + 24	FG	179 + 16	GH	21+	18 EG	1424+	117.6	FG	146 + 08	FG	12+	na
		250-50	111 + 67	F	311 + 54	F	202 + 47	FG	38+	2.3 F	247 1 +	149.6	FG	157 + 15	F	02+	0.0
	CHAR	< 50	42 + 09	FG	244 + 42	G	$153 \pm 16$	н	10+	0.4 G	633+	18.3	G	$15,7 \pm 1,5$ $15.8 \pm 1.6$	F	0,2 ±	0,0
occ I F	OTIAN	4 00	4,2 ± 0,5	10	24,4 1 4,2	0	10,0 ± 1,0		1,0 ±	0,4 0	00,0 ±	10,5	0	10,0 1 1,0	'	0,0 ±	0,2
000. LI		2000-250	5,9 ± 2,4	FG	17,7 ± 3,4	н	13,7 ± 3,1	н	1,0 ±	0,5 G	82,0 ±	41,4	G	13,1 ± 1,4	GH		
	REF	250-50	9,1 ± 1,9	FG	30,3 ± 4,2	F	22,2 ± 4,4	F	2,7 ±	0,8 FG	200,2 ±	60,3	FG	13,9 ± 1,4	GH		
		< 50	4,1 ± 3,5	G	21,3 ± 2,4	GH	17,1 ± 1,9	GH	0,9 ±	1,0 G	70,1 ±	72,9	G	12,4 ± 0,7	н		
		2000-250	414.2 ± 127.6	J	0.9 ± 0.2	JK	0.9 ± 0.3	J	3.5 ±	1.2 J	403.6 ±	228.8	J	9.8 ± 2.4	к	0.3 ±	0.2
		250-50	221.6 ± 70.4	L	$1.0 \pm 0.2$	J	$0.8 \pm 0.2$	JKL	2.2 ±	0.6 KL	168.6 ±	33.4	KL	$13.1 \pm 3.2$	JK	0.2 ±	0.1
	CHAR	< 50	252.1 ± 56.1	JK	$0.9 \pm 0.1$	JK	$0.7 \pm 0.2$	JKL	2.2 ±	0.3 KL	183.5 ±	100.7	KL	$13.9 \pm 3.3$	J	0.4 ±	0.1
HF			,,.		-,,-		-,,-			-,	,-	,.				-,- =	- , -
		2000-250	337,5 ± 80,5	JK	0,8 ± 0,2	К	0,9 ± 0,4	JK	2,7 ±	1,0 JK	306,6 ±	168,9	JK	10,0 ± 2,8	К		
	REF	250-50	310,7 ± 79,6	JKL	0,8 ± 0,1	JK	0,6 ± 0,1	KL	2,6 ±	0,7 JK	178,3 ±	43,5	KL	14,5 ± 2,2	J		
		< 50	217,1 ± 25,0	L	0,7 ± 0,2	К	0,5 ± 0,2	L	1,5 ±	0,3 L	109,3 ±	25,8	L	14,0 ± 1,2	J		
h	and-picke	d PvOM			37.8 ± 7.9		5.4 ± 1.7							76.6 ± 27.4	ı		
	fresh P	VOM			79,6 ± 0.8		8,8 ± 0,6							90,6 ± 6.9			

419

#### 420

#### **#** Table 2: Elemental composition of fractions.

#### 421 4.3. <u>Differential Scanning Calorimetry</u>

422 Three reference materials and two bulk soils were analyzed to allow for the discrimination of various

423 forms of OM (Figure 4). Three distinct exotherms characterized hand-picked PyOM (top-Figure 4) with a

- 424 major peak at 443 ± 4 °C. Fresh PyOM was characterized by a single exotherm (485 ± 2 °C) and a higher
- 425 punctual heat flow. Hand-picked non-PyOM (crop residues) were characterized by a strong first
- 426 exotherm occurring at  $350 \pm 2$  °C and a second smaller at  $430 \pm 1$  °C.
- 427 The total heat flow of CHAR bulk soils were on average 84 ± 59 % greater than REF bulk soils (Table 4).
- 428 CHAR bulk soils were characterized by four exotherms occurring at temperatures similar to those
- 429 occurring in hand-picked PyOM and hand-picked non-PyOM combined (top-Figure 4). Similarly,

- exotherms of reference bulk soils occurred at similar temperatures to those which characterized hand-picked non-PyOM.
- 432 Figure 5 illustrates the thermograms of the three main types of fractions addressed in this study, the
- 433 free LF, occluded LF and HF (respectively row A, B, C Figure 5) as a function of the aggregate size class
- 434 in which they occur (col. 1: macroaggregate, col. 2: microaggregate, col. 3: free S&C-size Figure 5).
- 435 Thermograms of the free LF (Figure 5 A) showed more pronounced exotherms occurring between 400-
- 436 500 °C for CHAR than REF fractions whereas thermograms of REF fractions tended to be more spread
- 437 out throughout the thermal continuum than CHAR fractions. Nonetheless, 50 % burn-off temperatures
- 438 were not significantly smaller in REF than CHAR fractions (p-val. > 0.05). Finally, a decrease in total heat
- 439 flow as a function of fraction size was reported whereby heat flows of S&C-size free LF were significantly
- smaller (CHAR =  $232 \pm 60 \text{ Jg}^{-1}$ , REF =  $172 \pm 30 \text{ Jg}^{-1}$ ) than microaggregate-size free LF (CHAR =  $665 \pm 254 \text{ J}$
- 441  $g^{-1}$ , REF= 753 ± 160 J  $g^{-1}$ ), and macroaggregate-size free LF (CHAR = 821 ± 185 J  $g^{-1}$ , REF = 645 ± 262 J  $g^{-1}$ )
- 442 regardless of the studied soil (p-val. <0.01). However, total heat flows are equal between fractions when
- 443 normalized by their C content.
- The occluded LF (Figure 5 B) were characterized by two distinct exotherms visible as individual peaks or
- 445 marked shoulders occurring between 325-342 °C and 430-480 °C for both studied soils (Table 4). In
- 446 CHAR soils, the second exotherm (430-480 °C) had a higher relative importance as shown by the
- 447 increase of thermally stable:total peaks ratios by 42.6 % in macroaggregate occluded-LF, 44.3 % in
- 448 microaggregate occluded-LF and 50.0 % in S&C-size occluded-LF compared to REF soils (Table 4). No
- differences in 50 % burn-off temperatures were noted between studied soils within a size class yet
- 450 macroaggregate protected occluded LF have significantly smaller 50 % burn-off temperatures than
- 451 microaggregate protected or S&C occluded LF (p-val. < 0.05) (Table 4).

#### **#** Figure 4: DSC reference curves.



453

452

454 Figure 4 – DSC reference curves. DSC thermograms of reference material for the characterization of C forms. Top panel:

455 compares three types of OM forms, hand-picked (abbreviated h.p) non-PyOM, hand-picked PyOM and fresh PyOM. Bottom 456 panel: compares CHAR and REF bulk soils.

- 457 HF thermograms were characterized by a main exotherm occurring for both studied soils at ~323 °C.
- 458 CHAR fractions were differentiated by a marked shoulder in the DSC curves occurring at ~405 °C.
- 459 Thermograms of HF (Figure 5 C) were more systematic between repetitions as highlighted by the
- 460 average heat flow. The average heat flow occurring in HF was of  $187.1 \pm 42.3 \text{ Jg}^{-1}$  for CHAR soils and
- 461 147.5 ± 31.5 J g<sup>-1</sup> for REF soils where the standard deviation represented respectively 22.5 and 21.3 % of
- 462 the total heat flow. In occluded LF the average total heat flow was of 356.1 ± 159.0 J g<sup>-1</sup> for CHAR and
- 463 242.8 ± 147.1 J g<sup>-1</sup> for REF soils with the standard deviation representing 44.6 and 60.6 % for CHAR and
- 464 REF soils. In free LF, it represented 55.0 % for CHAR samples and 55.5 % in reference samples (CHAR<sub>free</sub>
- 465  $_{LF}$ =495.2 ± 272.6 J g<sup>-1</sup>, REF<sub>free LF</sub>=523.8 ± 291.228 J g<sup>-1</sup>). For HF, 50 % burn-off temperatures were always
- significantly higher for charcoal rich than reference soils (p-val. < 0.01) in all size classes and notably
- 467 smaller than in LFs (Table 4).

- 468 Finally, thermograms differed between hand-picked reference materials (Figure 4) and fractions isolated
- 469 by the fractionation protocol (Figure 5). Indeed the first (~386 ± 3 °C) and last (~ 520-528 °C) exotherms
- 470 of hand-picked PyOM (Figure 4) were not found in soil fraction thermograms (Figure 5).
- 471

#### **#** Figure 5: DSC signatures of soil fractions.



472

473 Figure 5 - DSC signatures of soil fractions. Graphs are presented as: *free LF* (A - top row) either macroaggregate-size (2000-250 μm), microaggregate-size (250-50 μm) or silt and clay size (50> μm). Middle (B) and bottom (C) rows show occluded LF or HF of
475 silt and clay size, either protected in macroaggregates or microaggregates or as free material. In each graph the three replicas
476 were represented, each one was plotted by pair with the adjacent samples of the opposite studied soil (CHAR/REF). Curves were
477 vertically shifted to facilitate the comparison between replicas. Vertical lines show the mean (n=3) 50 % burn-off temperature
478 per fraction (REF=dashed line, CHAR=solid line) and the horizontal line at the top of each graph represents the standard
479 deviation (REF= light grey, CHAR= dark grey).

#### 480 4.4. <u>Elemental analyses</u>

- 481 Aged hand-picked PyOM displayed much higher H:C (0.70 ± 0.10) and O:C (0.40 ± 0.10) atomic ratios
- than fresh PyOM (H:C =  $0.02 \pm 0.00$ ; O:C =  $0.10 \pm 0.00$ ) (Table 3). Differences in H:C and O:C ratios were
- 483 greater between soil fractions (free or occluded) than between CHAR and REF soils (Figure 6). Indeed,
- 484 free LF had lower ratios than occluded LF for both studied soils (Table 3). However, S&C-size free LF had
- 485 higher H:C and O:C ratios in REF soils than in CHAR soils. For occluded LF, differences in H:C ratios
- 486 between studied soils were not significant, and only O:C ratios of macroaggregate-size occluded LF were
- 487 greater in REF fractions than in CHAR fractions.

488

#### # Figure 6: Van Krevelen diagram of fractions.



489

490 Figure 6 - Van Krevelen diagram of fractions. Results show H:C and O:C atomic ratios of LF isolated by the fractionation protocol.

491 Squares illustrate free-LF (f-LF, encircled by solid line) and circles illustrate occluded LF (occ-LF, encircled by dashed line). The

492 point color depends on studied soils, white= REF, grey= CHAR. Crosses show ratios for fresh PyOM or hand-picked PyOM.

493 H:C atomic ratios increased from free LF, to occluded LF and HF for both studied soils (Table 3). For

494 microaggregate protected and free HF, H:C ratios were higher in REF (microaggregate protected HF: 3.22

 $\pm$  0.34, free HF: 4.24  $\pm$  0.39) than CHAR soils (microaggregate protected HF: 2.71  $\pm$  0.15; free HF: 3.44  $\pm$ 

496 0.43).

497 Inversely, C:N atomic ratios decreased from free LF ( $[15.7 \pm 1.8 - 27.5 \pm 3.0]$ ), to occluded LF ( $[12.4 \pm 0.7 \pm 1.8 - 27.5 \pm 3.0]$ ), to occluded LF ( $[12.4 \pm 0.7 \pm 1.8 - 27.5 \pm 3.0]$ ), to occluded LF ( $[12.4 \pm 0.7 \pm 1.8 + 27.5 \pm 3.0]$ ), to occluded LF ( $[12.4 \pm 0.7 \pm 1.8 + 27.5 \pm 3.0]$ ), to occluded LF ( $[12.4 \pm 0.7 \pm 1.8 + 27.5 \pm 3.0]$ ), to occluded LF ( $[12.4 \pm 0.7 \pm 1.8 + 27.5 \pm 3.0]$ ), to occluded LF ( $[12.4 \pm 0.7 \pm 1.8 + 27.5 \pm 3.0]$ ), to occluded LF ( $[12.4 \pm 0.7 \pm 1.8 + 27.5 \pm 3.0]$ ), to occluded LF ( $[12.4 \pm 0.7 \pm 1.8 + 27.5 \pm 3.0]$ ).

498 -  $15.8 \pm 1.6$ ]) and HF ([9.8 ± 2.4 - 14.5 ± 2.2]). In free LF, C:N ratios decreased as a function of size while

their values were similar in occluded LF of the three size classes. Furthermore, C:N ratios of HF were

500 lower in macroaggregate protected HF than in microaggregate protected and free HF materials (p-val. <

501 0.05), for both studied soils (Table 3).

502 Table 3 - H:C and O:C atomic ratios of soil fractions. Either free LF, occluded LF and HF as a function of treatment and of the size

503 classes in which they were contained. Different letters indicate significant differences ( $p \le 0.05$ ) between studied soils as a

504 function of fraction. "na" indicated that not enough fraction material was recovered for a complete set of analysis. No O:C ratios

505 were measured for mineral dominated fractions.

frac	soil	cizo (um)	H:C	0:C				
nac.	5011	size (µiii)	(-)	(-)				
	CHAR	2000-250	0,69 ± 0,11	С	0,30 ± 0,10 A			
free LF		250-50	$0,72 \pm 0,06$	С	0,31 ± 0,00 A			
		< 50	0,67 ± na	а	0,41 ± <i>na</i>			
		2000-250	0,95 ± 0,20	В	0,34 ± 0,00 A			
	REF	250-50	0,75 ± 0,13	С	0,41 ± 0,00 A			
		< 50	$1,55 \pm 0,11$	А	1,30 ± 0,50 B			
occ. LF		2000 250	1 0 4 + 0 10	F	0.20 + 0.04 - 0			
	CHAR REF	2000-250	$1,24 \pm 0,12$	F FO	$0.39 \pm 0.04$ G			
		250-50	$1,19 \pm 0,20$	FG	$0,44 \pm 0,03$ G			
		< 50	1,01 ± 0,12	G	0,38 ± 0,01 G			
		2000-250	1,32 ± 0,20	F	0,85 ± 0,00 F			
		250-50	1,31 ± 0,12	F	0,37 ± 0,00 G			
		< 50	1,18 ± 0,14	FG	$0,39 \pm 0,05$ G			
HF		2000-250	3 14 + 0 37	ĸı				
	CHAR	2000-200	$3,14 \pm 0,37$					
		200-00	$2,71 \pm 0,13$					
		< 50	$3,44 \pm 0,43$	ĸL				
	REF	2000-250	$3,58 \pm 0,50$	К				
		250-50	$3,22 \pm 0,39$	Κ				
		< 50	$4,24 \pm 0,39$	J				
hand	l-picked	РуОМ	0,70 ± 0,10		0,40 ± 0,10			
f	resh Py	ОМ	$0.02 \pm 0,00$		0,10 ± 0,00			

506

507

#### # Table 3: H:C and O:C atomic ratios of soil fractions.



509 Amongst the different fractions of both studied soils, H:C atomic ratios decreased (Table 3) alongside an 510 increase in stable:total heat flow ratios (Table 4). HF (i.e., S&C minerals) were characterized by most of 511 the heat flow occurring at low temperatures as opposed to LF. While H:C ratios were similar between 512 studied soils, HF of CHAR soils displayed higher stable:total heat flows than HF of REF soils (CHAR = 24.0 ± 3.0, REF = 16.1 ± 2.5 %). On average in CHAR soils, free LF showed lower H:C and higher stable:total 513 ratios (H:C =  $0.69 \pm 0.09$ ; stable:total =  $76.6 \pm 11.1$  %) than for occluded LF (H:C =  $1.14 \pm 0.16$ ; 514 stable:total = 68.2 ± 7.8 %) whereas in REF soils, average H:C ratios for occluded fractions were lower 515 516 than for free LF (occ LF= 1.27 ± 0.15, free LF= 1.08 ± 0.44) while stable:total ratios were similar (occ LF=

517 63.2 ± 4.8, free LF= 64.1 ± 6.2 %)(Table 4)

#### 518 4.6. PyOM and non-PyOM quantification in charcoal rich soils

- 519 Charcoal rich bulk soils contained between 5.8 and 11.9 g kg<sup>-1</sup> soil of PyOM. This amounts for between
- 520 29.9 and 41.3 % of the total SOC in bulk CHAR soils. In free LF, the PyOM accounted between 13.7 and
- 521 41.2 % of the total SOC in these fraction. Most of the PyOM was found as macroaggregate-size free LF
- 522 and accounted for up to 1.1 ± 0.7 g kg<sup>-1</sup> soil (Table 2). Similar amounts of PyOM were found in occluded
- 523 LF and accounted between 5.4 and 55.9 % of total SOC contained in occluded fractions. HF contained
- between 0.2  $\pm$  0.1 and 0.4  $\pm$  0.1 g kg<sup>-1</sup> soil of PyOM. This represented between 7.3 and 16.2 % of the SOC
- 525 contained in HF and was highest for free HF ( $0.4 \pm 0.1 \text{ g kg}^{-1}$  soil).
- 526 A higher heat of reaction occurring at thermally labile temperatures in CHAR soils than REF soils show
- 527 that differences in C content did not solely occur as thermally stable OM but also as labile PyOM or non-
- 528 PyOM. Bulk charcoal rich soils accounted 0.4 to 2.3 g C kg<sup>-1</sup> soil of additional thermally labile OM
- 529 compared to reference soils. This represented between 5.8 and 16.2 % of the additional C content of
- 530 CHAR soils. The combined heat flows of occluded LF were on average 38.7 % higher in CHAR than REF
- 531 fractions (CHAR<sub>occ</sub> =  $9.8 \pm 4.3 \text{ W g}^{-1}$ , REF<sub>occ</sub> =  $7.1 \pm 1.8 \text{ W g}^{-1}$ ) although non-significantly different due to
- the variability between CHAR repetitions. However, an increase in thermally labile peak height was not
- 533 systematic between samples and hence not statistically significant for occluded LF (p-val. > 0.05) nor HF
- 534 (p-val. > 0.05).

Table 4 - DSC curve characteristics of soil fractions. 50 % burn-off T, peak T and heat flows of visible exotherms as well as ratios
 between labile:total and stable:total heat flow (mean ± SD) are represented followed by results of analysis of variance. For a
 given fraction (frac.) identical letters indicate equal means.

					exother	m 1	exother	m 2				
6			50% Burno	ff T	peak T	Heat flow	peak T	Heat flow	Labile : Total	Stable : Total		
frac.	SOII	size (µm)	(°C)		(°C)	(W g <sup>-1</sup> )	(°C)	(W g <sup>-1</sup> )	Heat flow (%)	Heat flow (%)		
free LF	CHAR	2000-250	436,3 ± 35,9	AB			442,3 ± 38,6 A	22,9 ± 3,7 A	28,5 ± 15,2 BC	72,0 ± 14,9 AB		
		250-50	443,3 ± 33,5	5 AB			448,7 ± 24,0 A	16,7 ± 5,6 AB	24,2 ± 13,3 C	76,2 ± 13,0 A		
		< 50	462,3 ± 3,1	А			471,0 ± 2,0 A	6,8 ± 2,6 C	22,6 ± 1,0 C	77,5 ± 1,0 A		
	REF	2000-250	413,5 ± 21,9	9 В			442,3 ± 38,6 A	11,0 ± 7,3 BC	41,2 ± 7,5 B	59,3 ± 7,4 B		
		250-50	439,8 ± 9,9	AB			454,0 ± 6,2 A	10,5 ± 3,5 BC	32,4 ± 2,4 BC	67,9 ± 2,4 AB		
		< 50	446,8 ± 23,2	2 AB			483,0 ± 4,4 A	3,2 ± 0,6 C	34,0 ± 6,2 BC	66,2 ± 6,2 AB		
occ. LF	CHAR	2000-250	414,3 ± 16,6	6 G	335,0 ± 1,4 F	3,8 ± 3,7 F	449,7 ± 15,0 G	6,7 ± 6,3 F	40,8 ± 6,7 FG	59,5 ± 6,8 GH		
		250-50	458,3 ± 2,0	F	328,7 ± 1,5 H	3,0 ± 0,4 F	469,7 ± 4,6 F	8,5 ± 3,1 F	26,6 ± 2,8 H	73,6 ± 2,8 F		
		< 50	449,5 ± 18,8	3 F	342,0 ± 0,0 F	3,0 ± 2,1 F	472,3 ± 5,7 F	7,9 ± 5,0 F	28,7 ± 7,0 H	71,5 ± 6,9 F		
	REF	2000-250	418,0 ± 20,3	2 G	335,0 ± 1,4 F	2,5 ± 0,4 F	471,7 ± 1,2 F	2,9 ± 0,8 F	42,8 ± 5,4 F	57,5 ± 5,4 H		
		250-50	447,3 ± 8,8	F	332,0 ± 4,0 G	3,5 ± 1,7 F	464,3 ± 8,3 F	6,8 ± 3,2 F	31,7 ± 1,5 GH	68,5 ± 1,5 FG		
		< 50	441,7 ± 18,9	9 FG	342,0 ± 0,0 F	1,1 ± 0,5 F	475,7 ± 4,0 F	2,1 ± 1,3 F	38,8 ± 6,2 FG	61,4 ± 6,2 GH		
HF	CHAR	2000-250	338,2 ± 7,8	JK	324,0 ± 1,7 K	0,4 ± 0,1 J	405,0 ± 0,0	0,2 ± 0,0 JKL	76,9 ± 4,6 KL	23,5 ± 4,6 JK		
		250-50	341,8 ± 3,8	J	329,7 ± 2,1 J	0,4 ± 0,1 J	405,0 ± 0,0	0,2 ± 0,0 JK	77,0 ± 4,2 KL	23,4 ± 4,2 JK		
		< 50	339,2 ± 4,2	J	322,0 ± 4,4 K	$0,4 \pm 0,0 J$	405,0 ± 0,0	$0,2 \pm 0,0 J$	75,3 ± 2,2 L	25,1 ± 2,2 J		
	REF	2000-250	330,8 ± 3,4	KL	322,3 ± 1,5 K	0,4 ± 0,1 J	405,0 ± 0,0	0,2 ± 0,0 KL	81,7 ± 2,7 JK	18,6 ± 2,7 KL		
		250-50	327,0 ± 2,6	KL	319,0 ± 3,6 K	0,4 ± 0,0 J	405,0 ± 0,0	0,1 ± 0,0 L	85,3 ± 1,2 J	15,1 ± 1,2 L		
		< 50	325,2 ± 2,6	KL	319,3 ± 3,8 K	$0,4 \pm 0,1 J$	405,0 ± 0,0	0,1 ± 0,0 L	85,6 ± 1,6 J	14,7 ± 1,6 L		

538

# Table 4: DSC curve characteristics of soil fractions.

## 540 5. Discussion

#### 541 5.1. <u>Physico-chemical characteristics of charcoal rich soils</u>

542 Our results show close to a twofold increase in C contents as a result of century-old PyOM accumulation 543 in cultivated soils as previously reported for similar sites (Hernandez-Soriano et al., 2016b; Kerré et al., 544 2016; Hardy et al., 2017a). As both studied soils were sampled in the same field, they have received 545 similar amendments and field works in identical climatic conditions. We can therefore be confident such 546 an increase in C content is induced by the presence of PyOM and corresponds to increases in both PyOM 547 and non-PyOM contents in kiln soils. Accumulation of non-PyOM in kiln soils have been previously 548 shown to originate from a decreased mineralization rates of recent non-PyOM (Hernandez-Soriano et 549 al., 2016a; Kerré et al., 2016) and from the adsorption of dissolved organic compounds onto charcoal 550 particles (Hernandez-Soriano et al., 2016b; Kerré et al., 2017). A relative greater aboveground biomass 551 production on kiln soils (Heidarian Dehkordi et al., 2020) may also be responsible for a positive feedback 552 loop through an increase in non-PyOM inputs to the charcoal rich soils.

553 Previous studies on similar kiln sites in Wallonia reported significant increases in CEC related to the

presence of charcoal (Kerré et al., 2016; Hardy et al., 2017a). Our results show slight yet non-statistically

555 significant increases. Furthermore, higher CEC has been estimated for century-old PyOM fragments

556 (PyOM= 414 cmol<sub>c</sub> kg<sup>-1</sup>) compared to non-PyOM (non-PyOM = 213 cmol<sub>c</sub> kg<sup>-1</sup>) explained by an increase in

the amounts of functional groups with charcoal ageing and cultivation combined with the high specific

surface area of PyOM (Hardy et al., 2017a). Additionally, CHAR soils displayed greater bioavailable Ca<sup>2+</sup>
 contents than adjacent REF soils as previously reported (Hardy et al., 2017a, 2017b). Increased Ca<sup>2+</sup>

560 contents in CHAR soils result from the high affinity between Ca<sup>2+</sup> and carboxylate groups found on aged

561 PyOM thus potentially serving as a mediator for PyOM interaction with soil constituents (Kalinichev and

562 Kirkpatrick, 2007).

563 5.2. Element C:N, H:C and O:C ratios support charcoal-induced organo-mineral associations 564 The presence of PyOM in charcoal enriched soils resulted in a greater macroaggregate formation at the 565 expense of free microaggregates in comparison to reference soils. Macroaggregate turnover is known to 566 be relatively rapid particularly in cropped ecosystems where tillage damages soil structure (Six et al., 567 2000; Chenu et al., 2019). In CHAR soils, where macroaggregates are more abundant than in REF soils, it 568 is likely that PyOM acts as an additional binding agent between soils constituents and microaggregates 569 (Six et al., 2000). Furthermore, a larger macroaggregate fraction in CHAR soils may also result from 570 higher aggregate stability (Pituello et al., 2018) caused by increased inter-particular cohesion (Sun and 571 Lu, 2014) or faster aggregate formation as a result of the specific surface area of PyOM. Century-old 572 PyOM therefore improves soil aggregation which is a key property for soil structure, essential in 573 maintaining soil fertility and which may also further increase the stability of OM through increased 574 physical disconnection with decomposers (Schmidt et al., 2011; Lehmann and Kleber, 2015).

575 Our results showed significant differences in O:C and H:C ratios between free and occluded LF for both

576 studied soils thereby showing that the position of PyOM and non-PyOM in the soil matrix and their

577 chemical composition are strongly linked. Lower O:C and H:C atomic ratios suggest a greater abundance

of large aromatic structures in free LF than in occluded LF whereas the latter may be enriched in either

579 PyOM with more functional entities such as carboxyl groups or additional non-PyOM (Brodowski et al.,580 2005; Cheng et al., 2008a).

581 Long-term residence of PyOM in soils results in higher O:C and H:C atomic ratios (Cheng et al., 2008a; Nguyen et al., 2008; Hardy et al., 2017b) compared to fresh PyOM (Hardy et al. 2017b). Ageing of PyOM 582 583 is known to occur mainly through oxidation of the particles and results in the development of phenol, 584 carboxyl and carbonyl functional groups on the PyOM aromatic structures (Cheng et al., 2008a). 585 Although free LF are highly weathered compared to fresh PyOM, their lower H:C and O:C ratios in 586 comparison to occluded LF suggests free LF may be less oxidized than occluded LF. In turn, this higher 587 functionalization of PyOM in occluded LF compared to free LF supports its increased reactivity towards 588 surrounding soil constituents. As all the PyOM in CHAR soils is of similar age range and as most of the oxidation occurs rapidly following the addition of PyOM to the soil, it is expected that particles would 589 590 have faced similar oxidative agents (Nguyen et al., 2008). Therefore, differences in O:C and H:C ratios 591 between free and occluded LF may originate from products with varying initial properties. Indeed, slight 592 changes in pyrolysis conditions throughout the kiln during charcoal production may result in varying 593 degrees of aromaticity or aromatic condensation (B. P. Singh et al., 2012; Wiedemeier et al., 2015) 594 resulting in different degrees of hydroxylation and carboxylation (Lehmann et al., 2005) and in turn 595 leading to charcoal fragments of varying persistence. However, it may be that when first produced, the 596 PyOM was physically and chemically homogeneous in composition and that, with time and farming 597 practices, the PyOM fragments were broken down to different size classes which resulted in different 598 degrees of oxidation. In addition, higher H:C and O:C atomic ratios of occluded LF than free LF in 599 charcoal enriched soils may also originate from higher amounts of non-PyOM such as microbial related 600 biomass (Lehmann et al., 2011) or stabilized fresh non-PyOM residues (Hernandez-Soriano et al., 2016a). 601 In HF, differences in H:C and O:C ratios result either from mineral phases or changes in OM. However, as 602 texture and mineralogy are similar throughout the study site, differences in H:C and O:C ratios between 603 studied soils are mainly attributed to the presence of PyOM. Lower H:C ratios in HF of charcoal enriched 604 soils than in reference soils demonstrate the presence of PyOM (C-rich) associated with minerals 605 through S&C-size microaggregation or adsorption.

606 Similar C:N ratios between studied soils, despite generally higher C content in CHAR, indicate that larger 607 amounts of N occur in charcoal enriched soils than in adjacent REF soils. This may result from 608 significantly higher nitrate contents retained by the biochar (Hagemann et al., 2017) or N-rich organic 609 compounds derived from root and microbial activities (Brodowski et al., 2005; Meier et al., 2017). 610 Indeed, microbial-derived proteins and root exudates are known to strongly bind to minerals with 611 possibilities for their polar chains to serve as binding sites (Kleber et al., 2007; Vogel et al., 2014; Poirier 612 et al., 2018) and to interact with PyOM (Brodowski et al., 2005). In HF this is supported by C:N ratios 613 similar to those expected for microbial-derived OM sorbed onto mineral surfaces ranging [7-14]

614 (Aufdenkampe et al., 2001; Bell et al., 2014).

#### 615 5.3. <u>Thermal analysis confirms organo-mineral associations</u>

- 616 The presence of charcoal in the bulk soil of kiln sites is distinguished from reference soils by three
- 617 distinct exotherms (Figure 4). These exotherms occur at higher temperatures than in reference
- 618 thermograms and correspond to peak temperatures characterizing hand-picked charcoal particles

619 (Hardy et al., 2017a). At first, fresh PyOM is characterized by a single sharp exotherm. With time, as the

- 620 PyOM particle oxidizes from its surface inwards (Brodowski et al., 2005; Cheng et al., 2008a) the
- aromatic structures are broken down and functional groups appear (Plante et al., 2009). The occurrence
- of these functional groups decreases the thermal stability of PyOM as shown by the occurrence of a new
- exotherm at ~386 °C (Figure 4), which corresponds to the combustion of O-rich compounds (Hardy et al.,
  2017b). Hardy et al. (2017b) suggested that Ca<sup>2+</sup> acted as a thermal catalyst thereby resulting in a
- 2017b). Hardy et al. (2017b) suggested that Ca<sup>2+</sup> acted as a thermal catalyst thereby resulting in a
   decrease of thermal stability of functional groups visible in thermograms as a decrease in peak
- 626 temperatures towards more thermally labile OM. Interestingly, hand-picked PyOM is characterized by
- 627 the occurrence of a third peak (~528 °C) with higher thermal stability (Figure 4) than fresh PyOM as
- 628 previously reported by Hardy et al. (2017b). In this study authors showed an increase in this peak height
- 629 with time of cultivation thereby suggesting that amounts of highly thermally stable C increase relatively
- to other, less stable forms which are preferentially altered or lost from the system. The third type of OM
- used as a reference curve is hand-picked non-PyOM which is characterized by the occurrence of both
- 632 thermally labile (336  $\pm$  2 °C) and thermally stable (442  $\pm$  2 °C) OM (Figure 4). These peak temperatures
- 633 correspond to those of cellulose and lignin like molecules (Lopez-Capel et al., 2005). The occurrence of
- these exotherms in both studied soils highlight that PyOM in soils can be determined as the additional
- heat flow occurring at thermally stable temperatures in CHAR as opposed to REF soils.
- 636 In both studied soils, LF (i.e. occ. or free) are characterized by higher thermal stability than HF. Free LF in 637 CHAR soils are characterized by a punctual heat flow comparable to that of pristine fresh PyOM which 638 suggests little functionalization of PyOM particles in these fractions. Such a recalcitrance to oxidation for 639 CHAR free LF is explained by a greater aromaticity or degree of condensation of the PyOM (Wiedemeier 640 et al., 2015). Thermograms of REF free LF on the other hand show more diversity in the type of OM composing the fraction as shown by a multitude of peaks. In agricultural soils, after biomass inputs, it 641 642 may be expected that free LF would be quickly deprived of small and energy rich forms of OM due to 643 rapid mineralization of thermally labile organic compounds more beneficial to decomposers (Leifeld, 644 2008; Rovira et al., 2008; Barré et al., 2016). As a result, following the preferential decomposition of 645 energy rich OM, free LF in CHAR soils are expected to be dominated by larger more persistent molecules 646 with higher binding energy and hence to be thermally more stable (Leinweber et al., 1992; Lopez-Capel 647 et al., 2005; De la Rosa et al., 2008). This is supported by higher 50 % burn-off temperatures in free LF 648 compared to other fractions (Leifeld, 2008)(Figure 5).
- 649 Occluded LF differ from free LF through the appearance of a second class of exotherms occurring around 650 ~330 °C (Figure 5). The occurrence of this thermally labile peak suggests an accumulation of energy-rich 651 OM resulting from physical inaccessibility of this OM to decomposers and organo-mineral associations 652 (Lehmann and Kleber, 2015). In occluded LF of REF soils significant heat flows occur at high thermal 653 stability. These exotherms coincide with peak temperatures attributed to PyOM (Figure 5) which 654 complicates a definite distinction between PyOM and non-PyOM (Leifeld, 2007). Such exotherms 655 highlight the importance of studying CHAR soils in comparison to thermograms of REF soils to determine 656 the PyOM pool as the additional heat flow occurring in CHAR soils. In CHAR occluded LF the presence of 657 PyOM is mainly underlined by a slightly higher 50 % burn-off temperatures (Figure 5) and higher
- 658 stable:total heat flow ratios (Table 4).

In HF the presence of an elbow occurring at ~410°C in CHAR and not in REF soils strongly suggests

- the presence of PyOM tightly associated with minerals. Indeed, although these fractions are dominated
   by thermally labile OM (Figure 5 C peaks ~330 °C), the sustained presence of PyOM in fractions
- 662 dominated by microaggregation and adsorption suggests that century-old charcoal displays strong
- 663 interactions with soil minerals (Chenu and Plante, 2006; Virto et al., 2010). The decrease in peak
- temperatures of PyOM originates from the creation of phenol, carbonyl and carboxyl functionalities at
- the surface of century-old charcoal particles (Hardy et al., 2017a). Our results support this assumption as
- this peak value in HF corresponding to PyOM (~410 °C) occurs between the two first peaks of hand-
- picked PyOM (p1 =  $386 \pm 2$  °C, p2 =  $443 \pm 4$  °C). Furthermore, because functional groups are needed for
- sorption to occur (Kleber et al., 2015) it is expected that PyOM particles tightly bound to mineral phases
- have been strongly oxidized through either biotic or abiotic processes (Cheng et al., 2006). As
- 670 microaggregation and adsorption are the main stabilization mechanisms for OM in soils, it is expected
- that beyond its chemical recalcitrance, PyOM is further stabilized by organo-mineral associations (von
- Lützow et al., 2006; Dungait et al., 2012; Lehmann and Kleber, 2015) yet that this additional physical
- 673 protection is not discernable by thermal analyses (Plante et al., 2011).

#### 5.4. <u>A major part of PyOM is associated with mineral phases</u>

- 675 DSC results show PyOM amounts for an important part of the total SOC [29.9 - 41.3 %] in bulk charcoal 676 rich soils. Due to the variability in chemical composition of PyOM no single method can correctly 677 quantify it (Lorenz and Lal, 2014; Kerré et al., 2016). Our methodology consisted in estimating the 678 amount of PyOM by comparing peak height ratios between CHAR and adjacent REF soil with respect to 679 differences in C content and is therefore not a direct quantification. Furthermore, our quantification 680 approach is developed on the assumption that no PyOM is found in REF soils. However, it may be 681 expected that small amounts of PyOM could be found in reference soils suggesting PyOM amounts are 682 slightly underestimated in charcoal rich soils. Using standard additions of PyOM to mineral soils free of 683 PyOM, Hardy et al., (2017a) reported that charcoal-C content in kiln sites amounts for 79.5 % of the 684 difference in C content between CHAR soils and REF soils. Using this relationship on our charcoal rich 685 soils we obtain an estimate of PyOM content ranging [25.3 – 39.2 %], slightly lower than our estimate 686 [29.9 - 41.3 %]. Using DSC on isolated soil fractions enabled an understanding of the distribution of 687 PyOM amongst soil fractions. Results show that after two centuries of residence in soils, PyOM is 688 located in all soil fractions and that, although a large amount occurred as free LF (43.6 ± 22.9 % of total 689 PyOM), the majority interacts with mineral phases ( $56.4 \pm 22.9$  % of total PyOM) through occlusion (29.6
- 690 ± 28.8 % of total PyOM) or sorption (26.78 ± 5.9 % of total PyOM) to mineral phases (Table 2).
- Despite decades of incorporation in the soil matrix and sustained farming practices, results show that a 691 692 large amount of PyOM is still found as free LF in CHAR soils. This result illustrates the importance of 693 PyOM composition for its stability in soils and the role of charring against oxidative agents through its 694 polycondensed aromatic structure (Wiedemeier et al., 2015) and against decomposers by making it a 695 hardly decomposable form of OM (Rovira et al., 2008; Wang et al., 2016). Nonetheless, in the presence 696 of specific decomposers, PyOM may decompose faster than often reported (Hamer et al., 2004). Recent 697 studies show that the persistence of PyOM in soils may be overestimated (Hammes et al., 2008; N. Singh 698 et al., 2012; Lehndorff et al., 2014; Lutfalla et al., 2017). Nonetheless, given the high temperatures and

- low oxygen contents found in kiln sites, it may be expected that the stability of the charcoal produced in
- such environment would be higher than wildfire residues (Bird et al., 2015). In the studied CHAR soils,
- the presence of PyOM amongst occluded LF and OM in the HF shows that its stability is not exclusively
- 702 explained by its chemical structure but further enhanced by a decreased accessibility for
- 703 microorganisms (Brodowski et al., 2006; Lützow et al., 2006). Charcoal-mineral associations likely
- 704 increase with PyOM ageing through increasing proportions of functional groups on the charcoal surfaces
- as well as the occurrence of biotic or abiotic binding agents (Brodowski et al., 2006; Kögel-Knabner et al.,
- 2008; Kopittke et al., 2020). Finally, our results show increased amounts of non-PyOM in charcoal rich
- soils which suggests PyOM may influence the stability of non-PyOM in soils by serving as a hotspot for its
- sorption onto already existing organo-mineral clusters (Kopittke et al., 2020) or by creating
- microaggregates in which it becomes physically inaccessible to decomposers.

## 710 6. Conclusion

711 Our work combined for the first time thermal and elemental analyses on soil fractions extracted from a

- vunique setting where PyOM from kiln sites was incorporated in conventionally cropped systems for over
- 713 two centuries.
- The C content of CHAR soils was significantly higher than that of adjacent REF soils thus confirming the
- potential of PyOM as a tool for C storage over centuries. Furthermore, our results show that such
- 716 increases in C content were not solely attributed to increases in PyOM content, but also to additional
- non-PyOM accumulation in CHAR soils. The presence of PyOM in all soil fractions showed that after
- centuries of incorporation in strongly anthropogenized environments PyOM still stands as free LF yet
- also strongly associates with mineral phases. This association occurs through occlusion into aggregates
- and sorption of PyOM onto mineral surfaces. Compared to fresh PyOM, century-old PyOM isolated from
- 721 charcoal rich soils showed higher H:C and O:C atomic ratios particularly in occluded LF vouching for a
- functionalization of the PyOM. As such its reactivity with other soil constituents increases as suggested
- by increased Ca<sup>2+</sup> concentrations in CHAR soils. Furthermore, similar C:N ratios in CHAR and REF
- 724 fractions suggest increased amounts of N-rich organic products in charcoal rich soils which probably
- serve as binding agents between PyOM and mineral phases. As a result of more binding sites, we
- observed increased amounts of water stable macroaggregate in CHAR soils. The related improved soil
- structure in charcoal rich soils is fundamental in maintaining fundamental ecosystems services such as
- 728 diminished erosion and enhanced plant primary production.
- 729 Our results highlight that beyond its chemical composition, PyOM is further stabilized by organo-mineral
- associations and can not only serve as a C sequestration tool but also as a mean to improve the soil
- 731 quality of conventionally cropped agroecosystems in temperate Luvisols.

## 732 7. Acknowledgments

- 733 We would like to thank colleagues from the water-soil-plant exchange lab and in particular F. de
- 734 Tombeur for their help and guidance during the soil fractionation phase. We also thank Robin Giger
- from Agroscope in Zurich for his help and guidance using the DSC and the elemental analyzer. Access to
- the research site was granted by Alexandre Godfrind who's help and availability we acknowledge. We
- thank colleagues from the CHAR project for the fruitful scientific discussions and the support during field
- work. This research was funded as a Concerted Research Action (ARC) by the French Community of
- 739 Belgium and written within the CHAR research project.

## 740 8. <u>References</u>

- Amelung, W., Zech, W., 1999. Minimisation of organic matter disruption during particle-size
  fractionation of grassland epipedons. Geoderma 92, 73–85. https://doi.org/10.1016/S00167061(99)00023-3
- Aufdenkampe, A.K., Hedges, J.I., Richey, J.E., Krusche, A. V., Llerena, C.A., 2001. Sorptive fractionation of
   dissolved organic nitrogen and amino acids onto fine sediments within the Amazon Basin. Limnol.
   Oceanogr. 46, 1921–1935. https://doi.org/10.4319/lo.2001.46.8.1921
- Awad, Y.M., Blagodatskaya, E., Ok, Y.S., Kuzyakov, Y., 2013. Effects of polyacrylamide, biopolymer and
   biochar on the decomposition of 14 C-labelled maize residues and on their stabilization in soil
   aggregates. Eur. J. Soil Sci. 64, 488–499. https://doi.org/10.1111/ejss.12034
- Barré, P., Plante, A.F., Cécillon, L., Lutfalla, S., Baudin, F., Bernard, S., Christensen, B.T., Eglin, T.,
  Fernandez, J.M., Houot, S., Kätterer, T., Le Guillou, C., Macdonald, A., van Oort, F., Chenu, C., 2016.
  The energetic and chemical signatures of persistent soil organic matter. Biogeochemistry 130.
  https://doi.org/10.1007/s10533-016-0246-0
- Baveye, P.C., 2020. Bypass and hyperbole in soil research: Worrisome practices critically reviewed
   through examples. Eur. J. Soil Sci. 1–20. https://doi.org/10.1111/ejss.12941
- Baveye, P.C., 2014. The Characterization of Pyrolysed Biomass Added to Soils Needs to Encompass Its
  Physical And Mechanical Properties. Soil Sci. Soc. Am. J. 78, 2112–2113.
  https://doi.org/10.2136/sssaj2014.09.0354l
- Bell, C., Carrillo, Y., Boot, C.M., Rocca, J.D., Pendall, E., Wallenstein, M.D., 2014. Rhizosphere
  stoichiometry: are C : N : P ratios of plants, soils, and enzymes conserved at the plant species-level?
  New Phytol. 201, 505–517. https://doi.org/10.1111/nph.12531
- Biederman, L.A., Harpole, S.W., 2013. Biochar and its effects on plant productivity and nutrient cycling: A
   meta-analysis. Glob. Chang. Biol. Bioenergy 5, 202–214. https://doi.org/10.1111/gcbb.12037
- Bird, M.I., Wynn, J.G., Saiz, G., Wurster, C.M., McBeath, A., 2015. The Pyrogenic Carbon Cycle. Annu.
   Rev. Earth Planet. Sci. 43, 273–298. https://doi.org/10.1146/annurev-earth-060614-105038

# Borchard, N., Ladd, B., Eschemann, S., Hegenberg, D., Möseler, B.M., Amelung, W., 2014. Black carbon and soil properties at historical charcoal production sites in Germany. Geoderma 232–234, 236–

- 768 242. https://doi.org/10.1016/j.geoderma.2014.05.007
- Brodowski, S., Amelung, W., Haumaier, L., Abetz, C., Zech, W., 2005. Morphological and chemical
   properties of black carbon in physical soil fractions as revealed by scanning electron microscopy
- and energy-dispersive X-ray spectroscopy. Geoderma 128, 116–129.
- 772 https://doi.org/10.1016/j.geoderma.2004.12.019
- Brodowski, S., John, B., Flessa, H., Amelung, W., 2006. Aggregate-occluded black carbon in soil. Eur. J.
  Soil Sci. 57, 539–546. https://doi.org/10.1111/j.1365-2389.2006.00807.x
- Cheng, C.-H., Lehmann, J., Engelhard, M.H., 2008a. Natural oxidation of black carbon in soils: Changes in
   molecular form and surface charge along a climosequence. Geochim. Cosmochim. Acta 72, 1598–
   1610. https://doi.org/10.1016/j.gca.2008.01.010
- Cheng, C.-H., Lehmann, J., Thies, J.E., Burton, S.D., 2008b. Stability of black carbon in soils across a
  climatic gradient. J. Geophys. Res. Biogeosciences 113, 1–10.
  https://doi.org/10.1029/2007JG000642
- Cheng, C.H., Lehmann, J., 2009. Ageing of black carbon along a temperature gradient. Chemosphere 75,
   1021–1027. https://doi.org/10.1016/j.chemosphere.2009.01.045
- Cheng, C.H., Lehmann, J., Thies, J.E., Burton, S.D., Engelhard, M.H., 2006. Oxidation of black carbon by
  biotic and abiotic processes. Org. Geochem. 37, 1477–1488.
  https://doi.org/10.1016/j.orggeochem.2006.06.022
- Chenu, C., Angers, D.A., Barré, P., Derrien, D., Arrouays, D., Balesdent, J., 2019. Increasing organic stocks
   in agricultural soils: Knowledge gaps and potential innovations. Soil Tillage Res. 188, 41–52.
   https://doi.org/10.1016/j.still.2018.04.011
- Chenu, C., Plante, A.F., 2006. Clay-sized organo-mineral complexes in a cultivation chronosequence:
   revisiting the concept of the "primary organo-mineral complex." Eur. J. Soil Sci. 57, 596–607.
   https://doi.org/10.1111/j.1365-2389.2006.00834.x
- Crane-Droesch, A., Abiven, S., Jeffery, S., Torn, M.S., 2013. Heterogeneous global crop yield response to
  biochar: a meta-regression analysis. Environ. Res. Lett. 8, 044049. https://doi.org/10.1088/17489326/8/4/044049
- 795 De la Rosa, J.M., Knicker, H., López-Capel, E., Manning, D.A.C., González-Perez, J.A., González-Vila, F.J.,
- 796 2008. Direct Detection of Black Carbon in Soils by Py-GC/MS, Carbon-13 NMR Spectroscopy and
- 797 Thermogravimetric Techniques. Soil Sci. Soc. Am. J. 72, 258–267.
- 798 https://doi.org/10.2136/sssaj2007.0031
- Dell'Abate, M.T., Benedetti, A., Sequi, P., 2000. Thermal methods of organic matter maturation
  monitoring during a composting process. J. Therm. Anal. Calorim. 61, 389–396.
  https://doi.org/https://doi.org/10.1023/A:1010157115211
- 802 Dungait, J.A.J., Hopkins, D.W., Gregory, A.S., Whitmore, A.P., 2012. Soil organic matter turnover is

- 803 governed by accessibility not recalcitrance. Glob. Chang. Biol. 18, 1781–1796.
- 804 https://doi.org/10.1111/j.1365-2486.2012.02665.x
- Fang, Y., Singh, B., Singh, B.P., 2015. Effect of temperature on biochar priming effects and its stability in
   soils. Soil Biol. Biochem. 80, 136–145. https://doi.org/10.1016/j.soilbio.2014.10.006
- Forbes, M.S., Raison, R.J., Skjemstad, J.O., 2006. Formation, transformation and transport of black
   carbon (charcoal) in terrestrial and aquatic ecosystems. Sci. Total Environ. 370, 190–206.
   https://doi.org/10.1016/j.scitotenv.2006.06.007
- Glaser, B., Balashov, E., Haumaier, L., Guggenberger, G., Zech, W., 2000. Black carbon in density fractions
  of anthropogenic soils of the Brazilian Amazon region. Org. Geochem. 31, 669–678.
  https://doi.org/10.1016/S0146-6380(00)00044-9
- Glaser, B., Birk, J.J., 2012. State of the scientific knowledge on properties and genesis of Anthropogenic
  Dark Earths in Central Amazonia (terra preta de Índio). Geochim. Cosmochim. Acta 82, 39–51.
  https://doi.org/10.1016/j.gca.2010.11.029
- Grunwald, D., Kaiser, M., Junker, S., Marhan, S., Piepho, H.-P., Poll, C., Bamminger, C., Ludwig, B., 2017.
  Influence of elevated soil temperature and biochar application on organic matter associated with
  aggregate-size and density fractions in an arable soil. Agric. Ecosyst. Environ. 241, 79–87.
  https://doi.org/10.1016/j.agee.2017.02.029
- Gulde, S., Chung, H., Amelung, W., Chang, C., Six, J., 2008. Soil Carbon Saturation Controls Labile and
- 821 Stable Carbon Pool Dynamics. Soil Sci. Soc. Am. J. 72, 605–612.
- 822 https://doi.org/10.2136/sssaj2007.0251
- Hagemann, N., Joseph, S., Schmidt, H.-P., Kammann, C.I., Harter, J., Borch, T., Young, R.B., Varga, K.,
- Taherymoosavi, S., Elliott, K.W., McKenna, A., Albu, M., Mayrhofer, C., Obst, M., Conte, P., Dieguez-
- Alonso, A., Orsetti, S., Subdiaga, E., Behrens, S., Kappler, A., 2017. Organic coating on biochar
- 826 explains its nutrient retention and stimulation of soil fertility. Nat. Commun. 8, 1089.
- 827 https://doi.org/10.1038/s41467-017-01123-0
- Hamer, U., Marschner, B., Brodowski, S., Amelung, W., 2004. Interactive priming of black carbon and
  glucose mineralisation. Org. Geochem. 35, 823–830.
- 830 https://doi.org/10.1016/j.orggeochem.2004.03.003
- Hammes, K., Schmidt, M.W.I., Smernik, R.J., Currie, L.A., Ball, W.P., Nguyen, T.H., Louchouarn, P., Houel,
  S., Gustafsson, Ö., Elmquist, M., Cornelissen, G., Skjemstad, J.O., Masiello, C.A., Song, J., Peng, P.,
  Mitra, S., Dunn, J.C., Hatcher, P.G., Hockaday, W.C., Smith, D.M., Hartkopf-Fröder, C., Böhmer, A.,
  Lüer, B., Huebert, B.J., Amelung, W., Brodowski, S., Huang, L., Zhang, W., Gschwend, P.M., FloresCervantes, D.X., Largeau, C., Rouzaud, J.-N., Rumpel, C., Guggenberger, G., Kaiser, K., Rodionov, A.,
  Gonzalez-Vila, F.J., Gonzalez-Perez, J.A., de la Rosa, J.M., Manning, D.A.C., López-Capél, E., Ding, L.,
  2007. Comparison of quantification methods to measure fire-derived (black/elemental) carbon in
- soils and sediments using reference materials from soil, water, sediment and the atmosphere.
- 839 Global Biogeochem. Cycles 21, n/a-n/a. https://doi.org/10.1029/2006GB002914

- Hammes, K., Torn, M.S., Lapenas, A.G., Schmidt, M.W.I., 2008. Centennial black carbon turnover
  observed in a Russian steppe soil. Biogeosciences 5, 1339–1350. https://doi.org/10.5194/bg-51339-2008
- Hardy, B., Cornelis, J.-T., Houben, D., Leifeld, J., Lambert, R., Dufey, J.E., 2017a. Evaluation of the longterm effect of biochar on properties of temperate agricultural soil at pre-industrial charcoal kiln
  sites in Wallonia, Belgium. Eur. J. Soil Sci. 68, 80–89. https://doi.org/10.1111/ejss.12395
- Hardy, B., Dufey, J., 2015. Les aires de faulde en forêt wallonne : repérage, morphologie et distribution
  spatiale. Forêt Nat. 135, 20–30.
- Hardy, B., Leifeld, J., Knicker, H., Dufey, J.E., Deforce, K., Cornélis, J.-T., 2017b. Long term change in
  chemical properties of preindustrial charcoal particles aged in forest and agricultural temperate
  soil. Org. Geochem. 107, 33–45. https://doi.org/10.1016/j.orggeochem.2017.02.008
- Heidarian Dehkordi, R., Denis, A., Fouche, J., Burgeon, V., Cornelis, J.T., Tychon, B., Placencia Gomez, E.,
  Meersmans, J., 2020. Remotely-sensed assessment of the impact of century-old biochar on chicory
  crop growth using high-resolution UAV-based imagery. Int. J. Appl. Earth Obs. Geoinf. 91, 102147.
  https://doi.org/10.1016/j.jag.2020.102147
- Herath, H.M.S.K., Camps-Arbestain, M., Hedley, M., Van Hale, R., Kaal, J., 2014. Fate of biochar in
  chemically- and physically-defined soil organic carbon pools. Org. Geochem. 73, 35–46.
  https://doi.org/10.1016/j.orggeochem.2014.05.001
- Hernandez-Soriano, M.C., Kerré, B., Goos, P., Hardy, B., Dufey, J., Smolders, E., 2016a. Long-term effect
  of biochar on the stabilization of recent carbon: soils with historical inputs of charcoal. GCB
  Bioenergy 8, 371–381. https://doi.org/10.1111/gcbb.12250
- Hernandez-Soriano, M.C., Kerré, B., Kopittke, P.M., Horemans, B., Smolders, E., 2016b. Biochar affects
  carbon composition and stability in soil: a combined spectroscopy-microscopy study. Sci. Rep. 6,
  25127. https://doi.org/10.1038/srep25127
- Hirsch, F., Raab, T., Ouimet, W., Dethier, D., Schneider, A., Raab, A., 2017. Soils on historic charcoal
  hearths: Terminology and chemical properties. Soil Sci. Soc. Am. J. 81, 1427–1435.
  https://doi.org/10.2136/sssaj2017.02.0067
- 867 IUSS Working Group WRB. 2014. World Reference Base for Soil Resources 2014. International soil
   868 classification system for naming soils and creating legends for soil maps. World Soil Resources
   869 Reports No. 106. FAO, Rome
- Jeffery, S., Verheijen, F.G.A., van der Velde, M., Bastos, A.C., 2011. A quantitative review of the effects of
   biochar application to soils on crop productivity using meta-analysis. Agric. Ecosyst. Environ. 144,
   175–187. https://doi.org/10.1016/j.agee.2011.08.015
- Kalinichev, A.G., Kirkpatrick, R.J., 2007. Molecular dynamics simulation of cationic complexation with
  natural organic matter. Eur. J. Soil Sci. 58, 909–917. https://doi.org/10.1111/j.13652389.2007.00929.x

876 Kerré, B., Bravo, C.T., Leifeld, J., Cornelissen, G., Smolders, E., 2016. Historical soil amendment with 877 charcoal increases sequestration of non-charcoal carbon: A comparison among methods of black 878 carbon quantification. Eur. J. Soil Sci. 67, 324–331. https://doi.org/10.1111/ejss.12338 879 Kerré, B., Willaert, B., Smolders, E., 2017. Lower residue decomposition in historically charcoal-enriched 880 soils is related to increased adsorption of organic matter. Soil Biol. Biochem. 104, 1–7. 881 https://doi.org/10.1016/j.soilbio.2016.10.007 882 Kleber, M., Eusterhues, K., Keiluweit, M., Mikutta, C., Mikutta, R., Nico, P.S., 2015. Mineral–Organic 883 Associations: Formation, Properties, and Relevance in Soil Environments, in: Advances in 884 Agronomy. Elsevier Ltd, pp. 1–140. https://doi.org/10.1016/bs.agron.2014.10.005 885 Kleber, M., Sollins, P., Sutton, R., 2007. A conceptual model of organo-mineral interactions in soils: Self-886 assembly of organic molecular fragments into zonal structures on mineral surfaces. 887 Biogeochemistry 85, 9–24. https://doi.org/10.1007/s10533-007-9103-5 888 Kögel-Knabner, I., Guggenberger, G., Kleber, M., Kandeler, E., Kalbitz, K., Scheu, S., Eusterhues, K., 889 Leinweber, P., 2008. Organo-mineral associations in temperate soils: Integrating biology, 890 mineralogy, and organic matter chemistry. J. Plant Nutr. Soil Sci. 171, 61–82. 891 https://doi.org/10.1002/jpln.200700048 892 Kopittke, P.M., Dalal, R.C., Hoeschen, C., Li, C., Menzies, N.W., Mueller, C.W., 2020. Soil organic matter is 893 stabilized by organo-mineral associations through two key processes: The role of the carbon to 894 nitrogen ratio. Geoderma 357, 113974. https://doi.org/10.1016/j.geoderma.2019.113974 895 Kuzyakov, Y., Bogomolova, I., Glaser, B., 2014. Biochar stability in soil: Decomposition during eight years 896 and transformation as assessed by compound-specific 14C analysis. Soil Biol. Biochem. 70, 229– 897 236. https://doi.org/10.1016/j.soilbio.2013.12.021 898 Lakanen, E., Erviö, R., 1971. A comparison of eight extractants for the determination of plant available micronutrients in soils. Acta Agral. Fenn. 123, 223–232. 899 900 Lal, R., 2004. Soil Carbon Sequestration Impacts on Global Climate Change and Food Security. Science 901 (80-.). 304, 1623. 902 Le Quéré, C., Raupach, M.R., Canadell, J.G., Marland, G., Bopp, L., Ciais, P., Conway, T.J., Doney, S.C., 903 Feely, R.A., Foster, P., Friedlingstein, P., Gurney, K., Houghton, R.A., House, J.I., Huntingford, C., 904 Levy, P.E., Lomas, M.R., Majkut, J., Metzl, N., Ometto, J.P., Peters, G.P., Prentice, I.C., Randerson, 905 J.T., Running, S.W., Sarmiento, J.L., Schuster, U., Sitch, S., Takahashi, T., Viovy, N., van der Werf, 906 G.R., Woodward, F.I., 2009. Trends in the sources and sinks of carbon dioxide. Nat. Geosci. 2, 831– 907 836. https://doi.org/10.1038/ngeo689 908 Lehmann, J., Gaunt, J., Rondon, M., 2006. Bio-char Sequestration in Terrestrial Ecosystems – A Review. 909 Mitig. Adapt. Strateg. Glob. Chang. 11, 403–427. https://doi.org/10.1007/s11027-005-9006-5 910 Lehmann, J., Hansel, C.M., Kaiser, C., Kleber, M., Maher, K., Manzoni, S., Nunan, N., Reichstein, M.,

911 Schimel, J.P., Torn, M.S., Wieder, W.R., Kögel-Knabner, I., 2020. Persitence of soil organic carbon

- 912 caused by functional complexity. Nat. Geosci. 13, 529–534. https://doi.org/10.1038/s41561-020 913 0612-3
- Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature 528, 60–68.
   https://doi.org/10.1038/nature16069
- Lehmann, J., Liang, B., Solomon, D., Lerotic, M., Luizão, F., Kinyangi, J., Schäfer, T., Wirick, S., Jacobsen,
- 917 C., 2005. Near-edge X-ray absorption fine structure (NEXAFS) spectroscopy for mapping nano-scale
- distribution of organic carbon forms in soil: Application to black carbon particles. Global
   Biogeochem. Cycles 19, 1–12. https://doi.org/10.1029/2004GB002435
- Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on
  soil biota A review. Soil Biol. Biochem. 43, 1812–1836.
  https://doi.org/10.1016/j.soilbio.2011.04.022
- Lehndorff, E., Roth, P.J., Cao, Z.H., Amelung, W., 2014. Black carbon accrual during 2000 years of paddyrice and non-paddy cropping in the Yangtze River Delta, China. Glob. Chang. Biol. 20, 1968–1978.
  https://doi.org/10.1111/gcb.12468
- Leifeld, J., 2008. Calorimetric characterization of grass during its decomposition. J. Therm. Anal. Calorim.
   93, 651–655. https://doi.org/10.1007/s10973-007-8852-7
- Leifeld, J., 2007. Thermal stability of black carbon characterised by oxidative differential scanning
   calorimetry. Org. Geochem. 38, 112–127. https://doi.org/10.1016/j.orggeochem.2006.08.004
- Leinweber, P., Schulten, H.R., Horte, C., 1992. Differential thermal analysis, thermogravimetry and
   pyrolysis-field ionisation mass spectrometry of soil organic matter in particle-size fractions and
   bulk soil samples. Thermochim. Acta 194, 175–187. https://doi.org/10.1016/0040-6031(92)80016 P
- Liang, B., Lehmann, J., Sohi, S.P., Thies, J.E., O'Neill, B., Trujillo, L., Gaunt, J., Solomon, D., Grossman, J.,
  Neves, E.G., Luizão, F.J., O'Neill, B., Trujillo, L., Gaunt, J., Solomon, D., Grossman, J., Neves, E.G.,
  Luizão, F.J., 2010. Black carbon affects the cycling of non-black carbon in soil. Org. Geochem. 41,
  206–213. https://doi.org/10.1016/j.orggeochem.2009.09.007
- Lopez-Capel, E., Sohi, S.P., Gaunt, J.L., Manning, D.A.C., 2005. Use of Thermogravimetry-Differential
  Scanning Calorimetry to characterize modelable soil organic matter fractions. Soil Sci. Soc. Am. J.
  69, 136–140.
- Lorenz, K., Lal, R., 2014. Biochar application to soil for climate change mitigation by soil organic carbon
   sequestration. J. Plant Nutr. Soil Sci. 177, 651–670. https://doi.org/10.1002/jpln.201400058
- Lutfalla, S., Abiven, S., Barré, P., Wiedemeier, D.B., Christensen, B.T., Houot, S., Kätterer, T., Macdonald,
  A.J., van Oort, F., Chenu, C., 2017. Pyrogenic Carbon Lacks Long-Term Persistence in Temperate
  Arable Soils. Front. Earth Sci. 5, 1–10. https://doi.org/10.3389/feart.2017.00096
- Lützow, M. V., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H.,

947 2006. Stabilization of organic matter in temperate soils: Mechanisms and their relevance under

- 948
   different soil conditions A review. Eur. J. Soil Sci. 57, 426–445. https://doi.org/10.1111/j.1365 

   949
   2389.2006.00809.x
- Meier, I.C., Finzi, A.C., Phillips, R.P., 2017. Root exudates increase N availability by stimulating microbial
   turnover of fast-cycling N pools. Soil Biol. Biochem. 106, 119–128.
   https://doi.org/10.1016/j.soilbio.2016.12.004
- Minasny, B., Malone, B.P., McBratney, A.B., Angers, D.A., Arrouays, D., Chambers, A., Chaplot, V., Chen,
  Z.-S., Cheng, K., Das, B.S., Field, D.J., Gimona, A., Hedley, C.B., Hong, S.Y., Mandal, B., Marchant,
  B.P., Martin, M., McConkey, B.G., Mulder, V.L., O'Rourke, S., Richer-de-Forges, A.C., Odeh, I.,
  Padarian, J., Paustian, K., Pan, G., Poggio, L., Savin, I., Stolbovoy, V., Stockmann, U., Sulaeman, Y.,
  Tsui, C.-C., Vågen, T.-G., van Wesemael, B., Winowiecki, L., 2017. Soil carbon 4 per mille. Geoderma
  292, 59–86. https://doi.org/10.1016/j.geoderma.2017.01.002
- Moni, C., Rumpel, C., Virto, I., Chabbi, A., Chenu, C., 2010. Relative importance of sorption versus
  aggregation for organic matter storage in subsoil horizons of two contrasting soils. Eur. J. Soil Sci.
  61, 958–969. https://doi.org/10.1111/j.1365-2389.2010.01307.x
- Nguyen, B.T., Lehmann, J., Kinyangi, J., Smernik, R., Riha, S.J., Engelhard, M.H., 2008. Long-term black
  carbon dynamics in cultivated soil. Biogeochemistry 89, 295–308. https://doi.org/10.1007/s10533008-9220-9
- Pituello, C., Dal Ferro, N., Francioso, O., Simonetti, G., Berti, A., Piccoli, I., Pisi, A., Morari, F., 2018.
  Effects of biochar on the dynamics of aggregate stability in clay and sandy loam soils. Eur. J. Soil Sci.
  69, 827–842. https://doi.org/10.1111/ejss.12676
- Plante, A.F., Fernández, J.M., Haddix, M.L., Steinweg, J.M., Conant, R.T., 2011. Biological, chemical and
   thermal indices of soil organic matter stability in four grassland soils. Soil Biol. Biochem. 43, 1051–
   1058. https://doi.org/10.1016/j.soilbio.2011.01.024
- Plante, A.F., Fernández, J.M., Leifeld, J., 2009. Application of thermal analysis techniques in soil science.
   Geoderma 153, 1–10. https://doi.org/10.1016/j.geoderma.2009.08.016
- Poeplau, C., Don, A., Six, J., Kaiser, M., Benbi, D., Chenu, C., Cotrufo, M.F., Derrien, D., Gioacchini, P.,
  Grand, S., Gregorich, E., Griepentrog, M., Gunina, A., Haddix, M., Kuzyakov, Y., Kühnel, A.,
- 975 Macdonald, L.M., Soong, J., Trigalet, S., Vermeire, M.-L., Rovira, P., van Wesemael, B., Wiesmeier,
- 976 M., Yeasmin, S., Yevdokimov, I., Nieder, R., 2018. Isolating organic carbon fractions with varying
- 977 turnover rates in temperate agricultural soils A comprehensive method comparison. Soil Biol.
- 978 Biochem. 125, 10–26. https://doi.org/10.1016/j.soilbio.2018.06.025
- Poirier, V., Roumet, C., Munson, A.D., 2018. The root of the matter: Linking root traits and soil organic
  matter stabilization processes. Soil Biol. Biochem. 120, 246–259.
- 981 https://doi.org/10.1016/j.soilbio.2018.02.016
- 982 Rasse, D.P., Rumpel, C., Dignac, M., 2005. Is soil carbon mostly root carbon? Mechanisms for a specific

- 983 stabilisation. Plant Soil 269, 341–356. https://doi.org/10.1007/s11104-004-0907-y
- Rillig, M.C., 2004. Arbuscular mycorrhizae, glomalin, and soil aggregation. Can. J. Soil Sci. 84, 355–363.
   https://doi.org/10.4141/S04-003
- 986 Rodionov, A., Amelung, W., Peinemann, N., Haumaier, L., Zhang, X., Kleber, M., Glaser, B., Urusevskaya,
- 987 I., Zech, W., 2010. Black carbon in grassland ecosystems of the world. Global Biogeochem. Cycles
- 988 24, 1–15. https://doi.org/10.1029/2009GB003669
- Rovira, P., Kurz-Besson, C., Coûteaux, M.M., Ramón Vallejo, V., 2008. Changes in litter properties during
   decomposition: A study by differential thermogravimetry and scanning calorimetry. Soil Biol.
   Biochem. 40, 172–185. https://doi.org/10.1016/j.soilbio.2007.07.021
- Sanderman, J., Hengl, T., Fiske, G.J., 2017. Soil carbon debt of 12,000 years of human land use. Proc.
   Natl. Acad. Sci. 114, 9575–9580. https://doi.org/10.1073/pnas.1706103114
- Schmidt, M.W.I., Noack, A.G., 2000. Black carbon in soils and sediments: Analysis, distribution,
   implications and current challenges. Global Biogeochem. Cycles 14, 777–793.
- Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., KögelKnabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E.,
  2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49–56.
  https://doi.org/10.1038/nature10386
- Schneider, A., Hirsch, F., Raab, A., Raab, T., 2019. Temperature Regime of a Charcoal-Enriched Land Use
   Legacy Soil. Soil Sci. Soc. Am. J. 83, 565–574. https://doi.org/10.2136/sssaj2018.12.0483
- Singh, B.P., Cowie, A.L., Smernik, R.J., 2012. Biochar Carbon Stability in a Clayey Soil As a Function of
   Feedstock and Pyrolysis Temperature. Environ. Sci. Technol. 46, 11770–11778.
   https://doi.org/10.1021/es302545b
- Singh, N., Abiven, S., Torn, M.S., Schmidt, M.W.I., 2012. Fire-derived organic carbon in soil turns over on
   a centennial scale. Biogeosciences 9, 2847–2857. https://doi.org/10.5194/bg-9-2847-2012
- Six, J., Elliott, E., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate formation: a
   mechanism for C sequestration under no-tillage agriculture. Soil Biol. Biochem. 32, 2099–2103.
   https://doi.org/10.1016/S0038-0717(00)00179-6
- Six, J., Elliott, E., Paustian, K., Doran, J.W., 1998. Aggregation and Soil Organic Matter Accumulation in
   Cultivated and Native Grassland Soils. Soil Sci. Soc. Am. J. 62, 1367–1377.
   https://doi.org/10.2136/sssaj1998.03615995006200050032x
- Sun, F., Lu, S., 2014. Biochars improve aggregate stability, water retention, and pore-space properties of
   clayey soil. J. Plant Nutr. Soil Sci. 177, 26–33. https://doi.org/10.1002/jpln.201200639
- 1015 Virto, I.C., Moni, C., Swanston, C., Chenu, C., 2010. Turnover of intra- and extra-aggregate organic
   1016 matter at the silt-size scale. Geoderma 156, 1–10.
- 1017 https://doi.org/10.1016/j.geoderma.2009.12.028

- Vogel, C., Mueller, C.W., Höschen, C., Buegger, F., Heister, K., Schulz, S., Schloter, M., Kögel-Knabner, I.,
   2014. Submicron structures provide preferential spots for carbon and nitrogen sequestration in
   soils. Nat. Commun. 5, 2947. https://doi.org/10.1038/ncomms3947
- von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner,
   B., 2007. SOM fractionation methods: Relevance to functional pools and to stabilization
- 1023 mechanisms. Soil Biol. Biochem. 39, 2183–2207. https://doi.org/10.1016/j.soilbio.2007.03.007
- von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa,
   H., 2006. Stabilization of organic matter in temperate soils: Mechanisms and their relevance under
   different soil conditions A review. Eur. J. Soil Sci. 57, 426–445. https://doi.org/10.1111/j.1365 2389.2006.00809.x
- Wang, J., Xiong, Z., Kuzyakov, Y., 2016. Biochar stability in soil: meta-analysis of decomposition and
   priming effects. Glob. Chang. Biol. Bioenergy 8, 512–523. https://doi.org/10.1111/gcbb.12266
- Wiedemeier, D.B., Abiven, S., Hockaday, W.C., Keiluweit, M., Kleber, M., Masiello, C.A., McBeath, A. V.,
   Nico, P.S., Pyle, L.A., Schneider, M.P.W., Smernik, R.J., Wiesenberg, G.L.B., Schmidt, M.W.I., 2015.
- 1032 Aromaticity and degree of aromatic condensation of char. Org. Geochem. 78, 135–143.
- 1033 https://doi.org/10.1016/j.orggeochem.2014.10.002
- WRB, I., 2014. World Reference Base for Soil Resources. World Soil Resources Report 103 . Rome: Food
   and Agriculture Organization of the United Nations (2006), pp. 132, US\$22.00 (paperback). ISBN
   92-5-10511-4. World Ref. Base Soil Resour. 3, 264–264.
- 1037 https://doi.org/10.1017/S0014479706394902
- 1038 Zimmerman, A.R., Gao, B., Ahn, M.-Y., 2011. Positive and negative carbon mineralization priming effects
- among a variety of biochar-amended soils. Soil Biol. Biochem. 43, 1169–1179.
- 1040 https://doi.org/10.1016/j.soilbio.2011.02.005