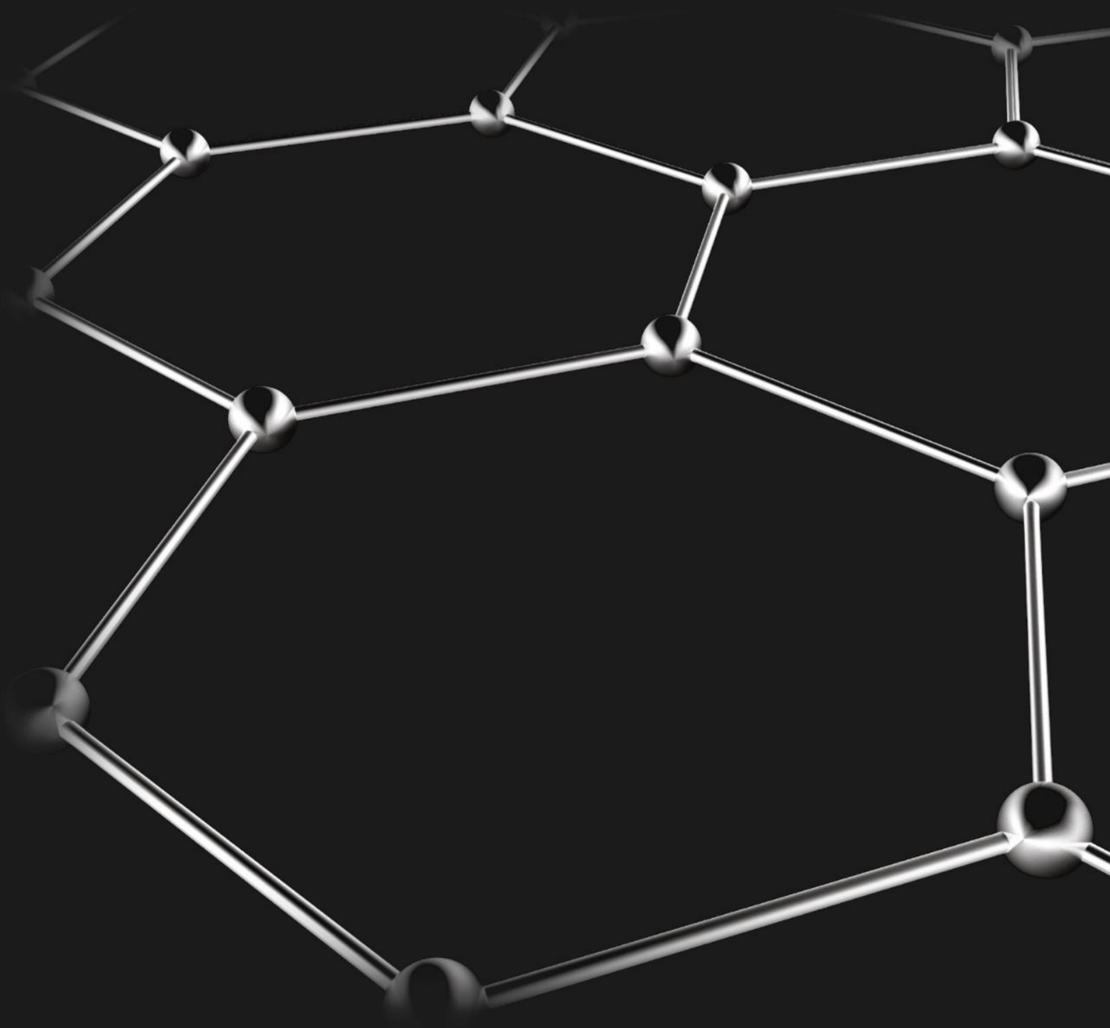
LONG-TERM PERSISTENCE OF SOIL ORGANIC MATTER IN AMAZONIAN DARK EARTH

TERRA PRETA PROGRAM



Carlos Francisco Brazão Vieira Alho

Propositions

1. Black carbon plays a minor role in explaining long-term persistence of soil organic matter in Amazonian Dark Earth. (this thesis)
2. Stability of soil organic matter depends on how stabilization is understood as a process. (this thesis)
3. Investigation of long-term effects of climate-smart agriculture is hampered by short-term scientific grants.
4. Scientists are better at raising new questions than answering old ones.
5. Perfectionism is procrastination in disguise.
6. Skipping breakfast is key for a longer and healthier life.

Propositions belonging to the thesis, entitled
Long-term persistence of soil organic matter in Amazonian Dark Earth

Carlos Francisco Brazão Vieira Alho
Wageningen, 11 November 2019

Long-term persistence of soil organic matter in Amazonian Dark Earth

Carlos Francisco Brazão Vieira Alho

Thesis committee

Promotors
Prof. Dr T.W.M. Kuyper
Personal chair at the Soil Biology Group
Wageningen University & Research

Co-promotors
Dr T. Hiemstra
Assistant professor, Soil Chemistry and Chemical Soil Quality
Wageningen University & Research

Dr W.G. Teixeira
Senior scientist
Brazilian Agricultural Research Corporation, Rio de Janeiro, Brazil

Other members
Prof. Dr A.A. Koelmans, Wageningen University & Research
Dr C.R. Stoof, Wageningen University & Research
Dr B. Jansen, University of Amsterdam
Dr J. Kern, Leibniz Institute for Agricultural Engineering and Bioeconomy, Hannover, Germany

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Long-term persistence of soil organic matter in Amazonian Dark Earth

Carlos Francisco Brazão Vieira Alho

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Contents

Chapter 1

General Introduction

Carlos Francisco Brazão Vieira Alho

1.1. Societal relevance

Soil organic matter (SOM) is the largest terrestrial carbon (C) pool (Scharlemann et al., 2014). Globally, soils contain 755 Pg C in the topsoil (0-30 cm) and up to 2060 Pg C if the subsoil (0–200 cm) is also considered (Batjes, 2016). SOM plays a major role in soil physical, chemical and biological processes. Therefore, SOM is considered a key function of soils (Wiesmeier et al., 2019), important for ecosystem services and agricultural production. Nonetheless, our limited understanding on the factors determining long-term persistence of SOM hampers our ability to better understand the role of SOM in the global C cycle (Stockmann et al., 2013).

Scientific consensus states that increased atmospheric concentration of greenhouse gases (GHG) such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) caused by anthropic activities (e.g. burning of fossil fuels and conversion of forested areas into agricultural fields) are linked to climate change (Cook et al., 2016). Raftery et al. (2017) reported that the global mean temperature is likely to increase more than 2°C by the year 2100. Global warming is expected to cause natural areas that are currently large C sinks (e.g. boreal forest soils and artic permafrost) into major C sources (Crowther et al., 2016). However, the net effects of climate change on SOM dynamics – if soils will become C sources or C sinks – is uncertain.

Soils in agricultural areas have lost substantial amounts of C due to land use change and poor management practices (Guo and Gifford, 2002). Sanderman et al. (2018) estimated that globally, soils have lost 116 Pg C (0–200 cm) due to land use change and cultivation. Climate-smart use of soils (Paustian et al., 2016) in agricultural areas may provide an opportunity to offset anthropic C emissions (Harden et al., 2018). For example, the 4 per 1000 initiative has been recently proposed to promote the increase in soil organic carbon (SOC) content in agricultural areas by 0.4% per year by means of sustainable management practices (e.g. minimum mechanical soil disturbance, permanent organic cover and diversification of crop species in rotations or intercropping) (Minasny et al., 2017).

On the one hand, there is an urgent need to increase SOM content to mitigate climate change (Chenu et al., 2018). On the other hand, SOM is most useful to soil quality when it decomposes (Janzen, 2006). Therefore, there is a societal and a scientific demand to better understand potential trade-offs between SOM decomposition and stabilization and how to fine-tune strategies based on local specificities to achieve the expected environmental and agricultural services provided by SOM. Therefore, understanding the mechanisms explaining long-term persistence of SOM is crucial to better understand the role of SOM in the global C cycle and soil-climate feedbacks (Schmidt et al., 2011).

1.2. Soil Organic Matter

SOM is a complex mixture of plant- and microbial-derived organic matter (OM) in different stages of biodegradation embedded in the soil mineral matrix. Despite its well-known importance to ecosystem functioning and agricultural productivity, views on the relative contribution of different mechanisms of SOM stabilization in explaining long-term persistence of SOM are controversial (Lehmann and Kleber, 2015).

Historically, studies have largely focused on SOM intrinsic chemical characteristics – often referred to as ‘inherent chemical recalcitrance’ – to explain long-term persistence of SOM (Kleber, 2010). This ‘traditional’ view postulates that a so-called ‘humification’ process generates ‘humic substances’ that persist in the soil due to their intrinsic chemical properties (Gerke, 2018). However, recent advances in analytical techniques, such as the use of specific molecular markers and compound-specific isotopic analysis (Amelung et al., 2009) indicate that SOM intrinsic chemical characteristics do not fully explain its long-term persistence in soils (Schmidt et al., 2011). Major organic compounds (e.g. lignin, cellulose, lipids and proteins) can be fully decomposed under optimal conditions (e.g. sufficient water, oxygen and in the presence of energy-rich OM), even though some may take longer to decompose than others (i.e. wood > leaves) (Ekschmitt et al., 2008). Therefore, the relative importance of inherent chemical recalcitrance in explaining long-term persistence of SOM has been challenged (Marschner et al., 2008). Currently, long-term persistence of SOM is interpreted as an ecosystem property (Schmidt et al., 2011). In this ‘new’ view, the role of soil minerals in organo-mineral associations that protect SOM from being further decomposed by the soil microbial community is highlighted (Baldock and Skjemstad, 2000; Torn et al., 1997). More information on the disputed ‘traditional’ and ‘new’ views on SOM is addressed in the discussion between Kleber and Lehmann (2019) and Olk et al. (2019). Moreover, whether this ‘new’ view is indeed a novel approach is addressed by Baveye and Wander (2019).

Still, advancements on the conceptual models of SOM stabilization need to be operationalized for the quantification of SOM pools that can be used to better estimate the relative contribution of different mechanisms in explaining long-term persistence of SOM (Harden et al., 2018; Stockmann et al., 2013). SOM complexity makes SOM fractionation procedures useful to quantify and characterize SOM pools for better estimation of the relative contribution of different mechanisms of SOM stabilization (Poeplau et al., 2018; von Lützow et al., 2007). SOM fractionation procedures are commonly divided into physical fractionation (i.e. aggregate, particle-size and density fractions) and chemical fractionation (i.e. solubility, hydrolysability, resistance to oxidizing chemicals and demineralization) or a combination of different procedures (Poeplau et al., 2018; von Lützow et al., 2007). However, since several mechanisms of SOM stabilization are likely to happen simultaneously, SOM stability is still difficult to be assessed operationally.

In general, three main mechanisms of SOM stabilization have been proposed: (i) physical protection (inaccessibility of OM compounds to decomposers); (ii) mineral association (adsorption of OM on the surface of reactive soil minerals) and (iii) inherent chemical recalcitrance (OM intrinsic chemical characteristics) (Sollins et al., 1996). However, the relative contribution of these mechanisms in explaining long-term persistence of SOM is poorly understood (Mikutta et al., 2006).

1.3. Amazonian Dark Earth

Amazonian Dark Earths (ADE) – also known as *Terras Pretas de Índio* in the Portuguese language – are anthropic soils (Anthrosols) that have intrigued scientists for decades. Despite tropical conditions, ADE are enriched in SOM and nutrients, particularly calcium (Ca) and phosphorus (P). For this reason, ADE contrast with the adjacent (ADJ) soils from the Amazon (Glaser et al., 2001). ADE also exhibit high pH and cation exchange capacity (CEC) and frequently other evidences of human activity (e.g. ceramic artefacts and charcoal fragments) (Glaser et al., 2001; Kämpf et al., 2003; Kern et al., 2017; Sombroek, 1966). Radiocarbon dating indicates that these soils were mostly formed between 2500 and 500 BP and are of pre-Columbian origin (Neves, 2008). Possible sources that caused soil enrichment have been linked to terrestrial/aquatic plant biomass, human/animal excrements/bones and charcoal/ash residues of incomplete combustion (Glaser, 2007). Current research indicates that the extent and intensity by which Amerindians occupied and transformed the Amazon is far more complex than previously assumed (Kern et al., 2017), when scientists took it for granted that the cultural and agricultural development of the Amerindians was limited by soil fertility. Therefore, studies on ADE empower the understanding of complex pre-Columbian cultural development in the Amazon and since ADE are man-made soils, understanding the processes that led to their formation (or creation) is likely to provide valuable insights for sustainable use of soils.

ADE are highly variable in site extension, depth of the anthropic horizon and soil physico-chemical characteristics, not only among different sites, but also within a single site. This variation is mainly caused by the diversity and complexity of pre-Columbian settlements (Costa et al., 2013; Costa and Kern, 1999; Kern et al., 2015). The persistence of anthropic markers likely depends on site frequency and intensity of occupation that may lead to a regime shift which manifests as ADE formation (Browne-Ribeiro, 2016). If ADE were formed intentionally (for agricultural purposes) or unintentionally (as the unintended consequence of waste deposition) is an ongoing debate. Nonetheless, scientists currently tend to perceive the formation of ADE (and other Anthrosols) as the inevitable outcome of daily activities throughout years of past human occupation (Fraser et al., 2014). The differentiation between ADE and ADJ is commonly done by non-quantitative field observations based on soil colour and the presence of archaeological remains (i.e. ceramic and charcoal fragments). Costa et al. (2013) reported that ADE characteristics could fit several qualifiers for Anthrosols. Recently, the pretic horizon has been proposed in an attempt to classify ADE systematically and better accommodate ADE within Anthrosols (IUSS Working Group WRB, 2015). This is important because it takes into consideration quantitative data rather than vague descriptive data.

Studies have suggested that higher SOM content in ADE compared to the adjacent soils is linked with the occurrence of black carbon (BC) in these soils (Glaser et al., 2001; Glaser, 2007). BC has been claimed to be one of the most stable forms of organic carbon (OC), highly resistant to decomposition due to its polycondensed aromatic structure caused by thermal alteration (Glaser, 2007; Haumaier and Zech, 1995; Novotny et al., 2007). Even though studies have shown that BC can be decomposed by soil microorganisms (Kuzyakov et al., 2009), BC turnover rate has been calculated to be in centennial scale (Singh et al., 2012). Furthermore, several studies have pointed to BC surface oxidation as a key property in ADE (Jorio et al., 2012; Lehmann et al., 2005; Liang et al., 2013; Mao et al., 2012) and BC has been claimed to be the main driver of ADE striking characteristics (Glaser, 2007; Novotny et al., 2009).

The occurrence of BC in ADE has inspired the biochar technology as an attempt to reproduce ADE desirable characteristics (Glaser et al., 2002). Nonetheless, biochar and ADE research became disconnected in the literature (Bezerra et al., 2016). Biochar is the charcoal-like material produced intentionally to be applied into soils (Sohi et al., 2010). Moreover, biochar application is envisioned in a multiple-win scenario to mitigate climate change via C sequestration in the soil, while co-producing bioenergy, reducing GHG emissions and boost plant productivity by increasing soil fertility (Lehmann, 2007). However, studies have shown that biochar lacked persistence in both tropical (Sagrilo et al., 2015) and temperate environments (Lutfalla et al., 2017) and that biochar application may boost crop yields in the tropics, but not in temperate regions (Jeffery et al., 2017). These findings indicate that simple addition of biochar does not reproduce ADE desirable characteristics. Therefore, understanding the role of other mechanisms that lead to ADE formation besides the occurrence of BC is crucial to better understand ADE as a model for sustainable use of soils.

1.4. Black Carbon

BC is a generic term used for a variety of thermally altered organic compounds derived from the incomplete combustion of biomass (pyrogenic) and fossil fuels (petrogenic), with no clear-cut boundaries (Schmidt and Noack, 2000). Globally, soils may contain up to 200 Pg BC in 0–200 cm (Reisser et al., 2016), which corresponds to ~10% of the total global C pool in soils (Batjes, 2016). However, BC sources, pools and fluxes across biomes and variation within soil profiles are poorly documented (Bird et al., 2015; Santín et al., 2016).

BC consists of polycondensed aromatic units, deficient in hydrogen (H) and oxygen (O), with different sizes and organizational levels (Kramer et al., 2004). Knicker et al. (2008) suggested that a large polycondensed aromatic structure (i.e. graphite-like) is unlikely for fire-derived BC. In fact, the size of the aromatic clusters in BC has been reported to be 2-8 nm (Jorio et al., 2012), consisting of 5-10 aromatic rings, heavily substituted with carboxylic groups due to surface oxidation (Mao et al., 2012). Due to its chemical characteristics, BC has been claimed to be one of the most stable forms of C and therefore, important for the long-term persistence of C in soils (Glaser et al., 2001). In addition, Knicker (2010) reported that thermally altered SOM also contains heterocyclic aromatic nitrogen (black N), which may be a source of long-term persistent N in soils.

BC encompasses a continuum from slightly charred to highly graphitic C-rich products with distinct molecular properties depending on the source and conditions by which BC was formed, with implications for its reactivity in the soil (Keiluweit et al., 2010; Knicker et al., 2008). For instance, BC produced in savannah wildfires (dry conditions) is likely to differ from BC produced in smouldering fires in the rainforest (humid conditions). This BC continuum imposes methodological difficulties to assess BC quantitatively. Due to specific molecular characteristics caused by thermal alteration (Keiluweit et al., 2010; Knicker et al., 2008), specific methods are required to assess the BC content in soils. Hammes et al. (2007) reviewed several analytical procedures that have been proposed to assess the BC content in environmental matrices and reported that the different methods provided great differences for the same set of samples. The use of benzene polycarboxylic acids (BPCA) as specific molecular markers has been proposed to assess the BC content in soils (Glaser et al., 1998). Briefly, BC polycondensed aromatic structure is converted into single BPCA compounds upon chemical oxidation with nitric acid (HNO_3) under high temperature and high pressure. Subsequently, single BPCA compounds are further separated and can be detected by different analytical techniques (Brodowski et al., 2005b; Cerqueira et al., 2015; Dittmar, 2008; Glaser et al., 1998; Hindersmann and Achten, 2017; Schneider et al., 2011). The BPCA method has been shown to yield conservative estimates of the BC content in a variety of environmental matrices and to cover a broad range of the BC continuum. One additional advantage of assessing single BPCA compounds is the possibility to investigate the molecular properties of BC (i.e. its degree of aromaticity) (Wiedemeier et al., 2015).

1.5. Scope

In the past decades, conceptual models on the mechanisms of SOM stabilization shifted from a perspective that focused on SOM chemical characteristics to explain its long-term persistence in soils to a perspective where the role of soil minerals in stabilizing SOM is highlighted (Baldock and Skjemstad, 2000; Torn et al., 1997). Nonetheless, despite the fact that BC can be of particular importance in some soils (i.e. ADE; biochar-amended soils and natural areas susceptible to wildfires), BC is often neglected in conceptual and mathematical models. Therefore, our understanding on the role of BC on the global C cycle is limited.

Studies often suggest that BC intrinsic chemical characteristics is the main mechanism explaining its long-term persistence in soils (Glaser et al., 2003; Krull et al., 2003). Nonetheless, several studies suggested that particulate BC is fragmented into smaller particles over time, resulting in high surface area (Brodowski et al., 2005a; Skjemstad et al., 1996). Furthermore, biotic and abiotic surface oxidation (Cheng et al., 2008a, 2006) enhances BC surface charge (Cheng et al., 2008b; Nguyen et al., 2009), which likely promotes the association of BC with soil minerals. Indeed, studies have shown BC in microaggregates (Brodowski et al., 2006, 2005a; Glaser et al., 2000; Lehmann et al., 2008; Skjemstad et al., 1996) surrounded by soil minerals (Archanjo et al., 2015; Chia et al., 2012; Jorio et al., 2012), suggesting organo-mineral associations involving BC.

Soil minerals are known to protect SOM against biodegradation via organo-mineral associations (Baldock and Skjemstad, 2000; Kleber et al., 2005; Mikutta et al., 2005a; Torn et al., 1997). Globally, soils may contain up to 600 Gt C of mineral-associated C in 0–200 cm (Kramer and Chadwick, 2018), which corresponds to ~30% of the total global C pool in soils (Batjes, 2016). SOM adsorption onto poorly crystalline iron (Fe) and aluminium (Al) oxides has been reported as the main mechanism of SOM stabilization in acidic to near neutral pH soils (Rumpel and Kögel-Knabner, 2011). Carboxylic groups in SOM are reported to play a major role in surface adsorption of OM compounds onto soil oxides through inner sphere complexation (Murphy et al., 1992). Reisser et al. (2016) reported that soils with clay content higher than 50% significantly contained more BC. Therefore, mineral association is likely to play an important role in explaining long-term persistence of BC in soils (Cusack et al., 2012; Czimczik and Masiello, 2007). However, the relative contribution of mineral association versus inherent chemical recalcitrance in explaining long-term persistence of BC in soils is poorly understood.

Despite the fact that the occurrence of BC in ADE inspired the biochar hype, very few studies have specifically assessed BC in ADE. Therefore, the relative contribution of BC in ADE is poorly understood. Besides BC, high input of Ca and P are important characteristic of ADE (Van Hofwegen et al., 2009). However, next to their well-known role in enhancing and sustaining soil fertility, their possible role in explaining long-term persistence of SOM in ADE has been largely neglected since studies often rely on BC inherent chemical recalcitrance to be responsible for ADE striking characteristics. Polyvalent cations (mainly Ca^{2+}) can form bridges between negatively charged OM compounds and soil minerals (Baldock and Skjemstad, 2000; Muneer and Oades, 1989). In this sense, high Ca content in ADE likely plays an important role in cation bridging. Furthermore, it has been suggested that adsorption of P and SOM onto soil oxides may inhibit their further growth (Eusterhues et al., 2008; Fukushi and Sato, 2005) and are likely to be the cause of nano-sized particles with high surface area in soils (Hiemstra et al., 2010b). Highly weathered soils from the Amazon generally exhibit high Fe and Al oxide content, which suggests that mineral association may be of great importance in these soils. In this sense, high P content in ADE could have led to high reactive surface area of Fe and Al oxides, thus enabling the adsorption of large amount of SOM. In addition, presence of Ca has been shown to act synergistically on P adsorption onto Fe and Al oxides (Weng et al., 2011). Therefore, besides BC, Ca and P are likely to play important roles in explaining long-term persistence of SOM in ADE.

Chemical oxidation procedures have been proposed to isolate a ‘stable’ pool of SOM (Eusterhues et al., 2005; Helfrich et al., 2007). The underlying assumption is that chemical oxidation mimics biological oxidation (Eusterhues et al., 2005) and that soil minerals may protect SOM from chemical oxidants (Mikutta et al., 2005a). Mikutta et al. (2006) suggested that chemical oxidation with sodium hypochlorite (NaOCl) combined with subsequent soil demineralization with hydrofluoric acid (HF) is a useful approach to separate and quantify the relative contribution of different mechanisms of SOM stabilization (i.e. mineral association and chemical recalcitrance). Other studies suggested that BC resists chemical oxidation with NaOCl and accounts for most of the ‘stable’ OC pool (i.e. NaOCl-resistant) (Bruun et al., 2008; Eusterhues et al., 2003; Leifeld and Zimmermann, 2006; Mikutta et al., 2005b; Simpson and Hatcher, 2004). Furthermore, studies that applied chemical oxidation with NaOCl and subsequent soil demineralization with HF, often suggest that the operationally defined ‘recalcitrant’ OC pool (i.e. HF-resistant) could be composed of BC (Bruun et al., 2008; Mikutta et al., 2005b). However, the BC content was not specifically assessed in these studies.

1.6. Objective

The overall objective of this PhD thesis was to investigate the interactive roles of BC, Ca and P in explaining long-term persistence of SOM in ADE. This information will shed light on importance of other elements (i.e. Ca and P) rather than BC alone (i.e. biochar) for the recreation of ADE desirable characteristics in other soils and on the processes underlying SOM stabilization, most importantly in soils where BC is likely to be of great importance to SOM (i.e. ADE; biochar-amended soils and natural areas susceptible to wildfires).

1.7. Outline

This PhD thesis is part of the Terra Preta Program (TPP), an international and interdisciplinary research program composed by European and Latin American partners, where social and natural sciences are combined to investigate ADE from different perspectives. The overall objective of the TPP is to provide information on the sustainable use and conservation of ADE and to provide insights on how to recreate ADE desirable characteristics in other soils.

This PhD research was developed in cooperation between Wageningen University and the Brazilian Agricultural Research Corporation (Embrapa). The study area (Experimental Research Station of Caldeirão) is located at Embrapa Western Amazon in Iranduba (Amazonas state) and most of the laboratory analysis were carried out at Embrapa Soils, in Rio de Janeiro (Rio de Janeiro state) in Brazil. Additional laboratory analyses were conducted at the National Institute of Metrology Standardization and Industrial Quality (Inmetro) in Brazil, Wageningen University & Research (WUR) in the Netherlands and the Martin-Luther-University Halle-Wittenberg (MLU) in Germany.

This PhD thesis contains a General Introduction (**Chapter 1**), 4 experimental chapters (**Chapters 2 to 5**) and a General Discussion (**Chapter 6**).

In **Chapter 2**, I combine spatial modelling with the pretic horizon criteria to systematically distinguish the pretic area (i.e. proxy for ADE) from the non-pretic area (i.e. proxy for ADJ).

In **Chapter 3**, I determine the number of consecutive treatments required to isolate a ‘stable’ pool of SOM upon chemical oxidation with NaOCl in soils with contrasting contents of OC, Fe and Al oxides and reactive surface area.

In **Chapter 4**, I evaluate the chemical composition of SOM pools upon chemical oxidation with NaOCl and soil demineralization with HF in ADE and ADJ with X-ray Photoelectron Spectroscopy (XPS).

In **Chapter 5**, I use BPCA as molecular markers to estimate the relative contribution of BC in SOM pools and infer on the molecular properties of BC in ADE and ADJ. Furthermore, I estimate the relative contribution of mineral association versus chemical recalcitrance in both areas and investigate the role of Ca and P in explaining long-term persistence of SOM in ADE.

Chapter 2

Spatial variation of carbon and nutrients stocks in Amazonian Dark Earth

Carlos Francisco Brazão Vieira Alho[a], Alessandro Samuel-Rosa[b], Gilvan Coimbra Martins[c], Tjisse Hiemstra[d], Thomas W. Kuyper[a], Wenceslau Geraldes Teixeira[e]

[a] *Soil Biology Group, Wageningen University and Research, 6700 AA, Wageningen, the Netherlands..*
[b] *Federal University of Technology - Paraná, 85892-000, Santa Helena, Brazil.*
[c] *Embrapa Western Amazon, 69010-970, Manaus, Brazil.*
[d] *Soil Chemistry and Chemical Soil Quality Group, Wageningen University and Research, 6700 AA, Wageningen, the Netherlands.*
[e] *Embrapa Soils, 22460-000, Rio de Janeiro, Brazil.*

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Abstract

Amazonian Dark Earths (ADE) are anthropic soils that are enriched in carbon (C) and several nutrients, particularly calcium (Ca) and phosphorus (P), when compared to adjacent soils from the Amazon basin. Studies on ADE empower the understanding of complex pre-Columbian cultural development in the Amazon and may also provide insights for future sustainable agricultural practices in the tropics. ADE are highly variable in size, depth and soil physico-chemical characteristics. Nonetheless, the differentiation between ADE and the adjacent soils is not standardized and is commonly done based on visual field observations. In this regard, the pretic horizon has been recently proposed as an attempt to classify ADE systematically. Spatial modelling techniques can be of great use to study the structure of the spatial variation of soil properties in highly variable areas. Here, we predicted the carbon and nutrients stocks in ADE by applying spatial modelling techniques using an environmental covariate (i.e. expected anthropic enrichment gradient) in our model. In addition, we used the pretic horizon criteria to classify pretic and non-pretic areas and evaluate their relative contribution to the total stocks. In this study, we collected soil samples from five 20-cm soil layers at n = 53 georeferenced points placed in a grid of about 10 to 60 m spacing in a study area located in Central Amazon (~9.4 ha). Ceramic fragments were weighed and quantified. Samples were analysed for: Total C, Total Ca, Total P, Exchangeable Ca + Mg, Extractable P, soil pH, potential CEC (pH = 7.0) and the clay content. The use of the pretic horizon criteria allowed us to clearly distinguish two unambiguous areas with a sharp transition, rather than a smooth continuum, in contrast to previous studies in ADE. Depth- and profile-wise linear regression model parameters indicated a greater importance of the chosen environmental covariate (i.e. expected anthropic enrichment gradient) to explain the spatial variation of Total Ca and Total P stocks than Total C stocks. The overall Total Ca and Total P stocks were twice as large in the pretic area when compared to the non-pretic area.

2.1. Introduction

Amazonian Dark Earths (ADE) are anthropic soils (Anthrosols) characterized by darker colour and enrichment in carbon (C) and other nutrients, particularly calcium (Ca) and phosphorus (P). These soils also exhibit higher pH and cation exchange capacity (CEC) and frequently evidences of human activity (e.g. ceramic artefacts and charcoal fragments) when compared to the carbon- and nutrient-poor adjacent soils from the Amazon basin (Glaser et al., 2001b; Kämpf et al., 2003; Kern et al., 2017; Sombroek, 1966). Radiocarbon dating indicated that these soils were formed between 2500 and 500 before present and are of pre-Columbian origin (Neves et al., 2003). Possible carbon and nutrients sources are: terrestrial/aquatic plant biomass, human/animal excrements/bones and charcoal/ash residues of incomplete combustion (Glaser, 2007).

Due to the prevalence of weathered clay minerals (e.g. kaolinite) and iron and aluminium oxides in these soils, their ability to retain nutrients depends mainly on soil organic matter (SOM). However, high temperature and precipitation in the tropics accelerate the decomposition of SOM. Studies have suggested that high carbon content in ADE is related to the black carbon (BC) content in these soils (Glaser, 2007; Glaser et al., 2001b). BC has been claimed to be one of the most stable forms of carbon found in soils due to its poly-condensed aromatic structure that makes it more difficult to be decomposed by soil microorganisms (Glaser, 2007; Haumaier and Zech, 1995; Novotny et al., 2007). In addition, high CEC in ADE is also likely to increase primary production due to higher soil fertility. Therefore, SOM input of non-BC origin is also expected to be increased in ADE. Despite tropical conditions, ADE have intriguingly remained highly fertile after abandonment of sites following European colonization. Current research indicates that the extent and intensity with which Amerindians occupied and transformed the Amazon is far more complex than previously assumed due to possible environmental limitations (Kern et al., 2017). Studies on ADE empower the understanding of complex pre-Columbian cultural development in the Amazon and may also provide insights for future sustainable agricultural practices in the tropics.

ADE are highly variable in size, depth and soil physico-chemical characteristics not only among different sites, but also within a single site. This variation is mainly caused by the diversity and complexity of pre-Columbian settlements (Costa et al., 2013; Costa and Kern, 1999; Kern et al., 2015). The debate whether ADE were formed intentionally (for agricultural purposes) or unintentionally (as the unintended consequence of waste deposition), seems to be diminishing in relevance. Currently, scientists tend to perceive the formation of ADE (and other Anthrosols) as the inevitable outcome of daily activities throughout years of past human occupations

(Fraser et al., 2014). The persistence of anthropic markers likely depends on frequency and intensity of occupation of the site that may lead to a regime shift which manifests as ADE formation (Browne-Ribeiro, 2016).

The differentiation between ADE and the adjacent soil is commonly done by non-quantitative field observations based on soil colour and the presence of archaeological remains (i.e. ceramic and charcoal fragments). Costa et al. (2013) reported that ADE characteristics could fit several qualifiers for Anthrosols. Recently, the pretic horizon has been proposed as an attempt to classify ADE systematically and better accommodate ADE within Anthrosols (IUSS Working Group WRB, 2015). This is important because it takes into consideration quantitative data rather than vague descriptive data. The pretic horizon is a dark surface horizon that among other criteria is characterized by high contents of carbon and nutrients.

Geochemical signatures reflect the variation within and beyond the limits of anthropic areas that were previously defined by visual evidence. Schmidt et al. (2014) reported a widespread pattern of past human occupation where terraces of domestic areas (e.g. houses or yards) are surrounded by waste disposal areas as middens that build up into mounds over time. Different geochemical signatures can be linked to past land use and occupation (Costa et al., 2013). However, interpretation of these patterns may be hindered by site-inherent complexity of past settlements and current land use and occupation (Kern et al., 2015). Moreover, anthropic soils likely exhibit these complexities both in small- and large-scale analyses. Hence, we still lack a solid understanding of the specific mechanisms that led to the formation and diversity of ADE (Schmidt et al., 2014). Limited and localized soil sampling is unlikely to elucidate those mechanisms in highly variable areas.

Spatial modelling techniques can be of great use to study the structure of the spatial variation of soil properties as it considers the continuity of spatial phenomena and the deterministic effect of environmental conditions. For soil scientists, it is a great tool to visualize how soil properties can vary greatly both horizontally and vertically. For archaeologists, it is a great tool to infer on the location of specific activities in the past. Significant progress in spatial modelling techniques was possible due to recent advances in data processing. However, several methodological hurdles are still evident, especially in large areas with high spatial variation between soil properties and environmental covariates (Song et al., 2016). These hurdles can be of great importance in anthropic areas where abrupt and gradual transitions can be expected horizontally and vertically due to the complexity of settlements. Therefore, it is important to include uncertainties of predictions when using these techniques.

Correlation between soil variables is not only dependent on the distance between sampling points, but also on their location. Therefore, environmental conditions may show a trend across a study area. Stochastic simulation of spatially distributed soil properties can be used for better predictions as it preserves the structure of the spatial variation, whereas kriging usually smoothens (Heuvelink and Webster, 2001). Predictions may be improved by using exhaustive environmental covariates (Lark and Webster, 2006). However, including several covariates is not always related to an increase in prediction accuracy (Samuel-Rosa et al., 2015).

Therefore, the aims of this study were to: (i) predict the Total C, Total Ca and Total P stocks using an environmental covariate (including the uncertainties of predictions) and (ii) use the pretic horizon criteria to classify pretic and non-pretic areas and evaluate their relative contribution to the total stocks.

2.2. Material and Methods

Study area

The study area (~9.4 ha) is situated on the north margin of the Solimões river (Amazon river), in the municipality of Iranduba, Amazonas state, Brazil (03º14’22’’ - 03º1547’’ S and 60º13’’02’’- 60º1350’’ W) (Fig. 1). Regional climate is classified as Aw according to Köppen classification (tropical rainy). Local annual mean temperature is 26.7°C, annual mean rainfall is 2100 mm and relative humidity is about 80%. The local slope is flat to undulated and the site is located above existing watercourses. The most common soil classes in the region are Xanthic Ferralsols, Plinthic Ferralsols, Pisoplinthic Plinthosols and Xanthic Acrisols (Macedo et al., 2017). The study area is locally known as the Experimental Research Station of Caldeirão - Embrapa Western Amazon. Part of the study area (~30%), located in the west (W) and southwest (SW) sectors, is composed of a forested area that has not been cultivated for over 40 years. The remaining part (~70%), was converted into an experimental field where several crops have been cultivated and soil management practices have been applied over the past 40 years.

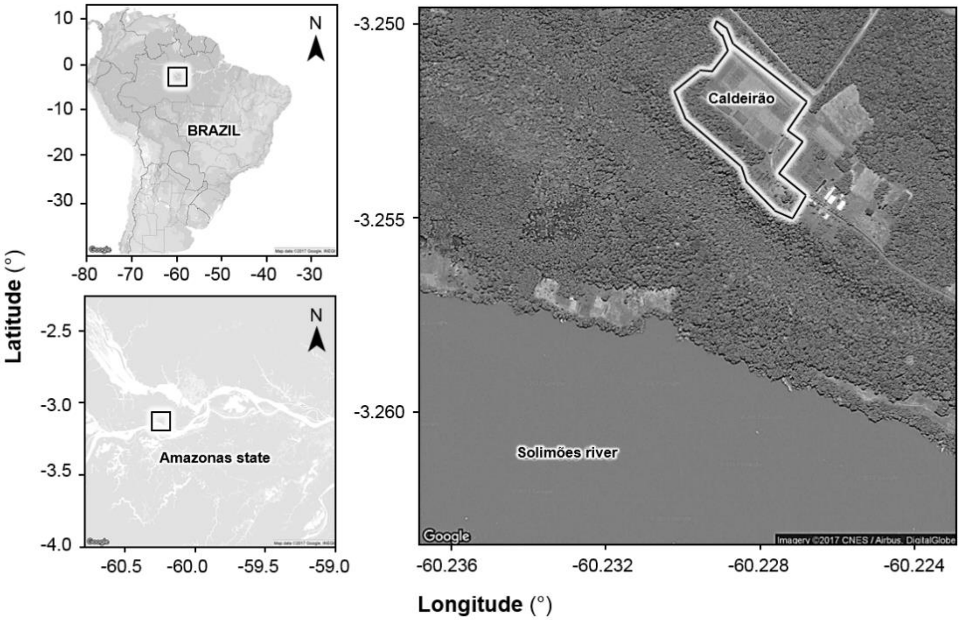

Fig. 1. Location of the study area (Experimental Research Station of Caldeirão - Embrapa Western Amazon) (Google Earth® images).

Soil sampling and analysis

Soil samples were collected from five 20-cm soil layers (from 0 to 100 cm) with a manual post hole digger (sample volume = 0.0063 m^3) at n = 53 georeferenced points (~5 m horizontal precision) placed in a grid of about 10 to 60 m spacing (265 soil samples). Ceramic fragments (> 2 mm) were weighed and quantified. Samples were air-dried, sieved through 2 mm mesh, homogenized and stored in plastic bags at room temperature prior to analyses. Samples were analysed for: Total C, Total Ca, Total P, Exchangeable Ca + Mg, Extractable P, soil pH, potential CEC (at pH = 7.0) and clay content. Total C was determined using an elemental analyser (PerkinElmer 2400 Series II) where acetanilide was used as reference material. Total Ca and Total P were determined at Geosol laboratories by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), where samples were previously digested with a multi-acid solution (HCl, HNO_3, HF and $HClO_4$). Extractable P, K, Na (Mehlich 1), Exchangeable Ca + Mg (1 M KCl) and H + Al (0.5 M calcium acetate at pH 7.0) were also determined. Soil pH was determined in water (soil:water ratio of 1:2.5), potential CEC (at pH = 7.0) was defined as the sum of exchangeable cations (K, Na, Ca + Mg) plus acidity (H + Al) and the clay content was determined by the pipet method after organic matter removal with hydrogen peroxide. Detailed description on the methods are described in Teixeira et al. (2017)

Spatial modelling

The spatial variation of soil properties was modelled as a function of fixed (deterministic) and random (stochastic) effects. The fixed effects describe the part of the spatial variation of a soil property that can be explained using spatially exhaustive covariates (Heuvelink and Webster, 2001). Spatial data covering the entire study area that can be related to the environmental conditions that likely influenced the observed large-scale patterns of spatial variation (> 50 m in our study area) can be used as covariates. Here, we assumed that past anthropic activities that caused enrichment of carbon and nutrients likely occurred closer to the river, despite specificity among activities. In addition, the current land use in the SW sector (forested area) is also expected to have caused some enrichment of organic matter due to SOM input, whereas the current land use in the NE (agronomic experimental field) is expected to have caused some impoverishment of organic matter due to cultivation. Therefore, the largest enrichment of carbon and nutrients likely occurred in the SW sector with decreasing enrichment gradient towards the NE sector. Because of the spatial association between these conditions, we chose to use one covariate to serve as their surrogate, which we defined as the expected anthropic enrichment gradient (Fig. 2a).

The understanding that this covariate could explain the large-scale spatial variation of soil properties was formalised by individually calibrating depth-wise linear regression models with soil property as dependent variable and the covariate as the independent variable. For an arbitrary soil depth (d), such linear regression model was defined as:

$$Y(\boldsymbol{s}_i, d) = \beta_{0d} + \exp[\boldsymbol{x}(\boldsymbol{s}_i, d)]^{\mathrm{T}}\beta_{1d} + \varepsilon(\boldsymbol{s}_i, d), \text{ with } i = 1, 2, \ldots, n, \quad (1)$$

where the β's are the estimated linear regression model coefficients conditional on the soil property (Y) and the covariate data ($\boldsymbol{x}$) at the observation locations ($\boldsymbol{s}_i, d$). The covariate is expressed in exponential form to emphasise the combined effect of past and current land use and occupation on enrichment of carbon and nutrients nearby the margin of the river.

In Eq. (1), $\varepsilon(\boldsymbol{s}_i, d)$ is the spatially auto-correlated difference between the fitted and observed values of the soil property (regression residuals) (Heuvelink and Webster, 2001). For an arbitrary soil depth (d), the structure of this spatial autocorrelation was analysed using the auto-variogram:

$$\gamma_d(\boldsymbol{h}) = 0.5 \text{ mean}\{[\varepsilon(\boldsymbol{s}_i, d) - \varepsilon(\boldsymbol{s}_i + \boldsymbol{h}, d)]^2\} \quad (2)$$

where $\boldsymbol{h}$ is a vector of separation distances between two observation locations ($\boldsymbol{s}_i$ and $\boldsymbol{s}_i + \boldsymbol{h}$). We used five exponentially spaced distance classes up to a maximum separation distance of about 315 m (half the diagonal of study area). Exponentially spaced distance classes were used because they deliver a better picture of the small-scale structure of spatial variation (< 50 m in our study area) (Pettitt and McBratney, 1993). The size of the two smallest distance classes (~20 m) starting at the variogram origin was determined by the sampling grid spacing (~10 to 60 m).

Regression residuals of a soil property from adjacent soil depths can be expected to have the same magnitude and/or sign. Such vertical correlation is characterized by the translocation of elements from upper to lower soil depths caused by natural and/or anthropic influences. For any two arbitrary soil depths, d_j and d_k, the structure of this spatial cross-correlation was analysed using the cross-variogram (Kyriakidis and Journel, 1999).

$$\gamma_{jk}(\boldsymbol{h}) = 0.5 \text{ mean}\{[\varepsilon(\boldsymbol{s}_i, d_j) - \varepsilon(\boldsymbol{s}_i + \boldsymbol{h}, d_j)][\varepsilon(\boldsymbol{s}_i, d_k) - \varepsilon(\boldsymbol{s}_i + \boldsymbol{h}, d_k)]\}, \text{ with } j \neq k \qquad (3)$$

By using the cross-variogram, we enforced the depth-wise auto-variograms of a soil property to be coherent among soil depths. For all soil properties, the shape of the variograms was defined using the sum of a nugget variance and the exponential covariance function:

$$\gamma(\boldsymbol{h}) = c_0 + c[1 - \exp(-\boldsymbol{h}/0.333a)], \qquad (4)$$

where c_0 is the nugget variance, c is the sill variance, and a is the correlation range (Supplementary Data).

Considering the phenomenon under study and the scale of analysis determined by the range of distances between nearest neighbouring observations (~10 to 60 m), the choice for using the exponential covariance function reflects our expectation that the spatial auto- and cross-correlation decrease rapidly with increasing separation distances. Generally, the nugget variance arises from measurements errors and very small-scale spatial variation. In this study, the latter corresponds to separation distances smaller than about 35 m (average distance between nearest-neighbouring observations).

The depth-wise empirical distribution of soil properties was transformed to Gaussian using the Box-Cox family of power transformations to meet statistical constraints and facilitate the estimation of model parameters. Then, the Box-Cox transformed variables were standardized to zero mean and unit standard deviation. The parameters of the depth-wise linear regression models were estimated using ordinary least squares, while the parameters of the auto- and cross-variogram models were estimated using iteratively reweighted least squares within the framework of the linear model of coregionalization (Pebesma, 2004).

For Total C, Total Ca and Total P, universal cokriging was used to make spatial predictions of the contents. Predicted values and prediction error variances were back-transformed using simulations as described elsewhere (Samuel-Rosa et al., 2015). For Total C, Exchangeable Ca + Mg and Extractable P data at the first depth (0–20 cm) used for the pretic horizon criteria, conditional sequential Gaussian simulation was used to produce 1000 equally probable realisations of their respective random fields (Pebesma, 2004). Spatial predictions and simulations were done in regular grids per soil depth containing 94,462 point-predictions at 1.0 m spacing. For further information on the geostatistical methods we used here, we refer to Goovaerts (1997) and Webster and Oliver (2007).

Computation of predicted carbon and nutrients stocks

Point-predicted values of Total C, Total Ca and Total P contents were taken to be equivalent to the average content of the respective element expected to be found in blocks of 0.2 m^3. This was possible since point and block universal cokriging should yield equivalent predictions given the existing sampling density and the chosen prediction grid spacing. Then, the risk of underestimating the true prediction errors is minimized since block kriging yields lower estimates of the prediction error variance than point kriging (Oliver and Webster, 2014).

Although it is often reported that ADE are rich in ceramic fragments, quantitative data is rarely shown. Therefore, the relative contribution of ceramics volume to the total soil volume is often unknown. In our study area, the volume of coarse fragments (i.e. ceramics fragments) per volume of soil was generally below 1% and never larger than 4%. In fact, ceramics were absent in 65% of our soil samples (Fig. 2b). Therefore, the volume of coarse fragments was assumed to be negligible and was not included to estimate the stocks.

Data on soil bulk density were obtained from three soil profiles located in our study area (P1, P2 and P3 in Fig. 2a) and described in previous studies (Macedo, 2012; Neves Júnior, 2008). These data could not be readily used as the soil depths intervals did not match the 20-cm soil layers of our study. Therefore, we fitted a model to the soil bulk density data using the depth at the centre of the sampled soil layers as predictor variable. To account for nonlinearities in the relation between soil bulk density and soil depth, the latter was transformed into *m* sets of new variables using natural spline basis functions (Hastie et al., 2009). These consisted of *m* cubic polynomial functions fitted to *m* adjacent subsets of the soil depth data, each subset being defined by breakpoints (knots) at the appropriate *m*-1 percentiles. The collection of polynomial functions was constrained to be continuous at the inner knots and linear beyond the boundary knots. Based on leave-one-out cross-validation results (data not shown), we found the best number of degrees of freedom of the natural cubic spline (the number of piecewise polynomials) to be $m = 5$. The fitted model was used to predict soil bulk density at the centre of the five 20-cm soil layers (i.e. 10, 30, 50, 70, and 90 cm) (Fig. 2c). Predicted values were then assumed to be equivalent to the average soil bulk density of the respective depths throughout the entire study area.

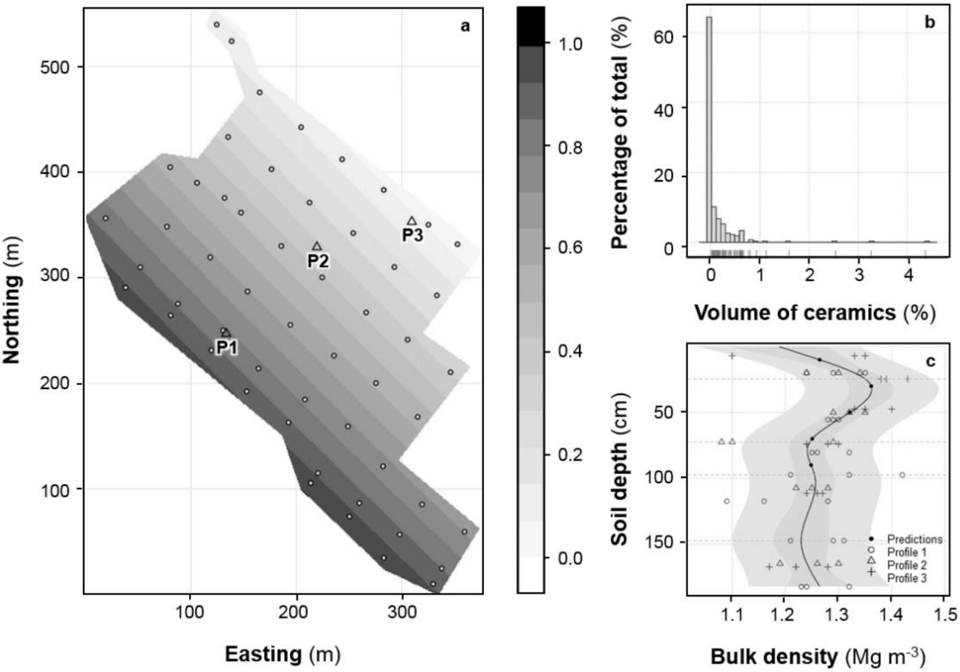

Fig. 2. (a) Expected anthropic enrichment gradient (covariate). Values range linearly from 0 to 1, where 1 means maximum enrichment. The location of the n = 53 sampling points and the three soil profiles (P1, P2 and P3) used to model soil bulk density are indicated as circles and triangles, respectively. (b) Frequency distribution of the volume of ceramics to the total volume of soil. (c) Soil bulk density modelled as a function of soil depth. The 90% confidence and prediction intervals around the fitted natural spline are shown in dark and light grey, respectively. Horizontal dashed lines indicate the position of the interior knots of the natural cubic spline.

Depth-wise predictions of Total C, Total Ca and Total P stocks (kg m^{-2}) were done by multiplying the predicted contents (g kg^{-1}) to the predicted soil bulk density (BD, Mg m^{-3}) and the known thickness of the soil layer (0.2 m). Depth-wise uncertainty of predicted stocks was approximated using a straightforward first-order Taylor series expansion (Heuvelink et al., 1989). For instance, the standard deviation (σ) of a total stock in an arbitrary block of 0.2 m³ was calculated as:

$$\sigma_{\mathrm{stock}} \approx 0.2 \cdot (\mathrm{BD}^2 \cdot \sigma^2_{\mathrm{content}} + \mathrm{content}^2 \cdot \sigma^2_{\mathrm{BD}})^{0.5} \tag{5}$$

Profile-wise prediction of the stocks (kg m^{-2}) was done by stacking all five depth-wise predictions and then computing the combined stocks in blocks of 1.0 m^3. Profile-wise uncertainty of predicted stocks was approximated by squaring the standard deviation (σ) of each of the 0.2 m³ stacked blocks, computing the sum and taking the square root of that value.

Classification of pretic and non-pretic areas

The pretic horizon, among other diagnostic criteria, is a dark surface horizon with one or more layers with a combined thickness of ≥ 20 cm, that has: (i) ≥ 10 g kg^{-1} Organic carbon; (ii) ≥ 2 $cmol_c$ kg^{-1} Exchangeable Ca + Mg and (iii) ≥ 30 mg kg^{-1} Extractable P (IUSS Working Group WRB, 2015). Here, we compute the probability of these three soil properties at the first soil depth (0–20 cm) to meet the diagnostic criteria by accounting the number of times the pretic horizon criteria were met in 1000 simulations at each of the 94,462 points of the simulation grid. The three probabilities were multiplied and the value was taken as the estimated pretic probability (p_{pretic}). Finally, a cut-off at $p_{\mathrm{pretic}} \geq 0.9$ was used to differentiate pretic from non-pretic areas. Total C, Total Ca and Total P stocks on each area were then computed separately to evaluate the relative contribution of pretic and non-pretic areas to the total stocks.

2.3. Results and Discussion

Soil properties

Depth-wise empirical distribution of soil properties is shown in Fig. 3. Total C, Total Ca and CEC exhibited a trend to decrease with soil depth, as well as their variation, although the distribution remained skewed. Total P and pH remained approximately constant with soil depth. However, the variance of Total P increased with soil depth, as well as its skewness. The variance of pH exhibited an approximately symmetric distribution. The clay content increased with soil depth with approximately constant variance and symmetric distribution.

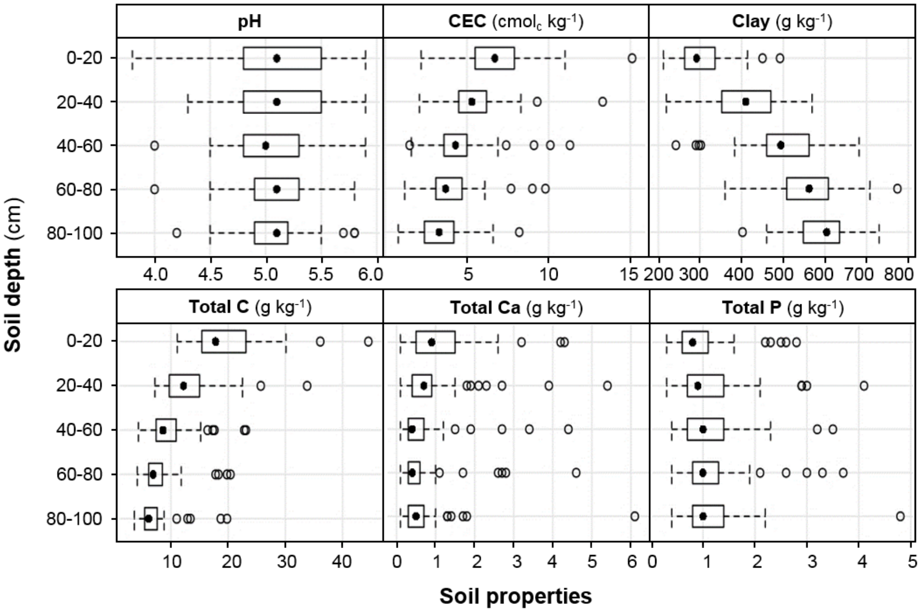

Fig. 3. Depth-wise empirical distribution of Total C, Total Ca, Total P, potential CEC (at pH = 7.0), pH and clay content (Clay) in n = 53 observation locations at five 20-cm soil layers (265 soil samples). The filled black dot in each box-and-whisker plot represents the median or second quartile (0.5), while the box range indicates the first and third quartiles (0.25, 0.75) defining the interquartile interval. The whisker length represents data points that are not distant from the box ≥ 1.5 times the box length, while the empty circles indicate extreme values.

Linear regression model parameters

Estimated depth-wise linear regression model parameters are shown in Table 1. The amount of variance explained given by the coefficients of determination (R^2) was overall low (0.01 to 0.34). Out of the three elements, Total C had the lowest R^2 values (0.01 to 0.08) and the data fit to the model was not significant in all soil depths ($p >$ 0.05). Compared to Total C, Total Ca had slightly higher R^2 values (0.07 to 0.24) and Total P had the highest R^2 values (0.24 to 0.34). Total Ca and P data fit to the model was significant in all soil depths ($p < 0.05$), except for Total Ca at 80–100 cm ($p >$ 0.05).

Table 1. Depth-wise linear regression model coefficients fitted to Total C, Total Ca and Total P and the amount of variance explained by the covariate as measured with the regression sum of squares and the coefficient of determination (R^2).

Soil depth (cm)	**Intercept**	**Covariate**	**Sum of Squares**	R^2	*p*
Total C					
0–20	-0.99 (0.51)	0.54 (0.26)	3.91	0.08	0.05
20–40	-0.78 (0.51)	0.42 (0.27)	2.44	0.05	0.12
40–60	-0.67 (0.52)	0.36 (0.27)	1.79	0.03	0.18
60–80	-0.40 (0.52)	0.22 (0.27)	0.65	0.01	0.43
80–100	-0.28 (0.52)	0.15 (0.27)	0.30	0.01	0.59
Total Ca					
0–20	-1.77 (0.46)	0.96 (0.24)	12.36	0.24	0.00
20–40	-1.47 (0.48)	0.79 (0.25)	8.52	0.16	0.00
40–60	-1.62 (0.47)	0.87 (0.25)	10.36	0.20	0.00
60–80	-1.60 (0.47)	0.86 (0.25)	10.12	0.19	0.00
80–100	-0.96 (0.51)	0.52 (0.26)	3.64	0.07	0.06
Total P					
0–20	-1.79 (0.46)	0.97 (0.24)	12.68	0.24	0.00
20–40	-1.97 (0.44)	1.07 (0.23)	15.43	0.30	0.00
40–60	-2.11 (0.43)	1.14 (0.22)	17.64	0.34	0.00
60–80	-2.07 (0.43)	1.12 (0.23)	16.94	0.33	0.00
80–100	-1.91 (0.45)	1.03 (0.23)	14.45	0.28	0.00

Data were Box-Cox transformed and standardized to zero mean and unit standard deviation prior to analysis. The approximate standard errors of the fitted coefficients are shown in brackets. The *p* values of the *F* statistics indicate the approximate significance of the covariate.

Predicted carbon and nutrients stocks

Depth- and profile-wise maps of predicted Total C, Total Ca and Total P stocks and maps of the associated standard deviation of predictions are shown in Fig. 4, Fig. 5 and Fig. 6, respectively. Stocks are commonly reported in Mg ha^{-1} for a certain soil depth. Here, given the point-prediction spacing (1.0 m), stocks are reported in kg m^{-2} in depth- (0.2 m) and profile-wise (1.0 m) maps.

Depth-wise maps of predicted Total C, Total Ca and Total P stocks indicated enrichments in the SW sector that decrease gradually with soil depth. One patch in the SW sector exhibited the highest Total C stocks (~10.0 kg m^{-2}) in the uppermost soil layer (0–20 cm). This evidence fades down to 40–60 cm soil depth (~4.0 kg m^{-2}) and diffuses linearly in the NE direction. The Total C stocks in the last two soil depths (60–80 and 80–100 cm) were rather homogenous (~1.0 to 3.0 kg m^{-2}) for the entire study area (Fig. 4a). Large patches enriched in Ca (~0.8 kg m^{-2}) were identified in the uppermost soil layer (0–20 cm). This evidence fades down with soil depth while diffusing in the NE direction. The Total Ca stocks were up to ~0.5 kg m^{-2} even at the lowest soil layer (80–100 cm) (Fig. 5a). The patches with the highest Total C and Total Ca stocks diffusing from the SW to the NE sector, can also be seen on the maps of predicted Total P stocks. The highest Total P stocks (~1.0 kg m^{-2}) were found in subsurface layers (20–40 and 40–60 cm). Nonetheless, Total P stocks were up to 0.6 kg m^{-2} even at the lowest soil layer (80–100 cm) (Fig. 6a). Profile-wise maps of predicted Total C, Total Ca and Total P stocks emphasized the highest stocks in the SW sector as well as the enrichment gradient that diffuses in the NE direction (Fig. 4c, Fig. 5c and Fig. 6c). Depth- (Fig. 4b, Fig. 5b and Fig. 6b) and profile-wise (Fig. 4d, Fig. 5d and Fig. 6d) maps of associated standard deviation of predictions showed that uncertainties were higher in the SW sector given the extreme values found in this part of the study area.

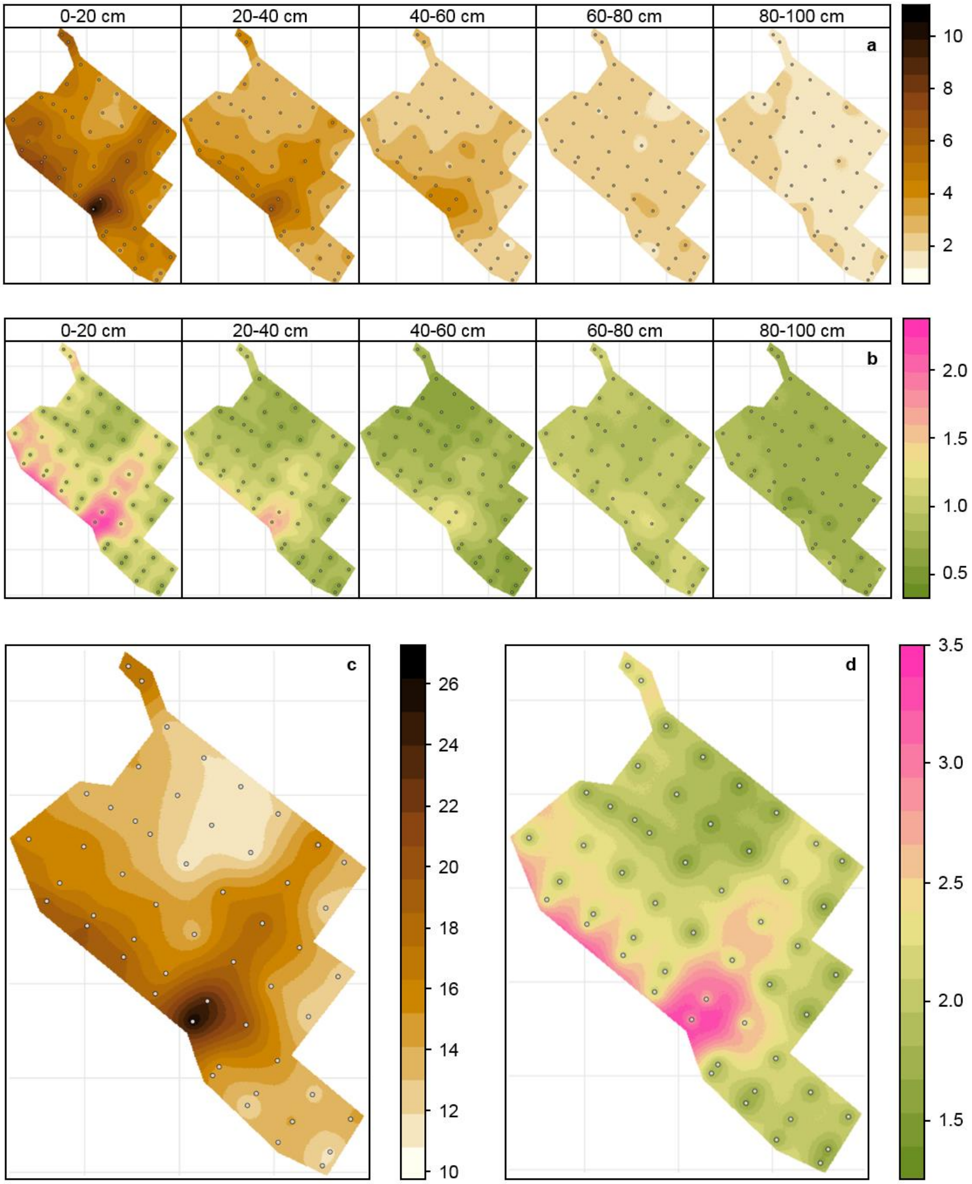

Fig. 4. Depth- (0.2 m) and profile-wise (1.0 m) maps of predicted Total C stocks (kg m^{-2}) (a and c, respectively) including the maps of the associated standard deviation (kg m^{-2}) (b and d, respectively).

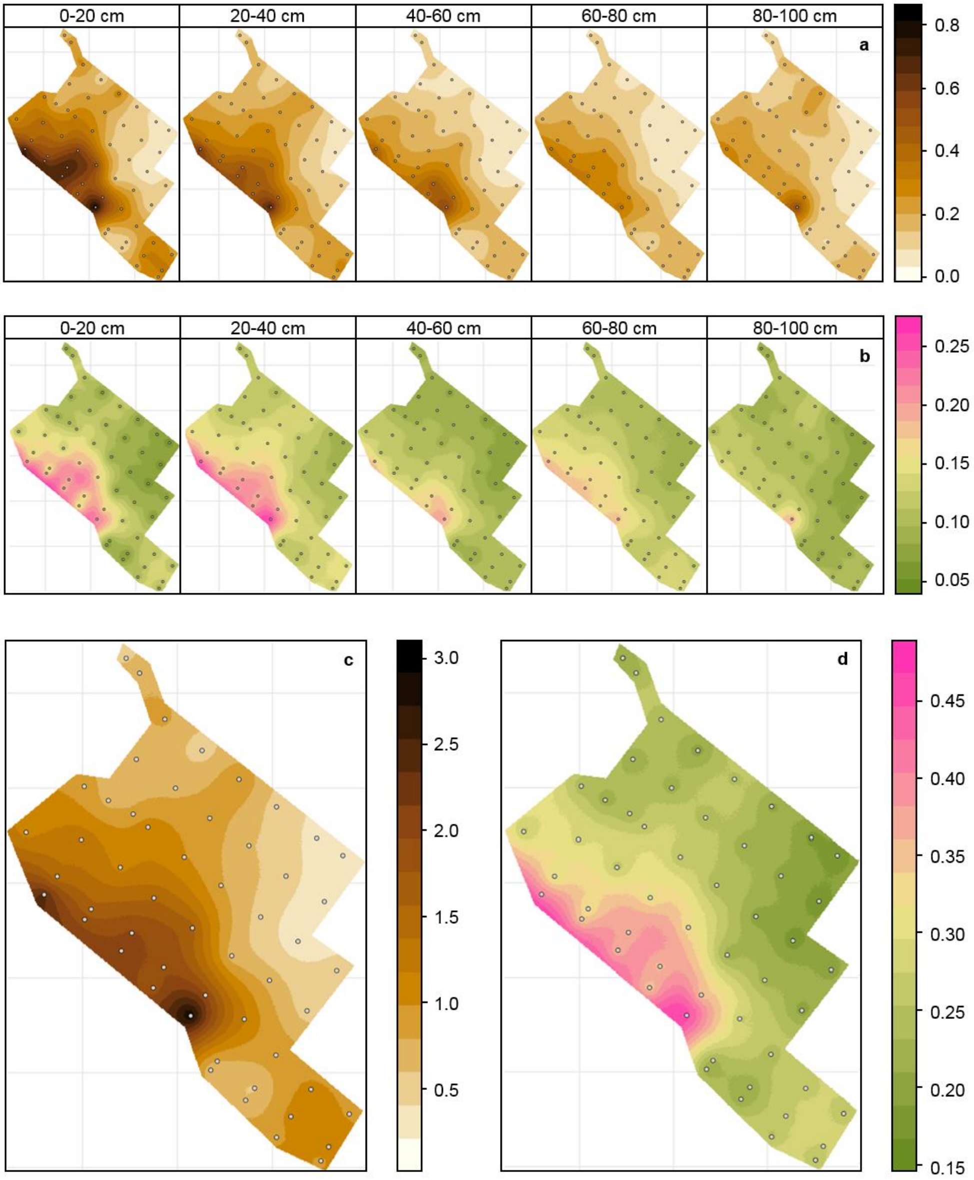

Fig. 5. Depth- (0.2 m) and profile-wise (1.0 m) maps of predicted Total Ca stocks (kg m^{-2}) (a and c, respectively) including the maps of the associated standard deviation (kg m^{-2}) (b and d, respectively).

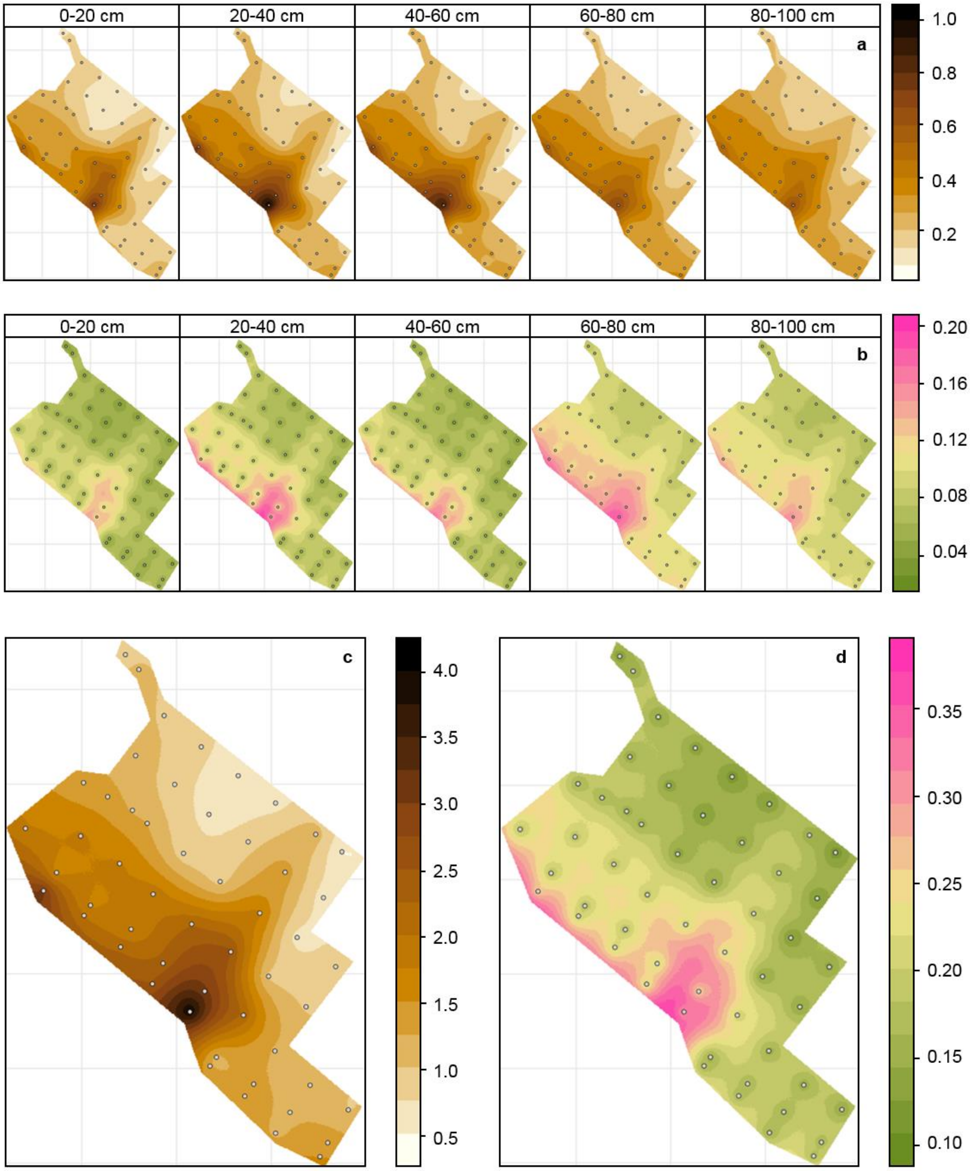

Fig. 6. Depth- (0.2 m) and profile-wise (1.0 m) maps of predicted Total P stocks (kg m^{-2}) (a and c, respectively) including the maps of the associated standard deviation (kg m^{-2}) (b and d, respectively).

Probability of point-predictions to meet the pretic horizon criteria (p_{pretic})

The probability map of point-predictions to meet the pretic horizon criteria (p_{pretic}) according to Total C, Exchangeable Ca + Mg and Extractable P is shown in Fig. 7. The pretic horizon criteria (and a cut-off at $p_{\text{pretic}} \geq 0.9$) allowed us to clearly distinguish two main areas with nearly half of the sampling points located on each side. One area was located in the SW sector (~4.2 ha) and exhibited the highest probability to find a pretic horizon (p_{pretic} values close to 1), whereas the other area was located in the NE sector of the study area (~5.2 ha) with the lowest probability to find a pretic horizon (p_{pretic} values close to 0). The transition between these two main areas of either very high or very low p_{pretic} was rather sharp.

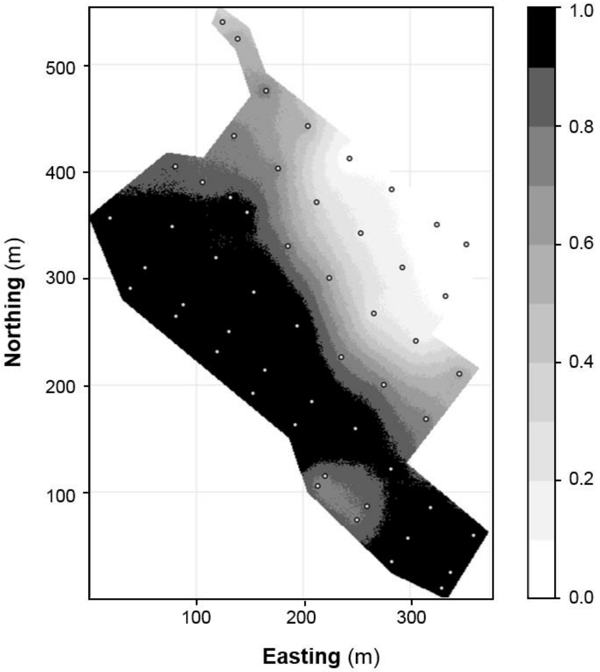

Fig. 7. Probability map of point-predictions to meet the pretic horizon criteria (p_{pretic}) according Total C, Exchangeable Ca + Mg and Extractable P. Soil properties were simulated separately and the lowest (most restrictive) probability was taken as the estimated pretic probability (p_{pretic}). Values vary from 0 to 1 and a cut-off at $p_{\text{pretic}} \geq 0.9$ was used to differentiate pretic from non-pretic areas.

Depth- and profile-wise linear regression model parameters did not indicate a great importance of the chosen covariate (i.e. expected anthropic enrichment gradient) to explain the spatial variation of Total C stocks (Table 1). Low R^2 values indicate low accuracy (how close a value is to the true value) and low precision (standard deviation to the true value). Nonetheless, the chosen covariate indicated a greater importance for Total Ca stocks and most importantly, Total P stocks (Table 1). Costa and Kern (1999) pointed out that high carbon turnover rates in the tropics likely hinders past interpretations. Therefore, Total Ca and Total P stocks are possibly better markers of the anthropic enrichment in ADE in contrast to Total C stocks. Costa et al. (2013) suggested that phosphorus can be an important indicator and delimiter of anthropic areas due to high affinity to bind to iron and aluminium oxides in the soil.

Depth- and profile-wise maps of predicted Total C, Total Ca and Total P stocks exhibited high spatial variation in our study area. Therefore, limited amount of soil samples selected on highly contrasting areas based on field observations (i.e. dark- versus light-coloured soils and presence versus absence of archaeological remains) are likely to give an unrealistic estimation of the overall carbon and nutrients stocks in ADE. Costa and Kern (1999) reported that only the surface horizon exhibited anthropic enrichments. However, here we showed that enrichments can be observed even in deep soil layers. One patch in the SW sector exhibited the highest stocks for all three elements (Fig. 4 to Fig. 6). Given the sampling density limitation to capture the structure of the spatial variation in the short-scale (< 50 m), the uncertainties of predictions are higher around this one patch with extreme values.

The probability map of point-predictions to meet the pretic horizon criteria (p_{pretic}) indicated that the p_{pretic} was higher in the SW sector (Fig. 7). Nonetheless, depth-wise maps of predicted total stocks showed that the non-pretic area can also exhibit stocks as high as the pretic area (Fig. 4a to Fig. 6a). In this sense, we reinforce that limited soil sampling is unlikely to capture the complexity of the spatial variation of soil properties in ADE. Studies with extensive soil sampling (Fraser et al., 2011; Schmidt et al., 2014), suggested that the transition from ADE to the adjacent soil is characterized by a soil fertility continuum. In addition, Fraser et al. (2011) pointed out that ADE are sometimes subcategorized into *Terra Preta* and *Terra Mulata*. The term *Terra Preta* refers to the typical carbon- and nutrient-enriched soils with archaeological remains, whereas *Terra Mulata* refers to a less enriched soil with few or no evidence of archaeological remains. Nonetheless, both soil categories would differ from the adjacent soil. Costa et al (2013) reported that it was not possible to identify a specific geochemical signature for *Terra Mulata*. In our study, we did not find indications to support a subcategory within ADE (i.e. *Terra Mulata*) nor did we find a smooth transition between ADE and the adjacent soil. Here, we showed that the pretic horizon criteria (and a cut-off at $p_{\text{pretic}} \geq 0.9$) allowed us to clearly differentiate two unambiguous areas with a sharp transition, rather than a smooth continuum, despite high spatial variation in our study area.

Depth-wise Total C, Total Ca and Total P stocks in pretic and non-pretic areas are shown in Fig. 8. Overall, the predicted carbon and nutrients stocks were higher in the pretic area. However, the additional stocks in the pretic area (enrichment factor) varied per element and soil depth (Fig. 8). The overall Total C stock was 162.9 Mg ha^{-1} m^{-1} in the pretic area and was on average only 14% larger than the non-pretic area. We used an average soil bulk density of 1.3 Mg m^{-3} to estimate the stocks in other studies and compare with our results. Overall, the range of carbon stocks reported for the soils of the Amazon basin is very broad. Moraes et al. (1995) reported an overall mean carbon stock of about 100 Mg ha^{-1} m^{-1} for soils in the Amazon basin, while Glaser et al. (2002) reported values in ADE as high as 500 Mg ha^{-1} m^{-1}. Lima et al. (2002) investigating A horizons in a toposequence in the Amazon, reported carbon stocks from 54.7 to 134.9 Mg ha^{-1} in ADE and 6.3 to 35.4 Mg ha^{-1} in the adjacent soil. In general, nutrient stocks in soils of the Amazon basin have been less investigated than carbon stocks. In our study, the overall Total Ca and Total P stocks were 14.2 and 19.0 Mg ha^{-1} m^{-1} in the pretic area as opposed to 6.6 and 11.0 Mg ha^{-1} m^{-1} in the non-pretic area. These values correspond to 2.1- and 1.7-fold larger stocks in the pretic area for Total Ca and Total P stocks, respectively. Costa et al. (2013) reported values corresponding to 2.8 Mg ha^{-1} and 2.3 Mg ha^{-1} for Total Ca and Total P stocks at 0–20 cm, respectively. In contrast, the adjacent soil exhibited values corresponding to only 0.4 and 0.5 Mg ha^{-1} for Total Ca and Total P stocks at 0–20 cm, respectively.

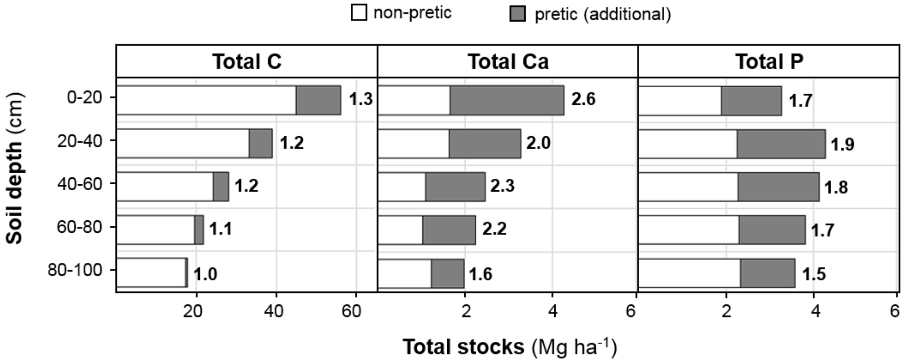

Fig. 8. Depth-wise Total C, Total Ca and Total P stocks in pretic and non-pretic areas. The stocks in the pretic area are the sum of the stocks in the non-pretic area (empty bars) plus the additional stocks in the pretic area (filled bars). The enrichment factors per soil depth are shown on the right side of each bar.

Besides the commonly reported high carbon and nutrients contents in ADE, one of the most remarkable characteristics of ADE is the higher CEC when compared to the adjacent soils. Liang et al. (2006) reported a high correlation between CEC and the carbon content in ADE. In our study, the correlation between the CEC and the Total C content was also higher in the pretic area ($R^2 = 0.82$, $p < 0.05$, 125 soil samples) than the non-pretic area ($R^2 = 0.57$, $p < 0.05$, 140 soil samples). Even though the Total C content decreased with depth, the ratio between CEC and the Total C content (CEC:C) increased with soil depth, most importantly in the pretic area (Fig. 9a). Data in Glaser et al. (2000) showed higher CEC:C in ADE when compared to the adjacent soil at surface horizons (0.33 against 0.08 in ADE and the adjacent soil at 0–10 cm, respectively). Here, we showed that the CEC:C is even greater in deeper soil layers (Fig. 9a). Data in Oliveira et al. (2014) also showed higher CEC:C with increasing soil depth in ADE under different land use (0.44 in ADE at 0–10 cm under corn and 2.46 in ADE at 100–130 cm under orchard). Even though the clay content increased with soil depth in our study area (Fig. 3), we did not observe differences in the soil texture between pretic and non-pretic areas (Supplementary Data). Therefore, the differences in CEC:C ratio between these areas may be attributed to carbon-related characteristics. Studies have shown that oxidation of black carbon (BC) is a key trait explaining high CEC in ADE (Glaser and Birk, 2012; Hiemstra et al., 2013; Liang et al., 2013; Novotny et al., 2007). Conceding that ADE is a model to sustainable agriculture in the tropics, higher CEC per unit of carbon is certainly a desirable characteristic to be reproduced.

There was a high significant correlation between Total Ca and Total P contents in the pretic area ($R^2 = 0.78$, $p < 0.05$, 125 soil samples). Conversely, the correlation between Total Ca and Total P contents in the non-pretic area was close to zero and not significant ($R^2 = 0.03$, $p > 0.05$, 140 samples) (Fig. 9b). This clearly indicates different sources and reaction pathways for these elements in pretic and non-pretic areas. Sato et al. (2009) investigating a chronosequence of ADE sites reported that biogenic calcium phosphate (i.e. bone-derived) disappeared after approximately 2000 years of ADE formation. Biogenic calcium phosphate transformations into more soluble fractions coincided with increased phosphorus adsorbed on soil oxides, organic phosphorus and occluded phosphorus. In addition, these authors reported that degradation of biogenic calcium phosphate occurred ten times faster than that of geogenic calcium phosphate (i.e. mineral-derived) under tropical conditions. Costa et al. (2013) also reported a high correlation between Ca and P values in ADE when compared to adjacent soils. In addition, these authors reported areas enriched in P where no ceramic was found, which may indicate the location of disposal areas, particularly of animal origin (i.e. bones). For instance, the one patch in our study area where the highest stocks were found (Fig. 4 to Fig. 6) could have been a former waste disposal area (Costa et al., 2013; Schmidt et al., 2014). Studies commonly describe ADE as rich in ceramics and some studies even suggested that ceramics may be an important source of nutrients in ADE (Valente and Costa, 2017). However, ceramics fragments were totally absent in the majority (> 65%) of the soil samples in our study area (Fig. 2b). Therefore, effects of ceramics on enrichment of nutrients are likely to be of minor importance and extremely localized.

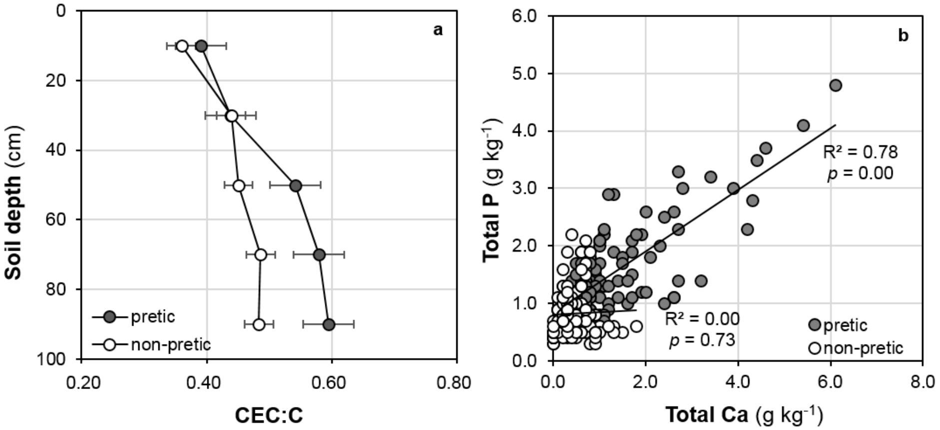

Fig. 9. (a) Depth-wise CEC (cmol$_c$ kg^{-1}) per Total C content (g kg^{-1}) (CEC:Total C). (b) Relation between Total Ca and Total P contents in pretic (125 samples) and non-pretic areas (140 soil samples).

Interdisciplinary studies are highly recommended to understand the complexity of ADE (Kern et al., 2017). Despite decades of research in ADE, direct comparison of data between studies is hindered by different analytical approaches. Soil samples can be analysed by different methods to determine either exchangeable or total contents. It is important to note that carbon stocks are commonly estimated with carbon content determined by the Walkley-Black (WB) method (Walkley and Black, 1934). However, the reliability of this method to account for BC has been questioned (Knicker et al., 2007) since the resistance of BC to sodium dichromate oxidation and its recovery is dependent on BC intrinsic level of oxidation and methodological reaction conditions (Hardy and Dufey, 2017). Therefore, if BC is of great importance in ADE (Glaser, 2007; Glaser et al., 2001b), then the WB method is unlikely to provide a realistic estimation of the carbon content in ADE or any other soil expected to be enriched in BC. Here, we determined Total C by elemental analysis to avoid underestimation of the carbon content.

It is possible that several mechanisms are simultaneously acting to explain the striking characteristics of ADE. For carbon, it is worth to note that Total C determined by elemental analysis accounts for carbon of different origins and different turnover rates. On the one hand, part of the carbon content likely exhibits a high turnover rate that hinders past interpretations (Costa and Kern, 1999). On the other hand, BC found in ADE is expected to remain in the soil for longer periods due to its inherent stability (Glaser, 2007; Haumaier and Zech, 1995; Novotny et al., 2007). In this sense, specific assessment of the BC content may contribute to better interpretations of the carbon content in ADE. For calcium, higher CEC derived from BC oxidation likely enhances Ca adsorption (Archanjo et al., 2014). For phosphorus, it has been suggested that adsorption of phosphate (PO_4^{-3}) and SOM to soil oxides may inhibit their further growth and polymerization (Eusterhues et al., 2008; Fukushi and Sato, 2005) and are likely to be the cause of nano-sized particles with high surface area in soils (Hiemstra et al., 2010b). A higher surface area likely increases the amount of adsorbed SOM that can be of BC and non-BC origin. However, since PO_4^{-3} may compete with SOM for adsorption sites on the surface of oxides (Antelo et al., 2007; Weng et al., 2008), the trade-offs are poorly understood. Therefore, further research is still needed to better understand the relative contribution of these different mechanisms in order to reproduce ADE remarkable characteristics that are desirable for sustainable agriculture.

2.4. Conclusion

The use of the pretic horizon criteria in combination with a cut-off at $p_{\text{pretic}} \geq 0.9$ permits an unambiguous identification of distinctive areas in ADE. In contrast to previous ADE studies, the presented method generates a sharp transition between two areas rather than a smooth continuum. Total Ca stocks and especially Total P stocks were better markers of anthropic enrichment in ADE than Total C stocks. Depth- and profile-wise linear regression model parameters indicated a greater importance of the chosen environmental covariate (i.e. expected anthropic enrichment gradient) to explain the spatial variation of Total Ca and Total P stocks than Total C stocks. The overall Total Ca and Total P stocks were twice as large in the pretic area when compared to the non-pretic area. In addition, there was a high significant correlation between Total Ca and Total P contents in the pretic area, whereas no correlation was found in the non-pretic area. This clearly indicates different sources and reaction pathways for these elements in pretic and non-pretic areas.

2.5. Acknowledgments

We acknowledge the contribution of G.B.M. Heuvelink and two anonymous reviewers for the improvement of our manuscript. This work was done under the scope of the Terra Preta Program and funded by the Interdisciplinary Research and Education Fund (INREF) of Wageningen University and Research (WUR) and the Brazilian Agricultural Research Corporation (Embrapa).

Supplementary Data

Linear models of coregionalization fitted to the auto- and cross-variograms for Total C, Total Ca and Total P are shown in Fig. S1, Fig. S2, and Fig. S3, respectively. The slope of the exponential covariance functions for Total C, Total Ca and Total P are shown subsequently in Table S1, Table S2 and Table S3, respectively. Soil texture and the respective average content of sand, silt and clay per soil depth in the pretic and non-pretic area are shown in Table S4.

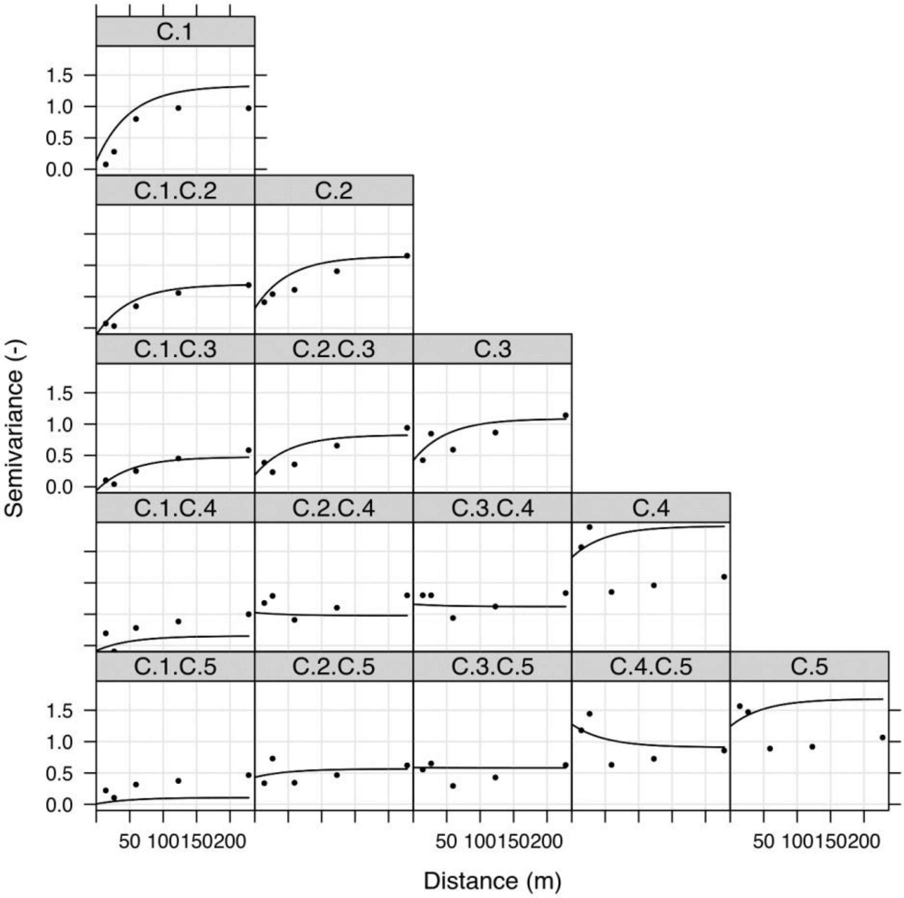

Fig. S1. Linear model of coregionalization fitted to auto- and cross-variograms of the Box-Cox transformed Total C for the five soil depths (1 = 0–20 cm, 2 = 20-40 cm, 3 = 40–60 cm, 4 = 60-80 cm, and 5 = 80–100 cm). The range parameter was fixed at 50 m because it minimized the leave-one-out cross-validation mean error (C.1 = 0.0051, C.2 = 0.0026, C.3 = -0.0015, C.4 = -0.0035, and C.5 = 0.0066).

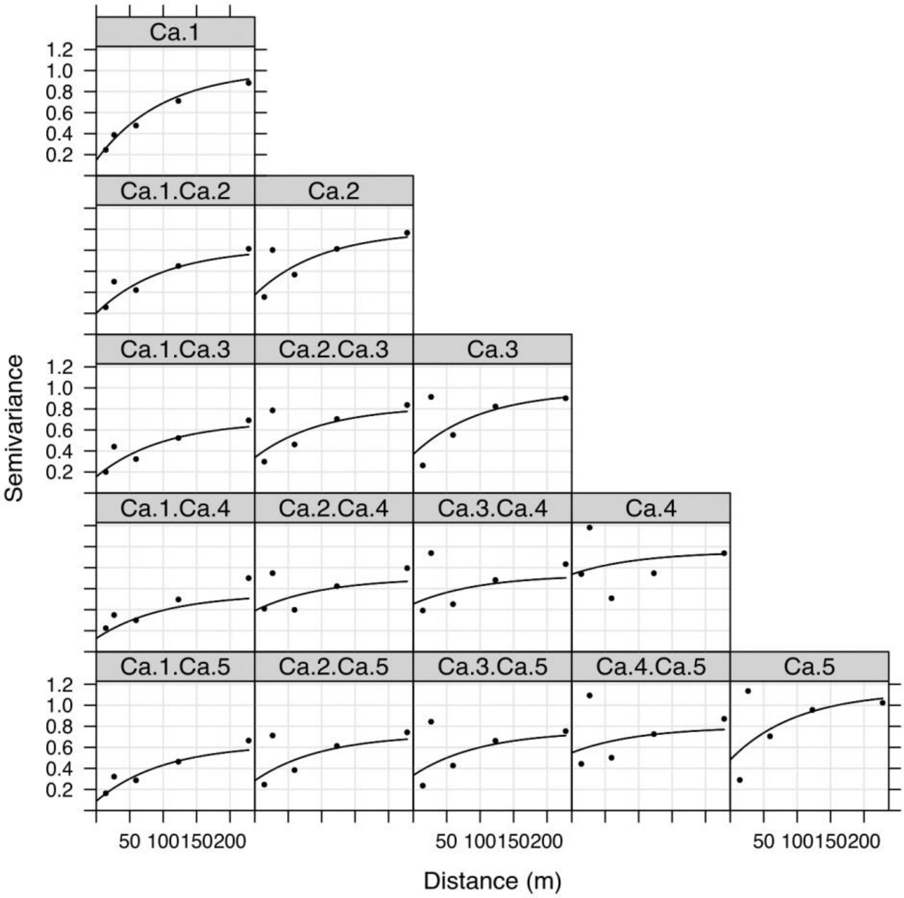

Fig. S2. Linear model of coregionalization fitted to auto- and cross-variograms of the Box-Cox transformed Total Ca for the five soil depths (1 = 0–20 cm, 2 = 20-40 cm, 3 = 40–60 cm, 4 = 60-80 cm, and 5 = 80–100 cm). The range parameter was fixed at 100 m because it minimized the leave-one-out cross-validation mean error (Ca.1 = 0.0012, Ca.2 = 0.0046, Ca.3 = 0.0034, Ca.4 = 0.0007, and Ca.5 = 0.0032).

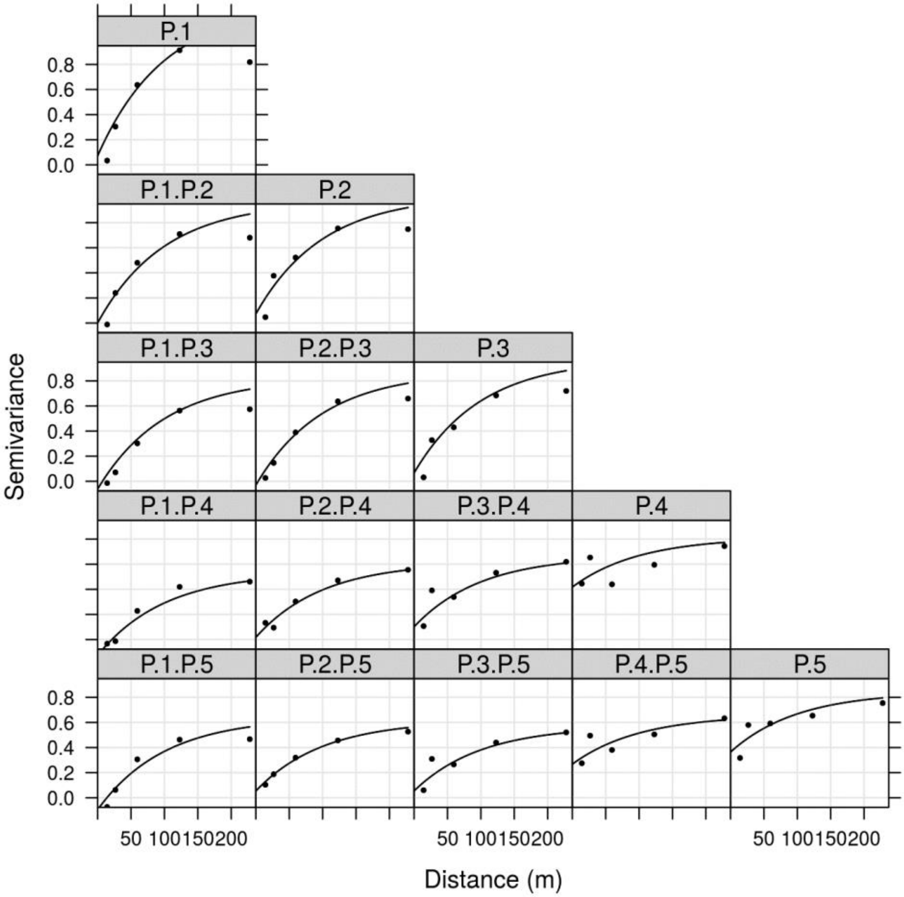

Fig. S3. Linear model of coregionalization fitted to auto- and cross-variograms of the Box-Cox transformed Total P for the five soil depths (1 = 0–20 cm, 2 = 20-40 cm, 3 = 40–60 cm, 4 = 60-80 cm, and 5 = 80–100 cm). The range parameter was fixed at 100 m because it minimized the leave-one-out cross-validation mean error (P.1 = 0.0150, P.2 = 0.0136, P.3 = 0.0119, P.4 = 0.0077, and P.5 = 0.0095).

Table S1. Slope of the exponential covariance function at the origin for Total C.

	C.1 (0.0800)	C.2 (0.0687)	C.3 (0.0652)	C.4 (0.1144)	C.5 (0.1010)
C.2	0.0417				
C.3	0.0285	0.0497			
C.4	0.0091	0.0287	0.0373		
C.5	0.0064	0.0339	0.0349	0.0546	0.0601
NA					0.0601

The numbers indicate the soil depth (1 = 0–20 cm, 2 = 20-40 cm, 3 = 40–60 cm, 4 = 60-80 cm, and 5 = 80–100 cm).

Table S2. Slope of the exponential covariance function at the origin for Total Ca.

	Ca.1 (0.0302)	Ca.2 (0.0297)	Ca.3 (0.0292)	Ca.4 (0.0286)	Ca.5 (0.0340)
Ca.2	0.0247				
Ca.3	0.0206	0.0248			
Ca.4	0.0165	0.0210	0.0220		
Ca.5	0.0189	0.0217	0.0227	0.0239	0.0300
NA					0.0300

The numbers indicate the soil depth (1 = 0–20 cm, 2 = 20-40 cm, 3 = 40–60 cm, 4 = 60-80 cm, and 5 = 80–100 cm).

Table S3. Slope of the exponential covariance function at the origin for Total P.

	P.1 (0.0382)	P.2 (0.0305)	P.3 (0.0293)	P.4 (0.0245)	P.5 (0.0254)
P.2	0.0291				
P.3	0.0248	0.0262			
P.4	0.0160	0.0185	0.0200		
P.5	0.0192	0.0185	0.0172	0.0198	0.0300
NA					0.0300

The numbers indicate the soil depth (1 = 0–20 cm, 2 = 20-40 cm, 3 = 40–60 cm, 4 = 60-80 cm, and 5 = 80–100 cm).

Table S4. Soil texture and the respective average content of sand, silt and clay contents (in g kg^{-1}) per soil depth in the pretic and non-pretic area.

Soil depth (cm)	Soil texture	pretic			non-pretic		
		Sand	Silt	Clay	Sand	Silt	Clay
0–20	Sandy clay loam	536.8	147.6	315.7	575.9	136.9	287.2
20-40	Sandy clay	447.7	128.5	423.8	490.5	113.2	396.3
40–60	Clay	375.0	111.1	513.9	409.0	108.2	482.7
60-80	Clay	327.6	95.5	577.0	356.5	102.7	540.7
80–100	Clay	305.8	91.1	603.1	320.4	97.6	581.9

Chapter 3

Isolating a stable pool of soil organic matter upon chemical oxidation with NaOCl

Carlos Francisco Brazão Vieira Alho[a], Jose Luis Munera-Echeverri[b], Wenceslau Geraldes Teixeira[c], Tjisse Hiemstra[d], Thomas W. Kuyper[a]

[a] Soil Biology Group, Wageningen University and Research, 6700 AA, Wageningen, the Netherlands.
[b] Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, NO-1432, Ås, Norway.
[c] Embrapa Soils, 22460-000, Rio de Janeiro, Brazil.
[d] Soil Chemistry and Chemical Soil Quality Group, Wageningen University and Research, 6700 AA, Wageningen, the Netherlands.

Abstract

Long-term persistence of soil organic matter (SOM) is currently interpreted as an ecosystem property, where the role of soil minerals in organo-mineral associations that protect SOM from being further decomposed by the soil microbial community is highlighted. SOM stability is still difficult to be assessed operationally since several mechanisms of SOM stabilization are likely to happen simultaneously. Chemical oxidation with sodium hypochlorite (NaOCl) has been suggested as a promising method to isolate a 'stable' pool of SOM. However, it is not clear how such a method can differentiate between a 'labile' and a 'stable' pool of SOM. In this study, we measured the change in organic carbon (OC) content in soils upon consecutive treatments with NaOCl. For this, we used a set of soil samples with a wide range in pH (4.5–7.2), OC content (20.7–56.4 g kg^{-1}), amounts of crystalline Fe and Al oxides (30.7–509.8 and 8.1–12.8 g mmol kg^{-1}, respectively), amounts of poorly-crystalline Fe and Al oxides (64.8–342.2 and 18.6–46.2 mmol kg^{-1}, respectively) and reactive surface area of soil oxides (4.5–32.7 m^2 g^{-1}). Our results showed that we were able to clearly distinguish a 'labile' OC pool (i.e. NaOCl-oxidizable OC) from a 'stable' OC pool (i.e. NaOCl-resistant OC) upon chemical oxidation with NaOCl. The 'labile' OC pool was rapidly oxidized with NaOCl within two consecutive treatments of 2h each and the remaining 'stable' OC pool resisted further consecutive treatments with NaOCl up to 8h. Our procedure is about five times faster than the procedure commonly applied in the literature of three repetitive treatments of 6 h each. Depending on the soil, the 'stable' OC pool varied from ~40–70% of the Total OC content. In contrast to the literature, we did not find a good correlation between the amount of the 'stable' OC pool and mineralogical properties (i.e. Fe and Al phases or reactive surface area). In fact, the 'stable' OC pool showed a significant positive correlation with the Total OC content, which may suggest that the chemical composition of SOM may play a significant role in determining the amount of OC left after NaOCl oxidation. Therefore, future studies should investigate the chemical composition of SOM pools upon chemical oxidation with NaOCl in soil samples with contrasting chemical composition of SOM.

3.1. Introduction

Soil organic matter (SOM) is the largest terrestrial carbon (C) pool (Scharlemann et al., 2014) and elucidating the mechanisms responsible for its the long-term persistence in soils is crucial to better understand the role of SOM in the global C cycle (Stockmann et al., 2013). Long-term persistence of SOM is currently seen as an ecosystem property (Schmidt et al., 2011), where the role of soil minerals and their role in organo-mineral associations that protect SOM from being further decomposed by the soil microbial community is highlighted (Baldock and Skjemstad, 2000; Torn et al., 1997). Three main mechanisms of SOM stabilization have been proposed, such as (i) occlusion in aggregates, (ii) association with soil minerals and (iii) inherent chemical recalcitrance (Sollins et al., 1996). However, the relative contribution of these mechanisms to SOM stabilization is poorly understood (Mikutta et al., 2006).

SOM stability is difficult to be assessed operationally since several mechanisms of SOM stabilization are likely to happen simultaneously. Due to chemical complexity of SOM, fractionation procedures can be a useful tool to distinguish SOM pools that can be linked to the mechanisms of SOM stabilization (Poeplau et al., 2018; von Lützow et al., 2007). Chemical oxidation procedures have been proposed to differentiate SOM pools based on the underlying assumption that chemical oxidation can reflect biological oxidation (Eusterhues et al., 2005). Commonly, two SOM pools are operationally defined (i.e. ‘labile’ and ‘stable’). The ‘labile’ pool refers to the SOM pool that can be oxidized to carbon dioxide (CO_2) upon chemical oxidation, whereas the ‘stable’ pool refers to the SOM pool that resists chemical oxidation due to several mechanisms of SOM stabilization, mainly mineral-association rather than chemical recalcitrance (Eusterhues et al., 2003; Mikutta et al., 2006).

Several chemical oxidants have been investigated for the purpose of isolating a ‘stable’ pool of SOM (Helfrich et al., 2007; Mikutta et al., 2005b) Chemical oxidation with sodium hypochlorite (NaOCl) has been suggested as a promising method among a few others (Poeplau et al., 2018; von Lützow et al., 2007). Anderson (1961) has originally proposed the use of 6% NaOCl for the removal of SOM prior to mineralogical analysis of clays. However, the procedure used to be performed in three short-time cycles (15 min) at high temperature (100 °C) and high pH (9.5), which can have detrimental effects on soil minerals (Siregar et al., 2005). In order to avoid this problem, more recent studies recommended chemical oxidation with NaOCl at room temperature (25 °C) and at a lower pH (8.0), but with extension of the reaction time to 6 h for each of the consecutive treatments (Kaiser and Guggenberger, 2003). Siregar et al. (2005) reported that chemical oxidation with NaOCl indeed removed SOM without affecting soil minerals and this procedure has been recommend to isolate a ‘stable’ pool of SOM (Kleber et al., 2005; Mikutta et al., 2005a). Mikutta et al. (2005b) reported that organic carbon (OC) removal upon chemical oxidation can be little due to strong binding of OC to mineral surfaces, most importantly in soils rich in poorly crystalline iron (Fe) and aluminium (Al) oxides due to their high reactive surface area for binding of OC (Kleber et al., 2005; Mikutta et al., 2005a). However, the number of consecutive treatments and reaction time varies among studies (Kaiser and Guggenberger, 2003; Mikutta et al., 2006) and authors often do not explain the rationale behind the chosen conditions.

Therefore, the aim of this work was to investigate the kinetics of chemical oxidation with NaOCl in order to determine the number of consecutive treatments required to differentiate a ‘labile’ OC pool (i.e. NaOCl-oxidizable OC) from a ‘stable’ OC pool (NaOCl-resistant OC). For this, we used a set of soil samples covering a wide range in soil pH, OC content, amounts of crystalline and poorly-crystalline Fe and Al oxides and reactive surface area of soil oxides. We hypothesized that the amount of NaOCl-resistant OC can be linked to the amount and reactive surface area of Fe and Al oxides.

3.2. Material and Methods

Soil samples and analysis

In this study, we used a subset of Dutch agricultural topsoils samples that had been previously investigated by Hiemstra et al. (2010). These samples exhibit a wide range in pH (4.5–7.2), OC content (20.7–56.4 g kg^{-1}), amounts of crystalline Fe and Al oxides (30.7–509.8 and 8.1–12.8 g mmol kg^{-1}, respectively), amounts of poorly-crystalline Fe and Al oxides (64.8–342.2 and 18.6–46.2 mmol kg^{-1}, respectively) and reactive surface area of soil oxides (4.5–32.7 m^2 g^{-1}) (Table 1). Samples were air-dried at room temperature, passed through a 2 mm sieve, visible roots were removed manually. For further analysis, a subsample was ground in a ball mill.

For statistical analysis, significant differences between mean values of NaOCl-resistant fractions were tested with analyses of variance (ANOVA) followed by HSD Tukey’s test ($p < 0.05$). Linear regression models were used to evaluate the relationship between selected parameters by interpreting the coefficient of determination (R^2) and the significance of the models (p).

Soil pH was determined in 0.01 M $CaCl_2$. The OC content was determined by a modified Walkley-Black procedure (Walkley and Black, 1934). Briefly, 7.5 mL of 12 M sulphuric acid (H_2SO_4) and 5.0 mL of 0.27 M potassium dichromate ($K_2Cr_2O_7$) were pipetted to glass tubes containing 0.3 g of soil. The tubes were homogenized and heated at 135 ˚C for 1h using a heating block. After cooling, the samples were transferred to 50 mL plastic tubes and centrifuged (at 3000 *g* for 10 min). The OC content was derived by determining the remaining dichromate content in the supernatant by measuring the absorbance at 585 nm using a spectrophotometer.

The amount of Fe and Al oxides and the reactive surface area of soil oxides were taken from Hiemstra et al. (2010). The amount of Fe and Al oxides were determined by extractions with dithionite-citrate-bicarbonate (DCB) and ammonium oxalate. Briefly, for the oxalate extraction, soil samples were reacted with a 0.2 M ammonium oxalate ($C_2H_8N_2O_4$) at pH 3.0 in the dark for 2h. For the DCB extraction, soil samples were reacted with 30 mL of 0.66 M sodium dithionite ($Na_2S_2O_4$) and 0.11 M sodium bicarbonate ($NaHCO_3$) solutions in polyethylene centrifuge tubes overnight. Samples were then centrifuged (at 3000 *g* for 10 min) and the supernatants analysed for Fe and Al by Inductively Coupled Plasma coupled with Atomic Emission Spectroscopy (ICP-AES). The amount of oxalate-extractable iron (Fe_o) and aluminium (Al_o) oxides is used as proxy for poorly-crystalline Fe and Al oxides with high reactive surface area, whereas the difference between the amount of DCB- and oxalate-extractable iron (Fe_{d-o}) and aluminium (Al_{d-o}) is used as a proxy for crystalline Fe and Al oxides with low reactive surface area.

The reactive surface area of soil oxides (A) was determined using phosphate (PO_4^{3-}) as a natural ion probe. Briefly, a 0.5 M sodium bicarbonate ($NaHCO_3$) solution adjusted to pH 8.5 by adding 2 M sodium hydroxide (NaOH) was used to desorb PO_4^{3-} at different solid-to-solution ratios. In addition, activated carbon was added to remove SOM released during the extraction. The suspensions were gently shaken for 10-15 days in a reciprocal shaker. After centrifugation, the supernatant was acidified to pH 2.0 by adding 1 M hydrochloric acid (HCl) and analysed for PO_4^{3-} with Segmented Flow Analysis (SFA). The PO_4^{3-} desorption isotherm was interpreted with the charge distribution model (Hiemstra and van Riemsdijk, 1996) to derive the reactive surface area of soil oxides.

Table 1. Soil properties

Sample	**pH**	**OC**	**Fe_d**	**Al_d**	**Fe_o**	**Al_o**	**Fe_{d-o}**	**Al_{d-o}**	**A**
	in $CaCl_2$	(g kg^{-1})	------------------- (mmol kg^{-1}) --------------------						(m^2 g^{-1})
1	5.6	46.6	852.0	27.0	342.2	18.6	509.8	8.4	32.7
2	4.6	39.1	242.0	36.0	211.3	27.5	30.7	8.5	27.3
3	4.9	56.4	318.0	59.0	252.2	46.2	65.8	12.8	18.9
4	4.5	42.9	233.0	29.0	114.4	20.9	118.6	8.1	23.3
5	7.2	20.7	178.0	32.0	64.8	32.2	113.2	n.a.	4.5
6	6.4	49.2	200.0	30.0	117.1	38.9	82.9	n.a.	9.8

Soil pH, Organic carbon (OC), Fe and Al oxides phases and reactive surface area of soil oxides (A) derived from Hiemstra et al. (2010). The amount of oxalate-extractable iron (Fe_o) and aluminium (Al_o) oxides is used as proxy for poorly-crystalline Fe and Al oxides with high reactive surface area, whereas the difference between the amount of DCB- and oxalate-extractable iron (Fe_{d-o}) and aluminium (Al_{d-o}) is used as a proxy for crystalline Fe and Al oxides with low reactive surface area. For details about the methods, please see text.

Kinetics of chemical oxidation with NaOCl

Briefly, 30 mL of 6% NaOCl solution adjusted to pH 8.0 by adding 32% HCl was added to plastic bottles containing 1.5 g of soil. The bottles were shaken in a reciprocal shaker during each reaction time (2h) at room temperature. Samples were then centrifuged (at 2574 *g* for 10 min) and the supernatants discarded. Residues were thoroughly washed with deionized water until electrical conductivity was < 40 µs cm^{-1}, oven-dried (at 70° for 24h) and a subsample was taken for the determination of the OC content in the residue. The NaOCl solution was renewed and the procedure repeated three times with a total of four consecutive treatments. The OC content in the residues was determined by a modified Walkley-Black procedure as previously described.

3.3. Results and Discussion

The OC content decreased rapidly in the first 2h of oxidation and further oxidation was much less pronounced or absent after 4h (Fig. 1). Based on the observed kinetic behaviour, a clear differentiation can be made for our samples between a ‘labile’ OC pool (i.e. NaOCl-oxidizable OC) and a ‘stable’ OC pool (i.e. NaOCl-resistant OC) upon chemical oxidation with NaOCl within 4h. The amount of NaOCl-oxidizable OC after 4h varied from 6.3 to 29.0 g kg^{-1} (Fig.1a). These values correspond to 30.6 to 59.1% of the Total C content (20.7–56.4 g kg^{-1}) (Fig.1b). The amount of NaOCl-resistant OC measured after 4h varied from 14.4 to 34.9 g kg^{-1} (Fig.1a). These values correspond to 40.9 to 69.4% of the Total C content (20.7–56.4 g kg^{-1}) (Fig. 1b). Our results are in agreement with other studies that also used chemical oxidation with NaOCl to isolate a ‘stable’ pool of SOM, but with three consecutive treatments of 6h each (Jagadamma et al., 2010; Mikutta et al., 2006; Thomsen et al., 2009). Nonethelsse, direct comparison among studies is not straightforward since soil type, soil depth, pre-treatments and reaction conditions vary greatly among studies. For further support, we provide an overview of the conditions investigated in some studies that used chemical oxidation with NaOCl in Table S1 (Supplementary Data).

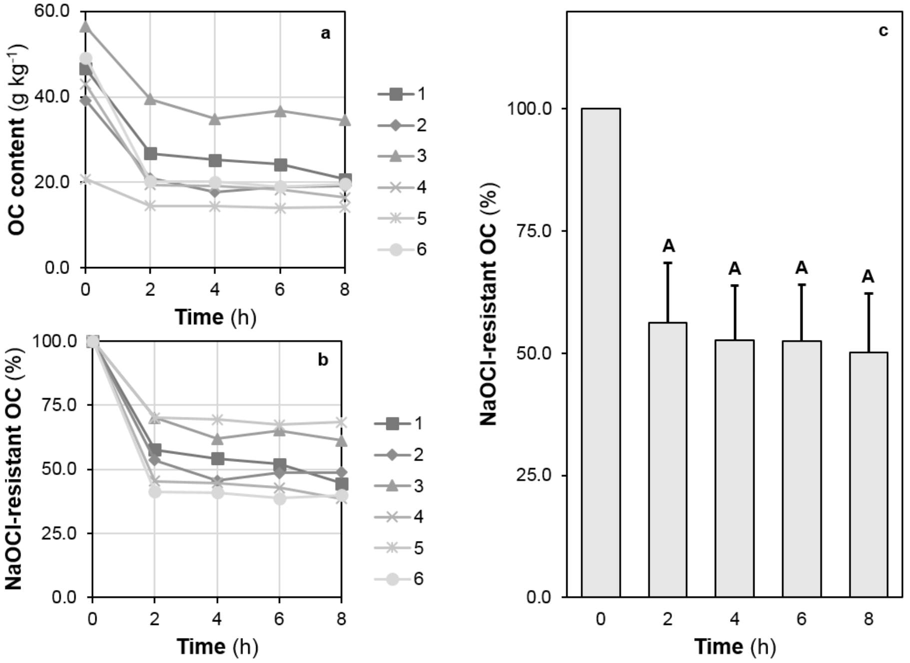

Fig. 1. (a) Organic carbon (OC) content in relation to the reaction time upon chemical oxidation with NaOCl, (b) percentage of NaOCl-resistant OC (i.e. ‘stable’ OC) in relation to the Total C content and (c) mean values of NaOCl-resistant OC (%) for each reaction time were calculated with n = 6 soil samples. Mean values followed by the same capital letter do not differ according to HSD Tukey’s test ($p < 0.05$). Small fluctuations in the OC content measured in the residues can be attributed to small analytical random variation in the OC analysis (coefficient of variation < 3%). Sample numbering refer to samples in Table 1.

The amount of NaOCl-oxidizable OC (i.e. ‘labile’ OC) and the amount of NaOCl-resistant OC (i.e. ‘stable’ OC) showed a significant positive correlation with the Total C ($R^2 = 0.69$, $p = 0.04$ and $R^2 = 0.66$, $p = 0.05$, respectively) in our study (Fig. 2a and

2b). However, the amount of NaOCl-resistant OC showed no correlation ($R^2 = 0.04$, $p = 0.72$) with the amount of $(Fe + Al)_{d\text{-}o}$ (i.e. proxy for crystalline Fe and Al oxides with low reactive surface area) (Fig. 3a). Even though we found a positive correlation ($R^2 = 0.52$) between the amount of NaOCl-resistant OC and the amount of $(Fe + Al)_o$ (i.e. proxy for poorly-crystalline Fe and Al oxides with high reactive surface area), it was not significant ($p = 0.11$). Furthermore, the reactive surface area of soil oxides (A) did not help better explaining the amount NaOCl-resistant OC ($R^2 = 0.10$, $p = 0.54$) in our study.

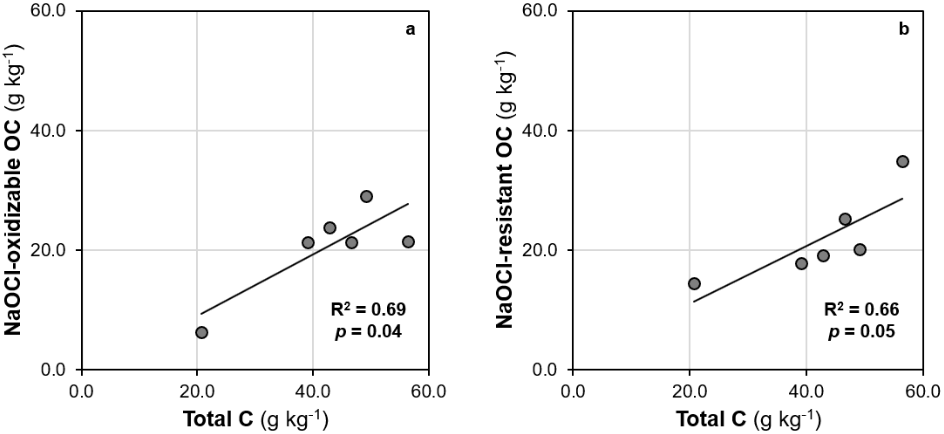

Fig. 2. (a) Relation between the Total C content and the NaOCl-resistant OC content (i.e. 'stable' OC) and (b) Relation between the Total C content and the NaOCl-oxidizable OC content (i.e. 'labile' OC). For details about the operationally defined OC pools, please see text.

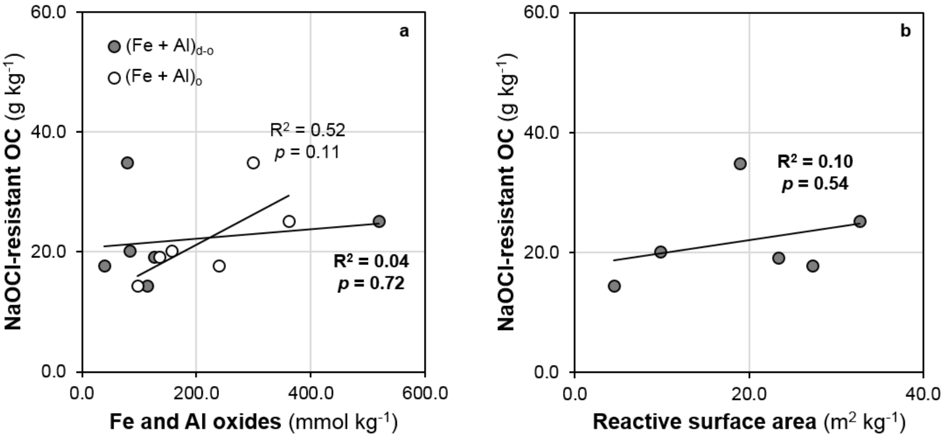

Fig. 3. (a) Relation between Fe and Al oxide phases and the NaOCl-resistant OC contents (i.e. ‘stable’ OC) and (b) Correlation between reactive surface area of soil oxides (A) and the NaOCl-resistant OC content. The amount of oxalate-extractable iron (Fe_o) and aluminium (Al_o) oxides is used as proxy for poorly-crystalline Fe and Al oxides with high reactive surface area, whereas the difference between the amount of DCB- and oxalate-extractable iron (Fe_{d-o}) and aluminium (Al_{d-o}) is used as a proxy for crystalline Fe and Al oxides with low reactive surface area. For details about the methods, please see text.

Our results contrast with other studies that found a good correlation between the ‘stable’ OC pool and mineralogical properties (Eusterhues et al., 2005, 2003; Kleber et al., 2005; Mikutta et al., 2006, 2005a). One possible explanation is the fact we investigated topsoil samples, while previous studies focused on subsoil samples. Compared to the subsoil, SOM in the topsoil is likely to be more particulate and less mineral-associated (Eusterhues et al., 2003; Kleber et al., 2005). Mikutta et al., 2006 proposed subsequent demineralization with hydrofluoric acid (HF) in NaOCl-treated soil samples to further separate ‘stable’ OC fractions and quantify the relative importance of mechanisms of SOM stabilization (i.e. mineral association and chemical recalcitrance). These authors reported that ‘mineral-associated’ OC accounted for the majority of the ‘stable’ OC pool (73% on average) and Fe and Al oxide contents phases explained 86% of the variance in the ‘mineral-associated’ OC pool. Hiemstra et al. (2013) estimated the OC loading in ‘mineral-associated’ OC pool for the samples in Mikutta et al. (2006) and reported that the OC loading corresponded to just ~0.3 mg C m^{-2} on average, contrasting with the much higher OC loading of ~1.4 mg C m^{-2} reported by Hiemstra et al. (2010) for sandy soils. Therefore, Hiemstra et al. (2013) suggested that chemical oxidation with NaOCl may underestimate the amount of OC that is associated with minerals in soils.

Furthermore, Mikutta and Kaiser (2011) reported that the amount of NaOCl-oxidizable OC (i.e. ‘labile’ OC) did not correlate with short-term (90 days) biological oxidation in an incubation experiment. For this reason, these authors pointed out that estimation of the ‘labile’ OC pool upon chemical oxidation with NaOCl is arguable. In addition, Lutfalla et al. (2014) reported that the amount of NaOCl-resistant OC (i.e. ‘stable’ OC) did not correlate with long-term (79 years) of biological oxidation in a long-term bare fallow (LTBF) experiment. For this reason, these authors pointed that estimation of the ‘stable’ OC pool upon chemical oxidation with NaOCl is also arguable. Nonetheless, whether the experimental design used by Mikutta and Kaiser (2011) and Lutfalla et al. (2014) provided a final answer to the suitability of chemical oxidation with NaOCl is still debatable since incubation and LTBF experiments have their own limitations. For example, the estimation of the ‘labile’ and ‘stable’ pool of SOM upon incubation and LTBF experiments likely depends on the duration of these experiments. Moreover, studies have shown that the NaOCl-resistant OC is less enriched in radiocarbon (^{14}C) than bulk soil samples prior to chemical oxidation (Helfrich et al., 2007; Jagadamma et al., 2010; Kleber et al., 2005; Zimmermann et al., 2007), which suggests that chemical oxidation with NaOCl preferentially removes ‘younger’ SOM and leaves behind ‘older’ SOM. However, Bruun et al. (2008) reported that chemical oxidation with NaOCl preferentially removed 40-year-old OC than 40-day-old OC. Nonetheless, Kleber et al. (2011) pointed out that ‘old’ and ‘stable’ SOM is not necessarily ‘recalcitrant’. Furthermore, von Lützow et al. (2007) pointed out that whether the isolated ‘stable’ pool can be considered stable due to a selective removal of more ‘labile’ compounds (Chefetz et al., 2002; Simpson and Hatcher, 2004) or due to interaction with the soil mineral matrix (Kleber et al., 2005; Mikutta et al., 2005a) is unclear and subjected to further investigations.

In our study, the amount and reactive surface area of Fe and Al oxides did not help explaining the amount of NaOCl-resistant OC (i.e. ‘stable’ OC). Therefore, we reject our initial hypothesis. Since we found a good correlation between the OC pools with the Total C content, our findings suggest that the chemical composition of SOM may also play a role in determining the amount of ‘stable’ OC that can be isolated via chemical oxidation with NaOCl. Other studies suggested that the ‘stable’ OC pool isolated upon chemical oxidation with NaOCl could be composed of black carbon (BC) that would resist chemical oxidation with NaOCl due to its poly-condensed aromatic structure (Bruun et al., 2008; Eusterhues et al., 2003; Leifeld and Zimmermann, 2006; Mikutta et al., 2005b; Simpson and Hatcher, 2004). Therefore, future studies should investigate the changes in chemical composition of SOM pools isolated upon chemical oxidation with NaOCl in samples with contrasting chemical composition of SOM (e.g. samples enriched in BC versus samples not enriched in BC).

3.4. Conclusion

Studies commonly apply three consecutive treatments of 6h each to isolate a ‘stable’ pool of SOM upon chemical oxidation with NaOCl. Here, we showed that for our soil samples with a wide range of soil properties (i.e. pH, OC content, amount of crystalline and poorly-crystalline Fe and Al oxides and reactive surface area of soil oxides), we were able to isolate a ‘stable’ pool of SOM with just two repetitive treatments of 2h each, which is about 5 times faster than the procedure commonly applied in the literature. Studies often suggest that soil minerals protect SOM against chemical oxidation. However, we did not find a good correlation between the ‘stable’ pool of SOM and mineralogical properties (i.e. amount and reactive surface area of Fe and Al oxides) in our study.

3.5. Acknowledgments

This work was done under the scope of the Terra Preta Program and funded by the Interdisciplinary Research and Education Fund (INREF) of Wageningen University and Research and the Brazilian Agricultural Research Corporation (Embrapa). Jose Luis Munera-Echeverri received funding from Colfuturo under the program Crédito Beca.

Supplementary Data

Table S1. Overview of the conditions investigated in some studies that used chemical oxidation with NaOCl.

Reference	Soil type	Land use	Soil depth	Soil fraction	Pre-treatment	No. of consecutive treatments with NaOCl	Reaction time (h) per treatment	NaOCl-oxidizable OC (%) range	NaOCl-resistant OC (%) range
Kaiser et al., 2002	temperate; sandy	forest	subsoils	density and particle size		5	6	> 87.0	< 13.0
Kaiser and Guggenberger, 2003	temperate and tropical; varying texture	forest	soil profiles	density		5	6	77.0 - 95.0	5.0 - 23.0
Mikutta et al., 2006	temperate and tropical; varying texture	forest	subsoils	bulk soil		3	6	12.3 - 72.0	28.0 - 87.7
Thomsen et al., 2009	temperate; sandy	agriculture	topsoils			3	6	19.0 - 62.0	38.0 - 81.0
Jagadamma et al., 2010	temperate	forest; agriculture	soil profiles	bulk soil	ball mill	3	6	22.2 - 62.2	37.8 - 77.8
Spohn and Giani, 2011	temperate; sandy	forest; agriculture	topsoils		ball mill	3	6	47.0 - 92.0	8.0 - 53.0

Chapter 4

Chemical composition of soil organic matter pools in Amazonian Dark Earth and Ferralsol upon chemical oxidation with NaOCl and soil demineralization with HF

Carlos Francisco Brazão Vieira Alho[a], Joyce R. Araújo[b], Carlos A. Achete[b], Wenceslau Geraldes Teixeira[c], Tjisse Hiemstra[d], Thomas W. Kuyper[a]

[a] *Soil Biology Group, Wageningen University and Research, 6700 AA, Wageningen, the Netherlands.*
[b] *Inmetro, 25250-020, Xérem, Brazil.*
[c] *Embrapa Soils, 22460-000, Rio de Janeiro, Brazil.*
[d] *Soil Chemistry and Chemical Soil Quality Group, Wageningen University and Research, 6700 AA, Wageningen, the Netherlands.*

Abstract

It has been suggested that the larger amount of soil organic matter (SOM) in Amazonian Dark Earths (ADE) compared to the adjacent (ADJ) Ferralsols is due to the occurrence of black carbon (BC) in ADE. BC has been claimed to be one of the most stable forms of organic carbon (OC) due to its chemical recalcitrance given its poly-condensed aromatic structure. However, the relative importance of chemical recalcitrance in explaining long-term persistence of SOM has been challenged and the importance of mineral associations highlighted. Chemical oxidation with sodium hypochlorite (NaOCl) has been used for isolating a 'stable' pool of SOM and subsequent demineralization with hydrofluoric acid (HF) has been proposed to further separate 'stable' OC fractions and quantify the relative importance of mechanisms of SOM stabilization (i.e. mineral association and chemical recalcitrance). Other studies suggested that BC could resist chemical oxidation with NaOCl and account for most of the 'stable' OC pool (i.e. NaOCl-resistant OC). Therefore, the aim of this study was to evaluate the chemical composition of SOM pools upon chemical oxidation with NaOCl and soil demineralization with HF in ADE (i.e. soils expected to be enriched in BC) in contrast with ADJ (i.e. soils not expected to be enriched in BC) by X-ray Photoelectron Spectroscopy (XPS). Despite larger SOM content in ADE compared to ADJ, our findings indicate that the vast majority (> 80%) of the Total C content has to be considered 'labile', while the vast majority (> 90%) of the 'stable' pool has to be considered 'mineral-associated', with minimal contribution of 'recalcitrant' OC in in both soils. High-resolution C 1s XPS spectra showed that chemical oxidation with NaOCl decreased the relative contribution of 'labile' components and increased the relative contribution of aromatic OC in the 'stable' OC pool (i.e. NaOCl-resistant OC). However, this relative increase in aromatic OC (from 0.9 to 5.6% in ADE and from 0.8 to 4.1% in ADJ) was not observed in the remaining 'recalcitrant' OC pool (i.e. HF-resistant OC), which contained very little (non-aromatic) OC. Therefore, our results suggest that the 'stable' aromatic OC (e.g. BC) is 'mineral-associated' and not 'chemically-recalcitrant'. Future studies should assess the BC content in SOM pools to infer on the relative contribution of BC in explaining the long-term persistence of SOM in ADE quantitatively. Furthermore, besides BC, studies should also investigate the role of other important inputs in ADE (i.e. Ca and P) in further explaining the long-term persistence of SOM in ADE.

4.1. Introduction

Amazonian Dark Earths (ADE) are pre-Columbian anthropic soils that have intrigued scientists for decades. Despite tropical conditions, ADE are enriched in soil organic matter (SOM) and nutrients, particularly calcium (Ca) and phosphorus (P). In both respects, ADE contrast to the adjacent (ADJ) soils in the Amazon (Glaser et al., 2001b). Previous studies have suggested that the large amount of SOM in ADE is due to the occurrence of black carbon (BC) in these soils (Glaser, 2007; Glaser et al., 2001b). BC has been claimed to be one of the most stable forms of organic carbon (OC) due to its poly-condensed aromatic structure (Glaser, 2007; Krull et al., 2003; Novotny et al., 2007). However, recent advancements in analytical techniques – such as the use of specific molecular markers and compound-specific isotopic analysis (Amelung et al., 2009) – indicate that the intrinsic chemical characteristics of organic matter (OM) do not fully explain its long-term persistence in soils (Schmidt et al., 2011). Therefore, the relative importance of inherent chemical recalcitrance in explaining long-term persistence of SOM has been challenged (Marschner et al., 2008), while the role of soil minerals in organo-mineral associations has been highlighted (Baldock and Skjemstad, 2000; Torn et al., 1997).

Despite recent advancement in conceptual models for SOM stabilization, SOM stability is still difficult to be assessed operationally since several mechanisms of SOM stabilization are likely to happen simultaneously to explain long-term persistence of SOM. Three main mechanisms of SOM stabilization have been proposed (occlusion in aggregates, association with soil minerals and chemical recalcitrance) (Sollins et al., 1996), but their relative contribution is still poorly understood (Mikutta et al., 2006). Chemical oxidation with NaOCl has been proposed to isolate a ‘stable’ pool of SOM leaving soil minerals largely unaffected (Siregar et al., 2005) and subsequent demineralization with hydrofluoric acid (HF) has been proposed to further separate ‘stable’ OC fractions and quantify the relative importance of mechanisms of SOM stabilization (i.e. mineral association and chemical recalcitrance) (Mikutta et al., 2006) leaving non-bound SOM largely unaffected (Eusterhues et al., 2003). Furthermore, studies suggested that BC could resist chemical oxidation with NaOCl and account for most of the ‘stable’ OC pool (Bruun et al., 2008; Eusterhues et al., 2003; Leifeld and Zimmermann, 2006; Mikutta et al., 2005b; Simpson and Hatcher, 2004).

Therefore, the aim of this study was to evaluate the chemical composition of SOM pools upon chemical oxidation with NaOCl and demineralization with HF in ADE (i.e. soils expected to be enriched in BC) in contrast with the adjacent (ADJ) Ferralsol (i.e. soils not expected to be enriched in BC) by X-ray Photoelectron Spectroscopy (XPS). In line with previous studies, we hypothesized that chemical oxidation with

NaOCl and subsequent soil demineralization with HF removes OC of non-BC origin and enables the investigation of the relative contribution of different mechanisms of SOM stabilization (i.e. mineral protection and chemical recalcitrance) in explaining the long-term persistence of BC in ADE.

4.2. Material and Methods

Soil samples and analysis

In this study, we compared topsoil samples (0–20 cm) collected in a pretic area (i.e. proxy for ADE) and non-pretic area (i.e. proxy for ADJ) (Fig. 1) as described in **Chapter 2** (Alho et al., 2019). These samples exhibit the typical contrast in soil properties between ADE and ADJ (e.g. pH, CEC and Total C, Ca and P) (Table 1). These samples correspond to ADE 1 and ADJ 1 in **Chapter 5**. The rationale to focus on these two samples was to evaluate the changes in chemical composition upon chemical oxidation with NaOCl between the samples where the largest difference between ADE and ADJ was expected. This is because ADE 1 is located where the largest enrichments in C, Ca and P were identified, whereas ADJ 1 is the sample furthest from ADE 1 within the same diagonal transect, where the p_{pretic} is zero (**Chapter 2**). Soil samples were air-dried at room temperature, passed through a 2mm sieve and visible roots were removed manually. For further analysis, a subsample was ground in a ball mill.

Soil pH was determined in water (soil:water ratio of 1:2.5) and potential CEC (at pH = 7.0) was defined as the sum of exchangeable cations (K, Na, Ca + Mg) plus acidity (H + Al). Total C was determined using an elemental analyser (PerkinElmer 2400 Series II), where acetanilide was used as reference material. Total Ca and Total P were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) after digestion with a multi-acid solution (HCl, HNO_3, HF and $HClO_4$).

Table 1. Soil properties in Amazonian Dark Earth (ADE) and adjacent (ADJ) Ferralsol.

Sample	pH	CEC	Total C	Total Ca	Total P
	in H_2O	(cmol$_c$ kg^{-1})	----------------- (g kg^{-1}) -----------------		
ADE	5.3	15.1	44.5	4.3	2.8
ADJ	4.5	2.2	25.7	0.2	0.9

For details about the methods to determine soil pH, cation exchange capacity (CEC), total carbon (Total C), total calcium (Total Ca) and total phosphorus (Total P), please see text. Samples correspond to ADE 1 and ADJ 1 in **Chapter 5**.

Chemical oxidation with NaOCl

Chemical oxidation with NaOCl was done in three experimental replicates according to a modified version of the method proposed by Mikutta et al. (2006) (**Chapter 3**). Briefly, 30 mL of 6% NaOCl solution adjusted to pH 8.0 by adding 32% hydrochloric acid (HCl) was added to plastic bottles containing 3.0 g of soil. The bottles were shaken in a reciprocal shaker for 2h at room temperature. Samples were then centrifuged (at 2574 *g* for 10 min) and the supernatants discarded. Residues were thoroughly washed with deionized water until electrical conductivity was < 40 µs cm^{-1}, oven-dried (at 70° for 24h) and a subsample was taken for the determination of the OC content. The NaOCl solution was renewed and the procedure repeated one more time.

Subsequent demineralization with HF

Subsequently, 20 mL of 10% HF solution was pipetted into plastic bottles containing 1.0 g of oven-dried NaOCl-treated soil. Samples were shaken for 2h at room temperature, centrifuged (at 2574 *g* for 10 min) and the supernatant discarded. The HF solution was renewed and the procedure repeated three times with a total of four repetitive treatments. Next, the residues were washed five times with 20 mL deionized water, oven-dried (at 70°C for 24h) and a subsample was taken for the determination of OC content.

The OC content was determined using an elemental analyser as previously described. OC pools were defined operationally as: ‘labile’ (i.e. NaOCl-oxidizable OC) and ‘stable’ (i.e. NaOCl-resistant OC). The ‘stable’ OC pool was further subdivided into ‘mineral-associated’ OC (i.e. HF-soluble OC) and ‘recalcitrant’ OC (i.e. HF-resistant OC) fractions. The ‘labile’ OC and ‘mineral-associated’ OC were obtained by difference (‘labile’ OC = initial OC - ‘stable’ OC and ‘mineral-associated’ OC = ‘stable’ OC - ‘recalcitrant’ OC).

X-Ray Photoelectron Spectroscopy (XPS)

X-ray Photoelectron Spectroscopy (XPS) was used to investigate the chemical composition of SOM pools upon chemical oxidation with NaOCl and subsequent soil demineralization with HF. XPS was performed under ultra-high vacuum (10^{-10} mbar) using a magnesium (Mg) X-ray source (K_{α} = 1,253.6 Ev) powered by an emission current of 16 mA and a voltage of 12.5 kV. High-resolution spectra were obtained for C (C 1s) using an analyser pass energy of 30 eV and 0.05 eV step. The binding energies were referenced to the C 1s level at 284.6 eV. Gaussian/Lorentzian (70/30) line shapes were used to fit C 1s peak components after Shirley background subtraction (Araujo et al., 2014).

4.3. Results and Discussion

OC pools

The amount of NaOCl-oxidizable OC (i.e. ‘labile’ OC) was 38.9 and 21.5 g kg^{-1} in ADE and ADJ, respectively (Fig. 1a). These values correspond to 87.4 and 83.5% of the Total C content for ADE and ADJ, respectively (Fig.1b). The amount of NaOCl-resistant OC (i.e. ‘stable’ OC) was 5.6 and 4.2 g kg^{-1} in ADE and ADJ, respectively (Fig.1a). These values correspond to 12.6 and 16.5% of the Total C content for ADE and ADJ, respectively (Fig.1b).

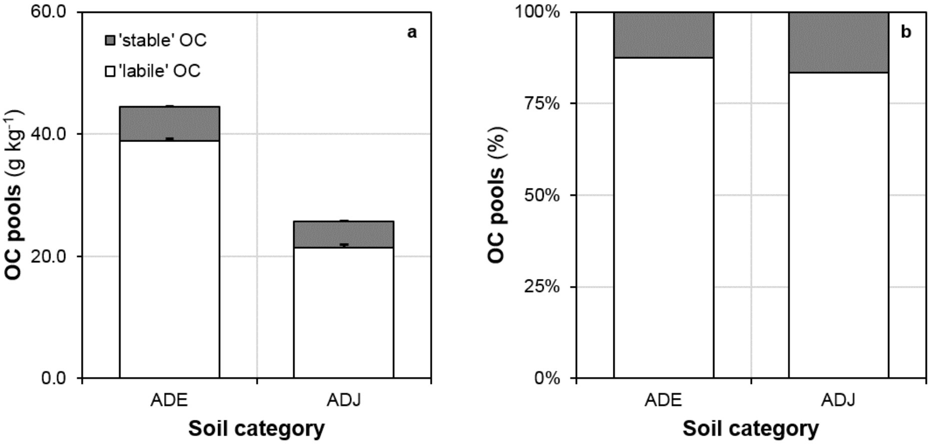

Fig. 1. (a) Organic carbon (OC) content and (b) Percentage of OC pools (i.e. ‘labile’ and ‘stable’) in relation to the Total C content in Amazonian Dark Earth (ADE) and the adjacent (ADJ) Ferralsol upon chemical oxidation with NaOCl. Standard errors derived from three experimental replicates were < 0.4 g kg^{-1} and therefore are not easily observed in the graph.

Our results are in agreement with to other studies that looked at the removal of SOM prior to surface area analysis (Kaiser et al., 2002; Kaiser and Guggenberger, 2003), where the authors applied five consecutive treatments with NaOCl. However, the amount of NaOCl-resistant OC in our samples was smaller than other studies that also used chemical oxidation with NaOCl to isolate a ‘stable’ pool of SOM, but where the authors used three consecutive treatments with NaOCl (Jagadamma et al., 2010; Mikutta et al., 2006; Thomsen et al., 2009). For further support, we provided an overview of the conditions investigated in some studies that used chemical oxidation with NaOCl in Table S1 (Supplementary Data) **in Chapter 3**. One plausible explanation for the larger amount of NaOCl-oxidizable OC in our samples compared to other studies is the fact we investigated topsoil samples. Compared to the SOM in subsoils, SOM in the topsoil is likely more particulate and less mineral-associated and therefore more easily oxidized (Eusterhues et al., 2003; Kleber et al., 2005).

However, some studies suggested that the amount of NaOCl-resistant OC in the topsoil can be large not because of mineral protection but due to OC chemical composition (i.e. inherent chemical recalcitrance). For example, aliphatic compounds (e.g. suberin- and cutin-derived OC) could resist chemical oxidation due to their hydrophobic properties (Eusterhues et al., 2005, 2003) and poly-aromatic compounds (e.g. BC) could resist chemical oxidation due to their poly-condensed aromatic structure (Simpson and Hatcher, 2004). Here, we showed that even though the Total C content in ADE (44.5 g kg^{-1}) was larger than ADJ (25.7 g kg^{-1}) (Table 1), the NaOCl-resistant OC content was very similar in both sample (5.6 and 4.2 for ADE and ADJ, respectively) (Fig. 1a), which shows that larger OC content in ADE compared to ADJ was composed of easily oxidizable OC rather than chemically-resistant OC (e.g. BC).

Subsequent soil demineralization with HF revealed that the amount of HF-soluble OC (i.e. ‘mineral-associated’ OC) was 5.2 and 3.9 g kg^{-1} in ADE and ADJ, respectively (Fig. 2a). These values correspond to 93.0 and 92.9% of the ‘stable’ OC content for ADE and ADJ, respectively (Fig. 2b). The amount of HF-resistant OC (i.e. ‘recalcitrant’ OC) was 0.4 and 0.3 g kg^{-1} in ADE and ADJ, respectively (Fig. 2a). These values correspond to 7.0 and 7.1% of the ‘stable’ OC content for ADE and ADJ, respectively (Fig. 2b).

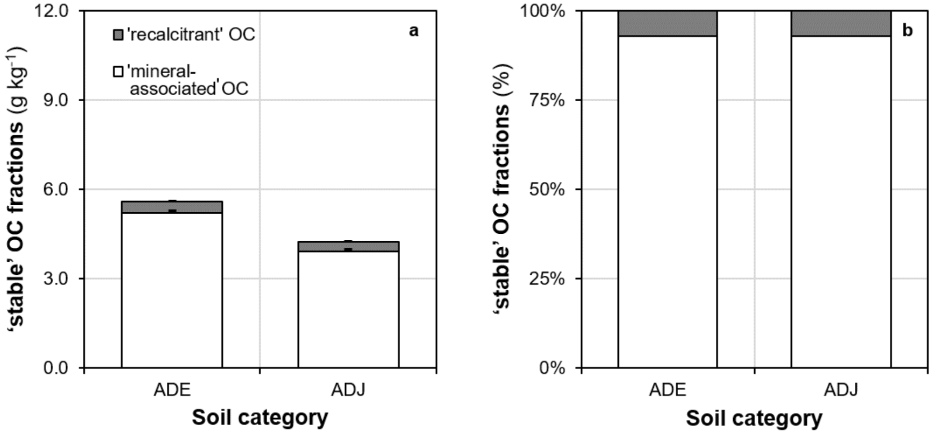

Fig. 2. (a) 'Stable' organic carbon (OC) fractions and (b) Percentage of 'stable' OC fractions (i.e. 'mineral-associated' and 'recalcitrant' OC) in relation to the 'stable' OC content in Amazonian Dark Earth (ADE) and the adjacent (ADJ) Ferralsol upon subsequent soil demineralization with HF. Standard errors derived from three experimental replicates were < 0.1 g kg^{-1} and therefore are not easily observed in the graph.

Our results are comparable to other studies that looked at the amount of HF-soluble OC (Mikutta et al., 2006; Yeasmin et al., 2017). Furthermore, our findings indicate that despite small amount of NaOCl-resistant OC in our samples, this 'stable' OC pool was mostly 'mineral-associated' and not chemically-recalcitrant. In the next section, we further discuss our results by evaluating the chemical composition of OC pools.

Chemical composition of OC pools

Soil samples contain a variety of elements, which can be observed in the XPS survey spectra (Fig. 3). Sodium (Na) peak in the NaOCl-treated samples can be attributed to use NaOCl as the chemical oxidant (Fig. 3c and 3d). Iron (Fe) and aluminium (Al) peaks were no longer detectable after soil demineralization with HF, confirming the efficacy of HF to solubilize soil minerals such as clays and Fe and Al oxides. However, quartz particles (SiO_2) and titanium oxides (TiO_2) resisted the HF treatment (Fig. S1 in Supplementary Data). Therefore, the silicon (Si) peak is relatively increased in NaOCl- and HF-treated samples (Fig. 3e and 3f) as well as the titanium (Ti) peak in the HF-treated samples (Fig. 3e and 3f).

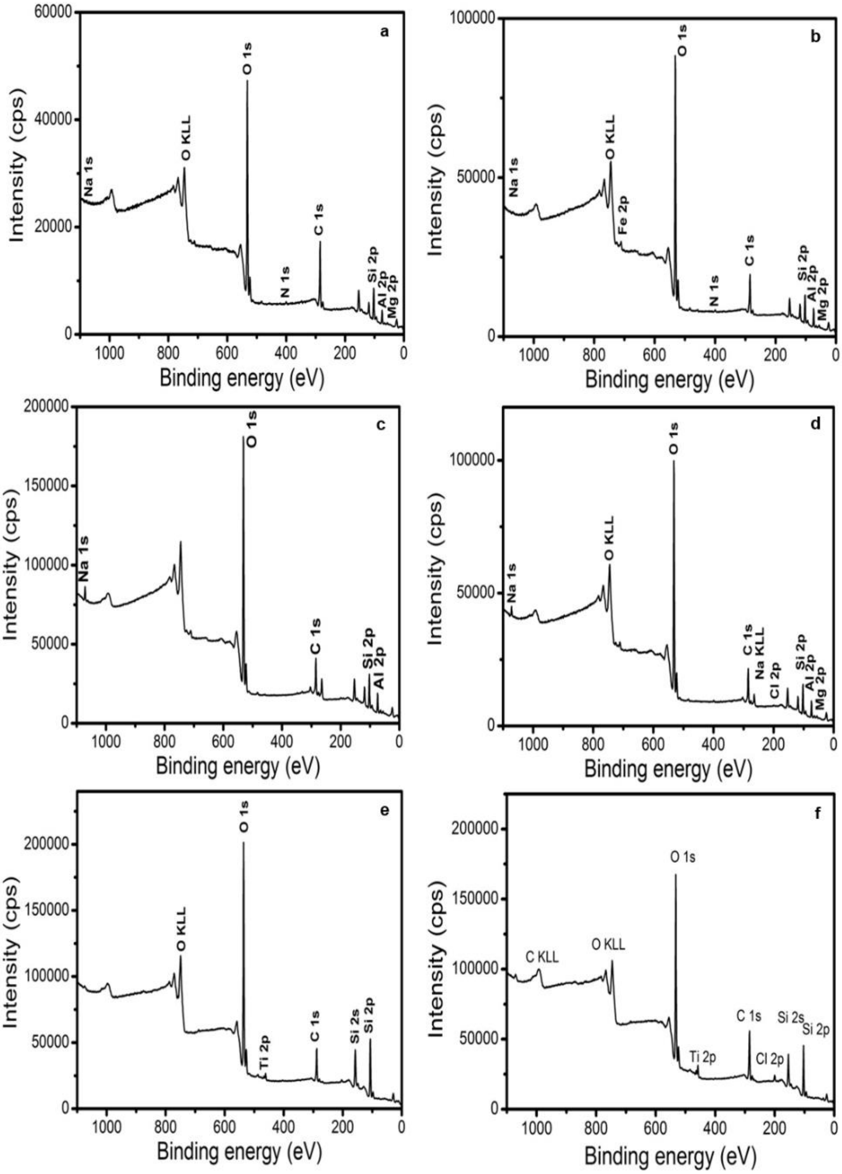

Fig. 3. X-ray Photoelectron Spectroscopy (XPS) survey spectra for untreated Amazonian Dark Earth (ADE) (a) and the adjacent (ADJ) Ferralsol (b), NaOCl-treated ADE (c) and ADJ (d) and NaOCl- and HF-treated ADE (e) and ADJ (f).

Based on the C 1s spectra (Fig. 4), the following functional groups can be distinguished: sp^2-hybridized C (C=C, 284.6 eV), sp^3-hybridized C (C–C/C–H, 285.5 eV), hydroxyl bound C (C–OH, 285.8-286.0 eV), epoxy/ether (C–O–C, 286.4 eV), carbonyl (C=O, 287.0-288.0 eV), carboxyl (HO-C=O, 288.5-289.5 eV), and a shake-up satellite peak ($\pi \rightarrow \pi^*$, 291.4 eV) characteristic for delocalized electrons of aromatic OC (Araujo et al., 2014).

Upon chemical oxidation with NaOCl, the C 1s spectra exhibited a relative decrease of oxidized C of C-OH, C-O-C and C=O (from 18.4 to 17.9% in ADE and from 26.8% to 16.5% in ADJ) and a relative increase in the shake-up peak (from 0.9 to 5.6% in ADE and from 0.8 to 4.1% in ADJ) (Fig. 4a to 4d). The decrease in the relative contribution of oxidized groups can be attributed to the degradation of ‘labile’ components (e.g. cellulose), whereas the increase in the shake-up peak can be attributed to aromatic components (e.g. BC). Therefore, our findings indicate that the ‘stable’ OC fraction was relatively enriched in aromatic OC.

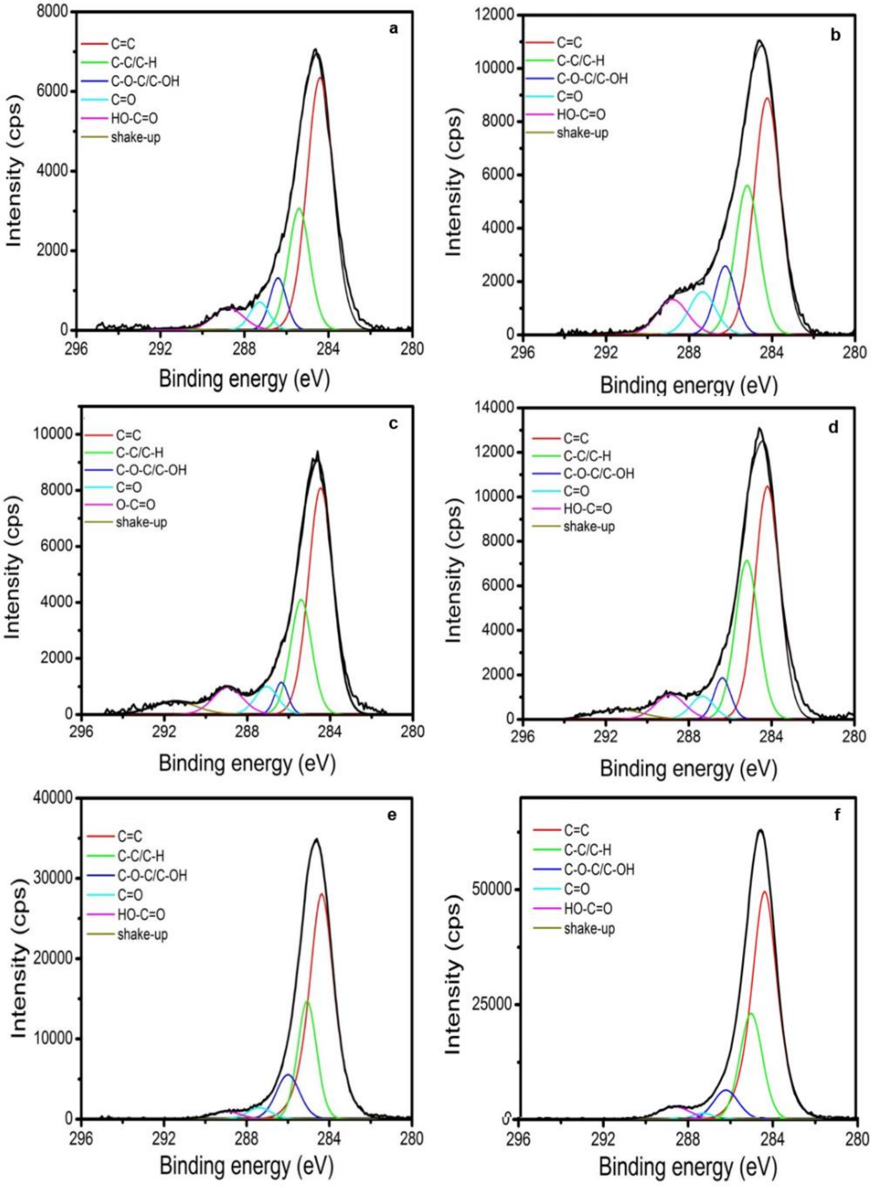

Fig. 4. X-ray Photoelectron Spectroscopy (XPS) C 1s spectra for untreated Amazonian Dark Earth (ADE) (a) and the adjacent (ADJ) Ferralsol (b), NaOCl-treated ADE (c) and ADJ (d) and NaOCl- and HF-treated ADE (e) and ADJ (f). C 1s functional groups: sp^2-hybridized C (C=C), sp^3-hybridized C (C–C/C–H), hydroxyl (C–OH), epoxy/ether (C–O–C), carbonyl (C=O), carboxyl (HO-C=O) and a shake-up satellite peak ($\pi \rightarrow \pi^*$) characteristic of electrons delocalized over aromatic OC.

Mikutta et al. (2005) suggested that specific OC compounds, e.g. aromatic OC, may be removed upon chemical oxidation with NaOCl. Indeed, Jagadamma et al. (2010) reported that NaOCl preferentially removed mono-aromatic C (e.g. lignin-derived OC) based on ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy. Therefore, the relative enrichment in aromatic OC that we found in the ‘stable’ OC fraction of our samples could be attributed to the presence of polyaromatic BC, in line with suggestions from other studies (Bruun et al., 2008; Eusterhues et al., 2003; Leifeld and Zimmermann, 2006; Mikutta et al., 2005b; Simpson and Hatcher, 2004).

However, the relative increase of aromatic OC in the ‘stable’ OC pool was not observed in the ‘recalcitrant’ OC pool (i.e. HF-resistant OC). The fraction remaining after removal of the soil minerals contained very little OC, which was non-aromatic. This observation has also been reported by Yeasmin et al. (2017) using XPS to study the SOM factions of Ferralsols upon chemical oxidation NaOCl and soil demineralization with HF. Since we only observed a relative increase in aromatic OC in the ‘stable’ OC pool, but not in the ‘recalcitrant’ OC pool, we conclude that the NaOCl-resistant aromatic OC is ‘mineral-associated’ and not ‘chemically-recalcitrant’. Our observed association of aromatic OC with soil minerals may be an important mechanism for long-term persistence of OC in soils as suggested by Kramer et al. (2012), which may be an important mechanism in ADE. However, caution should be taken while interpreting results after treatment with HF since ‘unprotected’ OC (i.e. not ‘mineral-protected’) can be lost, while ‘mineral-protected’ OC can persist in the solution and be accounted as ‘recalcitrant’ OC (Sanderman et al., 2017). Therefore, attributing the chemical composition of HF-resistant OC to inherent chemical recalcitrance is challenging.

Overall, our findings indicate that the vast majority (> 80%) of the Total C content has to be considered ‘labile’, while the vast majority (> 90%) of the ‘stable’ pool has to be considered ‘mineral-protected’, with minimal contribution of ‘recalcitrant’ OC in both soils. This conclusion also pertains for ADE, even though we expected it to be enriched in ‘recalcitrant’ OC (i.e. BC). These findings suggest that the relative contribution of different mechanisms of SOM stabilization (i.e. mineral protection versus chemical recalcitrance) did not differ between both soils despite differences in OC content and assumed differences in OC chemical composition in ADE and ADJ. In order to check the assumption that the ‘recalcitrant’ OC pool is enriched in BC, future studies should determine the BC content in SOM pools in ADE compared to ADJ. In this way, the relative contribution of BC in SOM pools may provide better insights on the overall relative contribution of different mechanisms of SOM stabilization in explaining long-term persistence of BC in ADE in a quantitative approach. Furthermore, besides BC, studies should also investigate the role of other important inputs in ADE (i.e. Ca and P) in further explaining the long-term persistence of SOM in ADE.

4.4. Conclusions

Evaluation of the chemical composition of SOM pools upon chemical oxidation with NaOCl in ADE and ADJ via XPS showed that chemical oxidation with NaOCl decreased the relative contribution of ‘labile’ components and increased the relative contribution of aromatic OC in the ‘stable’ OC pool (i.e. NaOCl-resistant OC). As argued, this aromatic OC is likely related to the presence of BC. However, this relative increase in aromatic OC was not observed in the ‘recalcitrant’ OC pool (i.e. HF-resistant OC), which contained very little (non-aromatic) OC. Therefore, NaOCl-resistant aromatic OC in these soils is ‘mineral-associated’ and not ‘chemically-recalcitrant’.

4.5. Acknowledgments

This work was done under the scope of the Terra Preta Program and funded by the Interdisciplinary Research and Education Fund (INREF) of Wageningen University and Research and the Brazilian Agricultural Research Corporation (Embrapa).

Supplementary Data

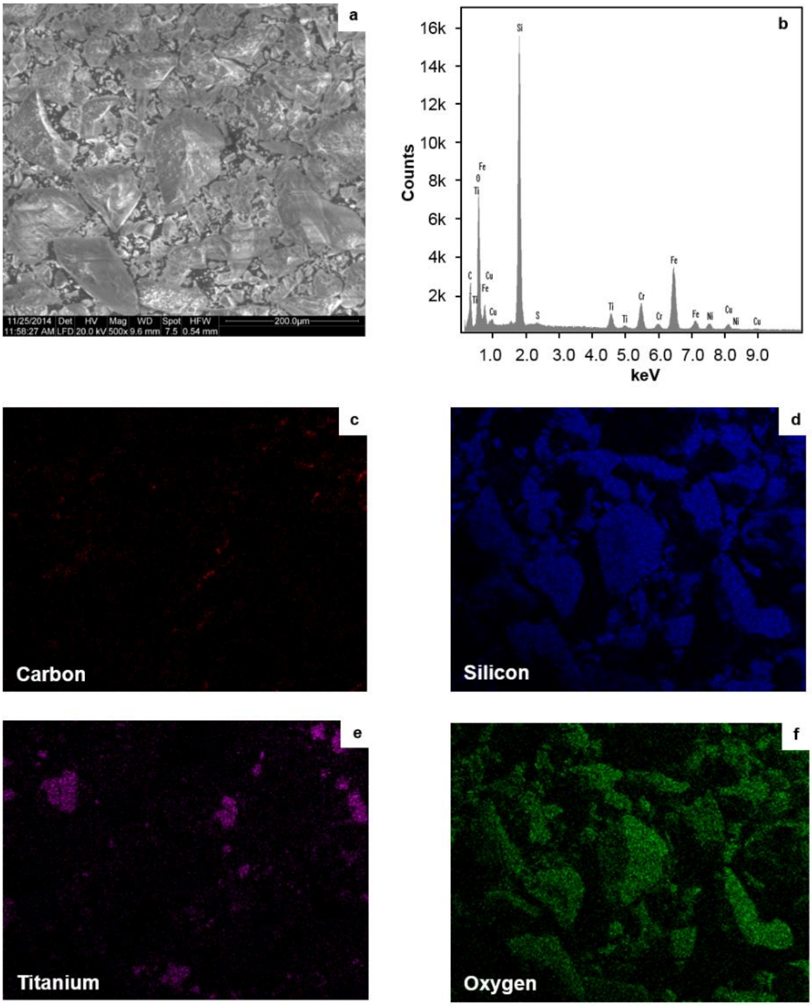

Fig. S1. (a) Scanning transmission electron microscopy (STEM) of the ‘recalcitrant’ organic carbon pool in Amazonian Dark Earth and (b) Energy dispersive X-ray spectroscopy (EDX) spectra and accompanied mapping for Carbon (c), Silicon (d), Titanium (e) and Oxygen (f). (EDX) microscopy were performed on a Cs-corrected FEI Titan 80/300 transmission electron microscope, equipped with a Gatan imaging filter Tridiem and an EDX analyser. The elemental mappings were obtained by integrating characteristic X-ray signals during a drift-corrected STEM spectrum imaging experiment. STEM images were acquired using a high-angle annular dark-field detector.

Chapter 5

The interactive roles of black carbon, calcium and phosphorus on the long-term persistence of soil organic matter in Amazonian Dark Earth

Carlos Francisco Brazão Vieira Alho[a], Bruno Glaser[b], Wenceslau Geraldes Teixeira[c], Tjisse Hiemstra[d], Thomas W. Kuyper[a]

[a] Soil Biology Group, Wageningen University and Research, 6700 AA, Wageningen, the Netherlands.
[b] Department of Biogeochemistry, Martin-Luther-University Halle-Wittenberg, 06120, Halle (Saale), Germany.
[c] Embrapa Soils, 22460-000, Rio de Janeiro, Brazil.
[d] Soil Chemistry and Chemical Soil Quality, Wageningen University and Research, 6700 AA, Wageningen, the Netherlands.

Abstract

Pre-Columbian anthropic soils found in the Amazon – known as Amazonian Dark Earths (ADE) – have intrigued scientists for decades. Despite tropical conditions, these soils exhibit a large amount of soil organic matter (SOM). The occurrence of black carbon (BC) in ADE has been suggested as the main driver of larger amount of SOM in ADE. Besides the occurrence of BC in ADE, large amounts of Ca and P are other important characteristics of ADE. However, their role in further explaining long-term persistence of large SOM content in ADE has been largely overlooked in previous studies. The aim of this study was to (i) determine the BC content in SOM pools in ADE and the adjacent (ADJ) Ferralsol upon chemical oxidation with NaOCl and subsequent demineralization with HF in combination with the BPCA method and (ii) investigate the role of Ca and P in further explaining long-term persistence of SOM in ADE. Contrary to previous studies, our results showed that the relative contribution of BC in ADE did not differ from the ADJ soil. Moreover, our results raised questions for the isolation of a so-called 'stable' OC pool upon chemical oxidation procedures. Contrary to previous assumptions, BC did not resist to chemical oxidation with NaOCl, which also raises questions on the 'inherent chemical recalcitrance' of BC. Furthermore, our results indicated that besides BC, Ca, and P are important in explaining high SOM contents in ADE. Here, we showed that the high P content in ADE led to a larger amount of poorly crystalline iron (Fe) and aluminium (Al) oxides, known for their importance on the stabilization of SOM via organo-mineral associations. Moreover, our results indicated that high Ca in ADE likely played an important role for the stabilization of SOM, most likely via cation bridging. In this study, we suggested possible mechanisms by which Ca and P may contribute to the long-term persistence of SOM in ADE, which brings implications for the sole use of biochar as a strategy to reproduce ADE desirable characteristics.

5.1. Introduction

Soil organic matter (SOM) is a complex mixture of plant- and microbial-derived organic matter (OM) in different stages of biodegradation embedded in the soil mineral matrix. Despite its well-known importance to ecosystem functioning and agricultural productivity, views on the relative contribution of different mechanisms of SOM stabilization in explaining its long-term persistence in soils are controversial (Kleber and Lehmann, 2019; Olk et al., 2019). Historically, studies have largely focused on the intrinsic chemical characteristics of SOM – often referred to ‘inherent chemical recalcitrance’ – to explain long-term persistence of SOM (Kleber, 2010). However, the relative importance of chemical recalcitrance has been recently challenged (Marschner et al., 2008) and currently, long-term persistence of SOM is seen as an ecosystem property (Schmidt et al., 2011), where the role of soil minerals in protecting SOM from biodegradation is highlighted (Baldock and Skjemstad, 2000; Kleber et al., 2005; Mikutta et al., 2005a; Torn et al., 1997). Rumpel and Kögel-Knabner (2011) pointed out that association of organic matter with soil minerals, most importantly poorly crystalline Fe and Al oxides, is likely to be the dominant mechanism of SOM stabilization. These authors also reported that the carbon (C) to nitrogen (N) ratio generally decreases with soil depth, indicating a larger contribution of microbial-derived OM. However, sources, chemical composition, as well as mechanisms of SOM stabilization are still poorly understood.

Pre-Columbian anthropic soils found in the Amazon – known as Amazonian Dark Earths (ADE) – have intrigued scientists for decades. This is because ADE exhibit large amounts of SOM despite tropical conditions that favour fast biodegradation of SOM. In addition, ADE are also enriched in nutrients, particularly calcium (Ca) and phosphorus (P). Therefore, ADE contrast with the carbon- and nutrient-poor adjacent soils (ADJ) from the Amazon (Glaser et al., 2001b). The occurrence of black carbon (BC) in ADE has been suggested as the main driver of larger amount of SOM in ADE compared to ADJ (Glaser, 2007; Glaser et al., 2001b). Studies often suggest that the intrinsic chemical characteristic of BC – given its poly-condensed aromatic structure – is the main mechanism explaining the long-term persistence of BC in soils (Glaser et al., 2003; Krull et al., 2003). Several studies indicated that particulate BC is fragmented over time, increasing its surface area (Brodowski et al., 2005a; Skjemstad et al., 1996). Furthermore, biotic and abiotic surface oxidation (Cheng et al., 2008a, 2006) create and enhance the surface charge of BC (Cheng et al., 2008b; Nguyen et al., 2009), which likely promotes the association of BC with soil minerals. Indeed, studies have shown that BC in microaggregates (Brodowski et al., 2006, 2005a; Glaser et al., 2000; Lehmann et al., 2008; Skjemstad et al., 1996) is surrounded by mineral particles (Archanjo et al., 2015; Chia et al., 2012; Jorio et al., 2012), indicating that BC is involved in organo-mineral associations (Cusack et al., 2012; Czimczik and Masiello, 2007). Therefore, besides the inherent chemical recalcitrance of BC, mineral association is likely to play an important role in its persistence in soils as well. However, the relative contribution of mineral association versus chemical recalcitrance in explaining long-term persistence of BC in soils is unknown.

Mikutta et al. (2006) suggested that chemical oxidation with sodium hypochlorite (NaOCl) combined with subsequent demineralization with hydrofluoric acid (HF) could be a useful approach to separate SOM pools and quantify the relative importance of mechanisms of SOM stabilization (i.e. mineral association and chemical recalcitrance). Other studies suggested that BC resists chemical oxidation with NaOCl and accounts for most of the ‘stable’ OC pool (i.e. NaOCl-resistant pool) (Bruun et al., 2008; Eusterhues et al., 2003; Leifeld and Zimmermann, 2006; Mikutta et al., 2005b; Simpson and Hatcher, 2004). Indeed, Simpson and Hatcher (2004) reported that mono-aromatic OC (i.e. lignin-derived) was successfully removed with NaOCl but poly-aromatic (i.e. BC) remained in the residue. Furthermore, studies that applied chemical oxidation with NaOCl and subsequent demineralization with HF mentioned that the operationally defined pool of ‘recalcitrant’ OC (i.e. HF-resistant) could be composed of BC (Bruun et al., 2008; Mikutta et al., 2005b), even though the BC content was not explicitly assessed in these studies.

Benzene polycarboxylic acids (BPCA) as specific molecular markers have been proposed to assess the BC content in soils (Brodowski et al., 2005b; Glaser et al., 1998). The BPCA method yields conservative estimates of the BC content in a variety of environmental matrixes and cover a broad range of the BC continuum (Dittmar, 2008; Glaser et al., 1998; Schneider et al., 2011). One additional advantage of assessing single BPCA compounds is the possibility to investigate the specific molecular properties of BC. For example, the ratio between B6CA (i.e. mellitic acid) to the sum of BPCA has been proposed to indicate the degree of aromaticity in BC samples (Wiedemeier et al., 2015).

Besides the occurrence of BC in ADE, large amounts of Ca and P are other important characteristics of ADE (Van Hofwegen et al., 2009). However, their role in further explaining long-term persistence of SOM in ADE has been largely neglected since studies often consider BC as the main driver of the striking properties of ADE. Polyvalent cations (mainly Ca^{2+}) form bridges between negatively charged OM compounds and soil minerals (Baldock and Skjemstad, 2000; Muneer and Oades, 1989). Therefore, a high Ca content in ADE likely plays an important role in cation bridging. Furthermore, it has been suggested that adsorption of P and SOM to iron Fe and Al oxides may inhibit crystal growth (Eusterhues et al., 2008; Fukushi and Sato, 2005) and are likely the cause of the presence of nano-sized oxide particles with high surface area in soils (Hiemstra et al., 2010b). Highly weathered soils from the Amazon generally exhibit high contents of Fe and Al oxides, being a prerequisite for mineral association in these soils. In combination with a high P content in ADE, it may have led to a high reactive surface area, thus enabling the adsorption of a large amount of SOM. The presence of Ca may further promote the binding of P and SOM onto these mineral surfaces (Weng et al., 2011). Therefore, besides BC, Ca and P are likely to play important roles in explaining long-term persistence of SOM in ADE.

The aim of this study was to (i) determine the BC content in SOM pools in ADE and ADJ upon chemical oxidation with NaOCl and subsequent demineralization with HF in combination with the BPCA method and to (ii) investigate the role of Ca and P in further explaining long-term persistence of SOM in ADE. In line with other studies, we hypothesized that BC resists chemical oxidation with NaOCl and subsequent demineralization with HF. In this approach, we attempt to estimate the relative contribution of BC in SOM pools and to derive the relative contribution of different mechanisms of SOM stabilization (i.e. mineral association and chemical recalcitrance) in explaining long-term persistence of BC in ADE. Furthermore, we hypothesized that high Ca and P contents in ADE may further explain larger SOM contents in ADE due to the role of Ca in cation bridging and P in creating a higher reactive surface area of soil minerals.

5.2. Material and Methods

Soil samples and analysis

In this study, we compared topsoil (0–20 cm), subsoil (40–60 cm) and deep subsoil (80–100) samples collected in three field replicates in the pretic area (i.e. proxy for ADE) and non-pretic area (i.e. proxy for ADJ (Fig. 1) as described in **Chapter 2** (Alho et al., 2019). Soil samples were air-dried at room temperature, passed through a 2mm sieve and visible roots were removed manually. For further analysis, a subsample was ground in a ball mill.

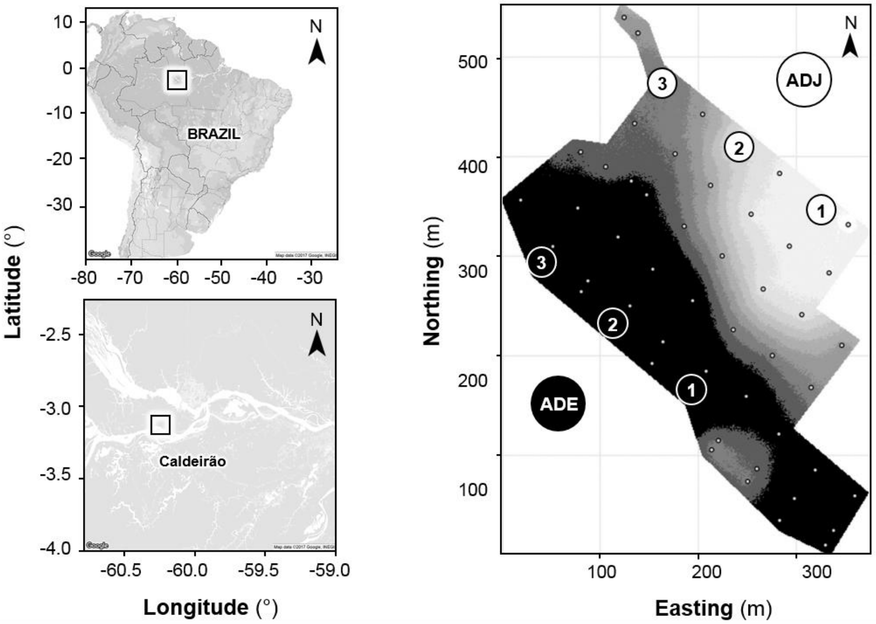

Fig. 1. Location of the study area (Experimental Research Station of Caldeirão - Embrapa Western Amazon) (Google Earth® images) and location of the three field replicates collected in the pretic (i.e. proxy for ADE) and non-pretic area (i.e. proxy for ADJ) according to **Chapter 2** (Alho et al., 2019).

For statistical analyses, significant differences between mean values in ADE versus ADJ per each soil depth were tested with analysis of variance (ANOVA) performed with three predefined orthogonal contrasts (C1–C3, Table 1). Data were tested and transformed to Gaussian using the Box-Cox family of power transformations to meet statistical assumptions (normality and homoscedasticity of residues). Matrices of correlation coefficients for selected parameters are shown in the Supplementary Data (Table S1 and S2) to support exploratory data analysis. Linear regression models were used to evaluate the relationship between selected parameters by interpreting the coefficient of determination (R^2) and the significance of the models (p).

Table 1. Orthogonal contrasts (C1-C3) used for the statistical analysis.

	1	**2**	**3**	**4**	**5**	**6**	**Orthogonal comparison**				
C1	1	-1	0	0	0	0	ADE	0-20	vs	ADJ	0-20
C2	0	0	1	-1	0	0	ADE	40-60	vs	ADJ	40-60
C3	0	0	0	0	1	-1	ADE	80-100	vs	ADJ	80-100

Samples were analysed for soil pH, cation exchange capacity (CEC), clay content, Total C, Ca, P, Fe, Al and the amounts of iron (Fe) and aluminium (Al) oxides. Soil pH was determined in water (soil:water ratio of 1:2.5), potential CEC (at pH = 7.0) was defined as the sum of exchangeable cations (K, Na, Ca + Mg) plus acidity (H + Al) and the clay content was determined by the pipette method after organic matter removal with hydrogen peroxide. The amount of Fe and Al oxides was determined by extractions with dithionite-citrate-bicarbonate (DCB) and ammonium oxalate. The amount of oxalate–extractable Fe (Fe_o) and Al (Al_o) is used as proxy for poorly crystalline Fe and Al oxides, whereas the difference between the amount of DCB- and oxalate-extractable Fe (Fe_{d-o}) and Al (Al_{d-o}) is used as a proxy for crystalline Fe and Al oxides. In addition, we determined the amount oxalate-extractable P (P_o), which is used as a proxy for P bound to the Fe and Al oxides. Detailed description of the methods are described in Teixeira et al. (2017). Total C was determined using an elemental analyser (PerkinElmer 2400 Series II) using acetanilide as reference material. Total Ca, P, Fe, and Al were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) after digestion with a multi-acid solution (HCl, HNO_3, HF, and $HClO_4$).

Chemical oxidation with NaOCl

Chemical oxidation with NaOCl was performed according to a modified version of the method described in Mikutta et al. (2006) (**Chapter 3**). Briefly, 30 mL of 6% NaOCl solution adjusted to pH 8.0 by adding 32% hydrochloric acid (HCl) was added to plastic bottles containing 3.0 g of soil. The bottles were shaken in a reciprocal shaker for 2 h at room temperature. Samples were then centrifuged (at 2574 *g* for 10 min) and the supernatants discarded. The residues were thoroughly washed with deionized water until electrical conductivity was < 40 µs cm^{-1} and oven-dried (at 70 °C for 24 h). Subsamples were taken for the determination of the OC and N content in the residues. The NaOCl solution was renewed and the procedure repeated one more time.

In **Chapter 4**, we showed that the vast majority (> 80%) of the Total C content had to be considered 'labile' (i.e. NaOCl-oxidizable) in both soils. Here, we hypothesized that some OC compounds could become more soluble upon chemical oxidation with NaOCl due to partial oxidation rather than complete oxidation to carbon dioxide (CO_2) and subsequent solubilisation in the washing procedure. Therefore, we determined the amount of dissolved organic carbon (DOC) in the supernatants after each treatment with NaOCl and after washing of the samples with deionized water. The rationale behind this procedure is to account for OC compounds that could have been discarded with the supernatants due to partial oxidation, increased pH, and addition of Na ions that may increase the solubility of OC compounds.

Subsequent demineralization with HF

Subsequently, 20 mL of 10% HF solution was pipetted into plastic bottles containing 1.0 g of oven-dried NaOCl-treated soil. Samples were shaken for 2h at room temperature, centrifuged (at 2574 *g* for 10 min), and the supernatants were discarded. The HF solution was renewed and the procedure was repeated three times with a total of four consecutive treatments. Next, the samples were centrifuged (at 2574 *g* for 10 min) and the supernatants discarded. The residues were washed five times with 20 mL deionized water, oven-dried (at 70 °C for 24 h), and a subsample was taken for the determination of the OC and N contents in the residues.

The OC and N contents were determined using an elemental analyser as previously described. OC pools were operationally defined as: ‘labile’ (i.e. NaOCl-oxidizable OC) and ‘stable’ (i.e. NaOCl-resistant OC). The sum of OC lost in the supernatants as DOC (C-DOC) was subtracted from the amount of ‘labile’ OC in order to estimate the amount of OC that was indeed fully oxidized to CO_2 (C-CO_2). The ‘stable’ OC pool was further subdivided into ‘mineral-associated’ OC (i.e. HF-soluble OC) and ‘recalcitrant’ OC (i.e. HF-resistant OC). The ‘labile’ OC and ‘mineral-associated’ OC were obtained by difference (‘labile’ OC = initial OC - ‘stable’ OC and ‘mineral-associated’ OC = ‘stable’ OC - ‘recalcitrant’ OC).

BPCA analysis

The BC content was determined according to the BPCA method (Brodowski et al., 2005b; Glaser et al., 1998). Briefly, 10 mL of 4 M trifluoroacetic acid (TFA) was pipetted into glass flasks containing 0.5 g of soil and reacted at 105 °C for 4h to remove polyvalent cations. Next, the samples were reacted with 2 mL 65% HNO_3 for 8h at 170 °C in a high-pressure apparatus and then purified with a cation exchange resin prior to trimethylsilyl derivatisation for the separation and detection of BPCA compounds by Gas-Chromatography (GC) coupled with Flame Ionization Detector (FID). BPCA standard solutions were used in combination with internal (2,2’-biphenyldicarboxylic acid) and recovery standards (phthalic acid) for the quantification of the BPCA compounds (i.e. B3-, B4-, B5- and B6CA represent BPCA with three, four, five and six carboxylic groups, respectively). The sum of B3-, B4-, B5- and B6CA is multiplied by the conversion factor of 2.27 as suggested by Glaser et al. (1998).

5.3. Results and Discussion

Soil properties

Depth-wise distribution of soil pH, CEC, clay content, Total C, Total Ca and Total P in ADE and ADJ are shown in Fig. 2. Selected samples exhibited the overall pattern in our study area discussed in **Chapter 2** (Alho et al., 2019). However, the ADE samples have a more pronounced enrichment of particularly Total Ca and Total P. The corresponding enrichment factors are given with numbers in Fig. 2e and 2f. The higher values than average underline the high spatial variation and illustrate the localized soil enrichment at the site studied (Alho et al., 2019). It implies that the comparison of highly contrasting samples is likely to overemphasize the general differences between ADE and ADJ, most importantly the enrichment in Ca and P.

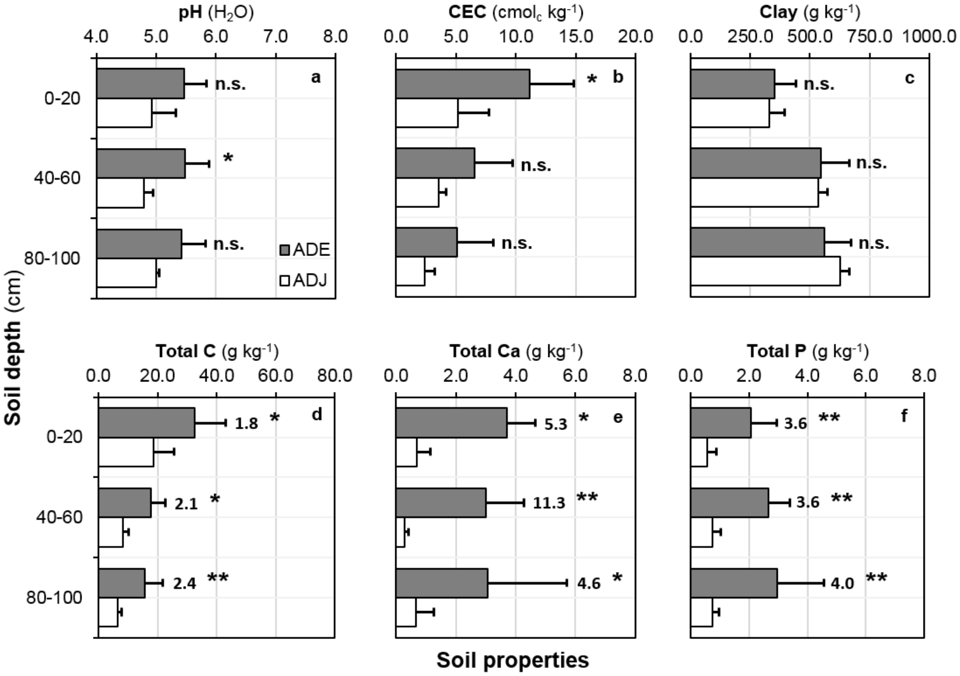

Fig. 2. Depth-wise distribution of soil pH (a), CEC (b), clay content (c), Total C (d), Total Ca (e) and Total P (f) in Amazonian Dark Earth (ADE) and the adjacent (ADJ) Ferralsol. Mean values and standard deviations were calculated from three field replicates in ADE (filled bars) and ADJ (empty bars). The enrichment factors for Total C, Total Ca and Total P in ADE are shown on the right side of the bars. Significant differences between mean values in ADE versus ADJ per soil depth were tested with orthogonal contrast and are indicated with * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) or n.s. (not significant).

OC pools

The distribution of 'labile' (i.e. NaOCl-oxidizable OC) and 'stable' (i.e. NaOCl-resistant OC) OC pools decreases with soil depth in both ADE (Fig. 3a) and ADJ (Fig. 3c). The amount of 'labile' OC was larger in ADE compared to ADJ, whereas the amount of 'stable' OC did not differ between ADE (Fig. 3a) and ADJ (Fig. 3c) as already pointed out in **Chapter 4** for topsoil samples. It implies that the relative contribution of the 'stable' OC pool is larger in ADJ (Fig. 3d) compared to ADE (Fig. 3b) and increases with soil depth in ADJ (Fig. 3d), whereas in ADE (Fig. 3b) it remains fairly constant with soil depth.

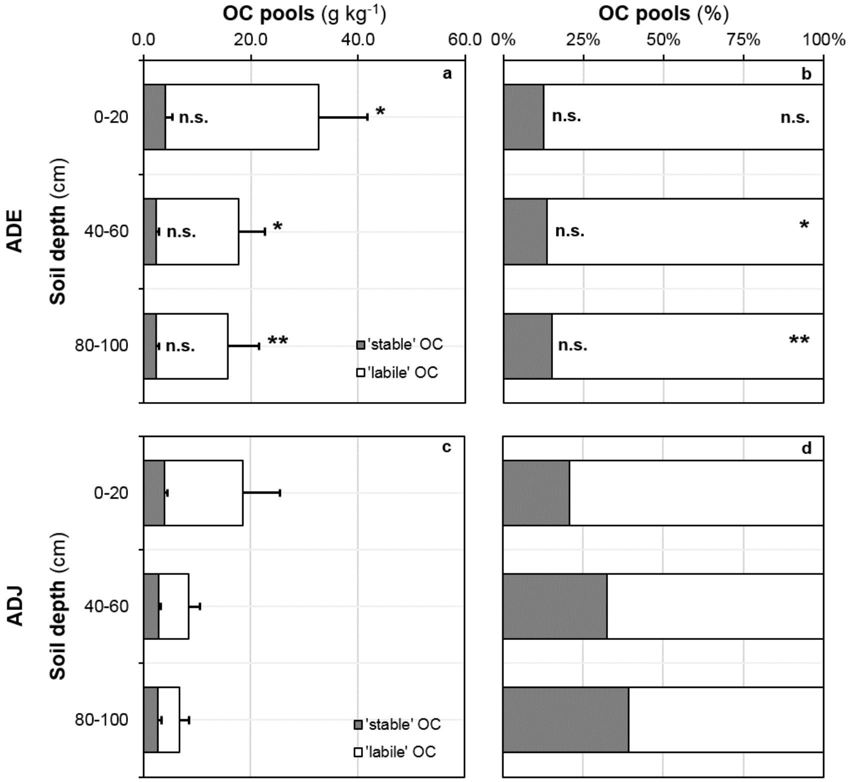

Fig. 3. Depth-wise distribution of organic carbon (OC) pools (i.e. ' labile' OC and ' stable' OC) in Amazonian Dark Earth (ADE) (a) and the adjacent (ADJ) Ferralsol (c) and their relative contribution to the Total C content in ADE (b) and ADJ (d). Mean values and standard deviations were calculated from three field replicates in ADE (filled bars) and ADJ (empty bars). Significant differences between mean values in ADE versus ADJ per soil depth were tested with orthogonal contrast and are indicated with * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) or n.s. (not significant).

In this study, we further differentiated the 'labile' OC pool into two fractions (i.e. C-DOC and C-CO_2) (Fig. 4). Compared to the amount of C-CO_2, the amount of C-DOC was very small in both ADE (Fig. 4a) and ADJ (Fig. 4c). The amount of C-DOC was on average 0.7 and 0.2 g kg^{-1} in ADE and ADJ, respectively. These values correspond to 4.2 and 6.2% of the 'labile' OC pool in ADE and ADJ, respectively. Other studies did not account for C-DOC upon chemical oxidation with NaOCl. However, our results indicate that operationally some of the 'labile' OC pool comprises OC that is soluble in the chemical oxidation procedure with NaOCl rather than being oxidized to CO_2. Fortunately, its contribution is little and the vast majority (> 90 %) of the 'labile' OC pool is indeed oxidizable C (C-CO_2).

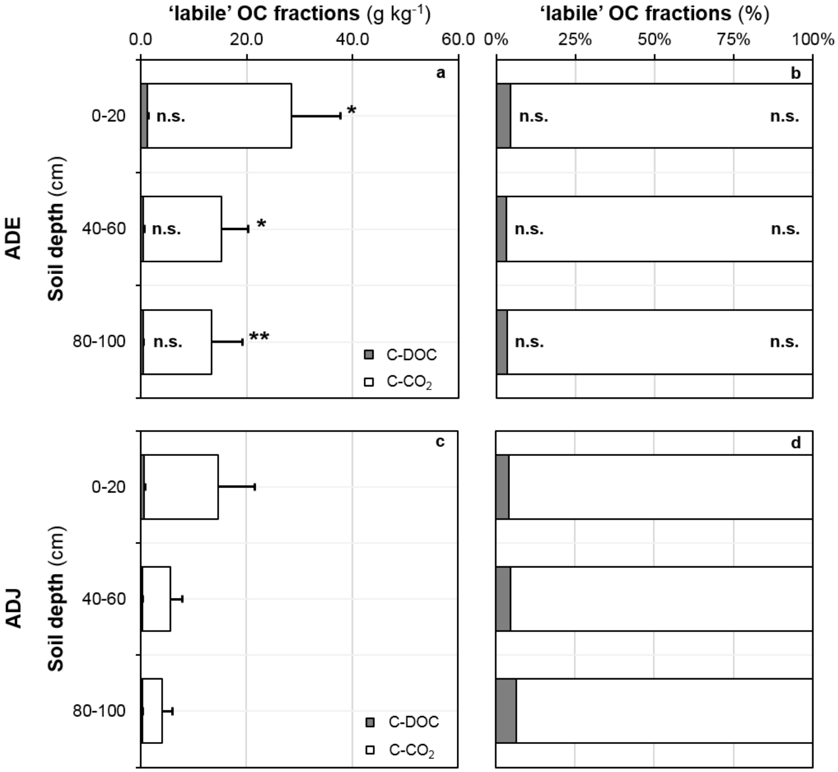

Fig. 4. Depth-wise distribution of 'labile' OC fractions (i.e. C-CO_2 and C-DOC) in Amazonian Dark Earth (ADE) (a) and the adjacent (ADJ) Ferralsol (c) and their relative contribution to the 'labile' OC pool in ADE (b) and ADJ (d). Mean values and standard deviations were calculated from three field replicates in ADE (filled bars) and ADJ (empty bars). Significant differences between mean values in ADE versus ADJ per soil depth were tested with orthogonal contrast and are indicated with * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) or n.s. (not significant).

Depth-wise distribution of ‘stable’ OC fractions (i.e. ‘mineral-associated’ OC and ‘recalcitrant’ OC) showed a decrease from the topsoil (0–20 cm) to the subsoil (40–60 cm), but did not differ from the subsoil to the deep subsoil (80–100 cm) in both ADE (Fig. 5a) and ADJ (Fig. 5c). Moreover, the absolute amounts of ‘stable’ OC did not differ between ADE (Fig. 5a) and ADJ (Fig. 5c), nor did the relative contribution of ‘mineral-associated’ and ‘recalcitrant’ OC pools (Fig. 5b and 5d for ADE and ADJ, respectively). However, the absolute amounts of ‘mineral- associated’ OC was slightly larger in ADJ compared to ADE. This suggests more binding of OC to the mineral surfaces which may be due to the presence of less P in ADJ than in ADE (Fig.1e) causing less competition of OC for binding to the oxide surfaces.

The amount of ‘recalcitrant’ OC was on average 0.20 and 0.24 g kg^{-1} in ADE and ADJ, respectively. These values correspond to as little as 6.6 and 8.0% of the ‘stable’ OC pool in ADE and ADJ, respectively. Our results indicate that the amount of ‘recalcitrant’ OC are essentially the same in both soils, contrary to other studies that suggested that the ‘recalcitrant’ OC pool is larger in ADE due to the occurrence of BC (Glaser, 2007; Glaser et al., 2003). Even though BC has been claimed to contribute to the ‘recalcitrant’ OC pool in ADE, the present results are in agreement with the claim that ‘mineral-association’ is more important for the long-term stabilization of SOM than ‘chemical recalcitrance’ (Marschner et al., 2008; Mikutta et al., 2006).

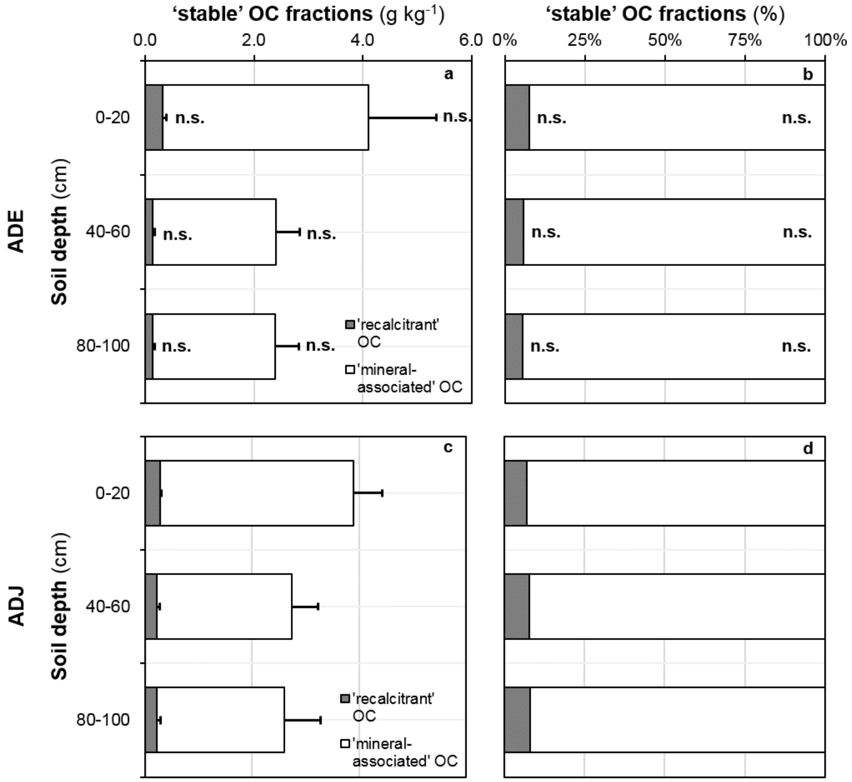

Fig. 5. Depth-wise distribution of 'stable' organic carbon (OC) fractions (i.e. 'mineral-associated' OC and 'recalcitrant' OC) in Amazonian Dark Earth (ADE) (a) and the adjacent (ADJ) Ferralsol (c) and their relative contribution to the 'stable' OC pool in ADE (b) and ADJ (d). Mean values and standard deviations were calculated from three field replicates in ADE (filled bars) and ADJ (empty bars). Significant differences between mean values in ADE versus ADJ per soil depth were tested with orthogonal contrast and are indicated with * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) or n.s. (not significant).

Changes in the C:N ratio upon chemical oxidation with NaOCl are shown in Fig. 6. The C:N ratio before (Fig. 6a) and after NaOCl (Fig. 6b) showed that the C:N ratio is higher after NaOCl in both soils. This indicates that N-rich compounds were preferentially removed upon chemical oxidation with NaOCl. This pattern has also been reported in other studies (Jagadamma et al., 2010; Kleber et al., 2005; Mikutta et al., 2006; Sleutel et al., 2009). However, it is not clear whether this is due to a preferential oxidation of N-rich compounds or less mineral-association of N-rich compounds (Kleber et al., 2005). The distribution of the C:N ratio of OM before NaOCl oxidation increases with soil depth in ADE, whereas it decreases in ADJ.

Both results are in agreement with literature. Rumpel and Kögel-Knabner (2011) reported that in general, the C:N ratio decreases with soil depth, indicating a larger contribution of microbial-derived OC in the subsoils. However, these authors also reported that in some cases, the C:N ratio can also increase with soil depth due to the occurrence of BC, which is expected for our ADE soil samples.

The C:N ratio after NaOCl remained fairly constant with soil depth in ADE, whereas in ADJ, it decreased with soil depth (Fig. 6d). The 'stable' OC pool in the deeper layers of ADJ is richer in N than in ADE, which could indicate a different origin of this OM. The 'stable' pool in ADJ – mostly 'mineral-associated' OC – may have a larger contribution of microbial-derived OC compared to ADE (Rumpel and Kögel-Knabner, 2011).

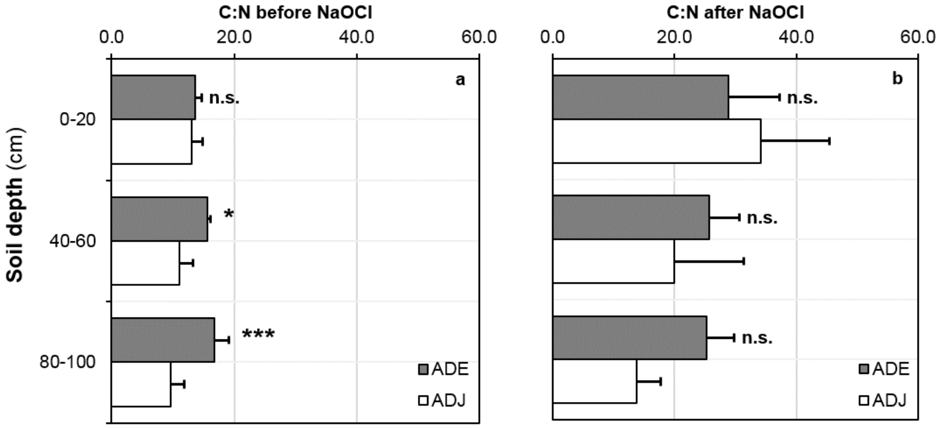

Fig. 6. Depth-wise pattern of carbon to nitrogen (C:N) ratio before (a) and after (b) chemical oxidation with NaOCl in Amazonian Dark Earth (ADE) and the adjacent (ADJ) Ferralsol. Mean values and standard deviations were calculated from three field replicates in ADE (filled bars) and ADJ (empty bars). Significant differences between mean values in ADE versus ADJ per soil depth were tested with orthogonal contrast and are indicated with * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) or n.s. (not significant).

For the 'recalcitrant' OC pool (i.e. HF-resistant OC), it was not possible to calculate the C:N ratio because the amount of N was below the detection limit in both soils. Nonetheless, one may conclude that the 'recalcitrant' OC pool is largely N-poor. Studies often suggest that the 'recalcitrant' OC pool upon chemical oxidation with NaOCl and demineralization with HF is mainly composed of aliphatic OC, most likely hydrophobic compounds (Eusterhues et al., 2005, 2003). However, chemical composition in relation to the stabilization of these hydrophobic compounds against microbial decomposition is not well known, which certainly deserves more attention in future studies. Some other studies suggested that the ‘recalcitrant’ OC pool could also be composed of poly-condensed aromatic compounds (e.g. BC) (Bruun et al., 2008; Mikutta et al., 2005b).

BC in OC pools

The absolute amounts of BC and non-BC and their relative contribution to the Total C content in the original ADE and ADJ soils without NaOCl treatment are shown in Fig. 6. The fractions of BC and non-BC decreased with soil depth in both soils. In ADE, the relative contribution of BC did not differ with soil depth and was on average 18.9%, whereas in ADJ, it increased with soil depth, reaching 22.9% in the deep subsoil (80–100 cm). Our results indicate that despite the fact that ADE showed larger absolute amounts of BC, their relative contribution to the Total C content did not differ greatly between ADE and ADJ. This is rather unexpected as ADE have been considered as highly enriched in BC. Our results contrast with previous studies on ADE where authors reported impressive BC contents in ADE (up to 70 times larger than ADJ) (Glaser, 2007; Glaser et al., 2000). However, it is important to note that these BC contents were determined according to the first version of the BPCA methodology (Glaser et al., 1998), in which hydrochloric acid (HCl) was used for the removal of polyvalent cations. Brodowski et al. (2005) showed that the use of HCl can generate BC-like compounds and overestimate the BC content up to 90%. Therefore, the impressive BC contents in ADE that has been previously reported in other studies (Glaser, 2007; Glaser et al., 2000) can be highly questioned.

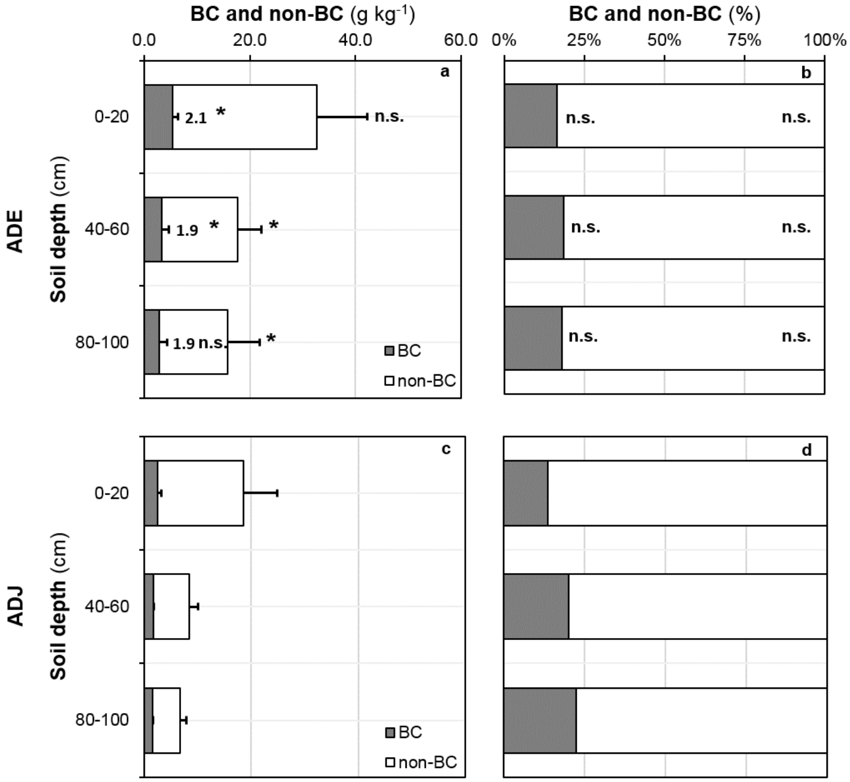

Fig. 7. Depth-wise distribution of black carbon (BC) and non-black carbon (non-BC) in untreated samples of Amazonian Dark Earth (ADE) (a) and the adjacent (ADJ) Ferralsol (b) and their relative contribution to the Total C content in ADE (c) and ADJ (d). Non-black carbon contents were estimated by the difference between the Total C and BC contents. Mean values and standard deviations were calculated from three field replicates in ADE (filled bars) and ADJ (empty bars). The enrichment factors for BC in ADE are shown on the right side of the bars. Significant differences between mean values in ADE versus ADJ per soil depth were tested with orthogonal contrast and are indicated with * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) or n.s. (not significant).

Depth-wise distribution of the various individual BPCA in ADE and ADJ are shown in Fig. 7. According to our measurements, BC is more polycondensed in ADE than in ADJ as the relative contribution of B6 in ADE is larger (Fig. 7b). Moreover, our results indicate that BC is more polycondensed in both topsoils (Fig. 7b). In the subsoils, the contribution of B3-B5 is higher which indicates that deeper in the soil profiles, the BC fraction is more oxidized which may be due to a smaller size of the initial polyaromatic molecules. The distribution as well as the absolute levels can be discussed in terms of the origin of BPCA in these soils.

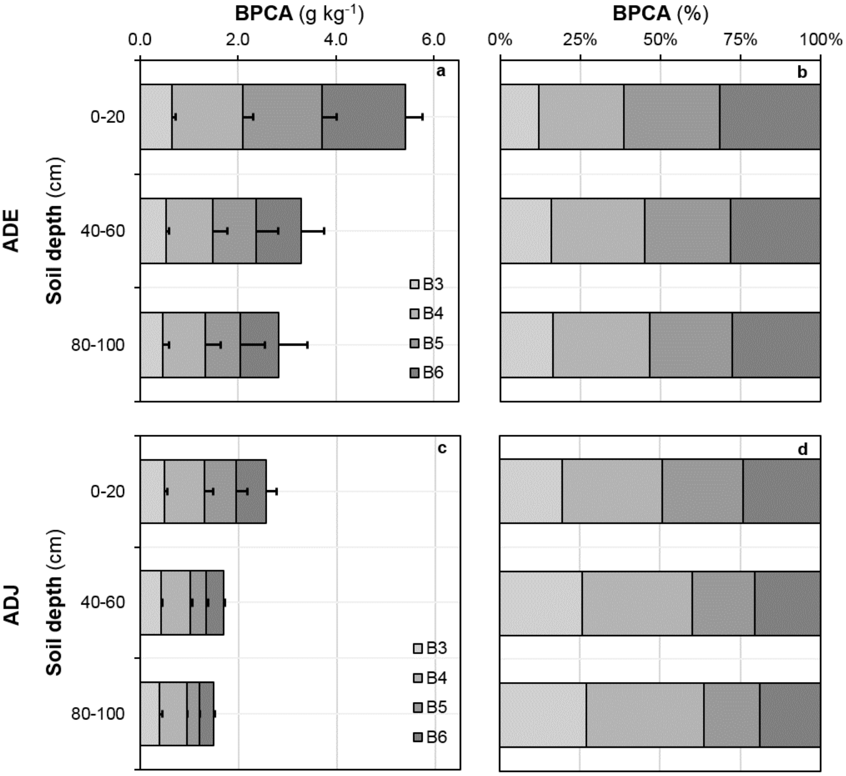

Fig. 8. Depth-wise distribution pattern of benzene polycarboxylic acids (BPCA) in untreated samples of Amazonian Dark Earth (ADE) (a) and the adjacent (ADJ) Ferralsol (b) and the relative contribution of individual BPCA in ADE (c) and ADJ (d). Mean values and standard deviations were calculated from three field replicates in ADE (filled bars) and ADJ (empty bars).

It is important to note that the origin of BC cannot be attributed exclusively to the remains of charred OM since studies have shown that BC is not necessarily only pyrogenic (Di Rauso Simeone et al., 2018; Glaser and Knorr, 2008). Di Rauso Simeone et al. (2018) reported that BPCA can naturally occur in soils as the microbial by-product of OC degradation. According to these authors, BPCA in Ferralsol may result from a biogenic source. Polyaromatic carbon is for instance found in the black pigment of the fungus *Aspergillus niger*. Biogenic BC has been considered either negligible (Brodowski et al., 2005b) or significant (Glaser and Knorr, 2008), but the possibility of biogenic sources of polyaromatic carbon has been largely overlooked in the literature. Therefore, more studies are certainly needed in order to better estimate the relative contribution of different sources of BC to the total BC content in soils. Combining isotopic labelling with BPCA analysis is likely to provide a better understanding of different sources of BC in environmental matrices (Di Rauso Simeone et al., 2018; Glaser and Knorr, 2008).

In line with suggestions from previous studies (Bruun et al., 2008; Eusterhues et al., 2003; Leifeld and Zimmermann, 2006; Mikutta et al., 2005b; Simpson and Hatcher, 2004), we had previously hypothesized that BC resists chemical oxidation with NaOCl and accounts for most of the ‘stable’ OC pool (i.e. NaOCl-resistant pool). However, BC was not detected in the NaOCl-resistant OC fraction via the BPCA analysis in both soils. Our data suggest that BC is part of the ‘labile’ OC pool rather than the ‘stable’ or ‘recalcitrant’ OC pools as suggested in other studies (Bruun et al., 2008; Mikutta et al., 2005b). It would imply that the removal of aromatic OC upon chemical oxidation with NaOCl is not limited to mono-aromatic OC (i.e. lignin) (Simpson and Hatcher, 2004) and that polycondensed aromatic OC (i.e. BC) can be oxidized too. However, the degree of aromatic polymerisation of BC may also play a role. Studies have shown that chemical oxidation of charcoal with NaOCl can enhance surface charge (Araujo et al., 2014; Linhares et al., 2012). In that process, carboxylic functional groups are created by oxidation. Our results bring implications for the isolation of a so-called ‘stable’ OC pool upon chemical oxidation with NaOCl and challenges the claims on the ‘inherent chemical recalcitrance’ of BC. Studies have shown that the resistance of BC to chemical oxidation decreases with time (i.e. BC ‘ageing’) (Hardy and Dufey, 2017), which imposes challenges to the usefulness of strong chemical oxidation procedures (Naisse et al., 2013), most importantly in soils where BC is expected to be ‘aged’, like in ADE.

Organo-mineral associations

Depth-wise distribution of Total Fe and Total Al as well as crystalline and poorly crystalline Fe and Al oxides are shown in Fig. 9. Total Fe and Total Al as well as the amount of crystalline Fe and Al oxides did not differ between ADE and ADJ (Fig. 9a to 9e). This is expected since ADE and ADJ share the same parent material. However, the amount of poorly-crystalline Fe and Al oxides was larger in ADE compared to ADJ (Fig. 9c and 9f).

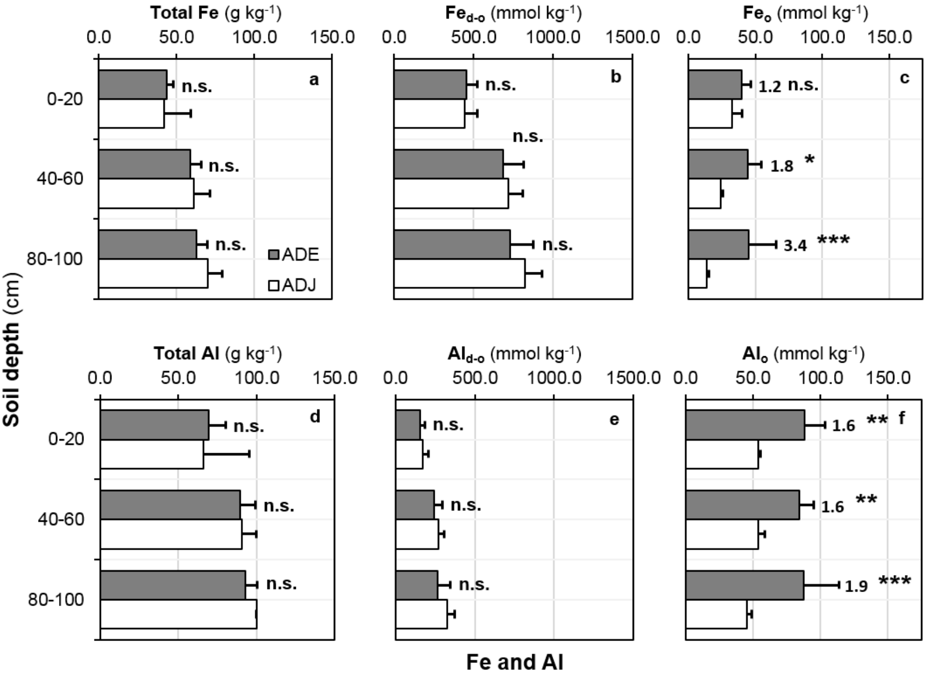

Fig. 9. Depth-wise distribution of Total Fe (a), Total Al (d), crystalline Fe (Fe_{d-o}) (d) and Al (Al_{d-o}) (e) and poorly-crystalline Fe (Fe_o) (c) and Al (Al_o) (f) oxides in Amazonian Dark Earth (ADE) and adjacent (ADJ) Ferralsol. Mean values and standard deviations were calculated from three field replicates in ADE (filled bars) and ADJ (empty bars). The enrichment factors for Fe_o and Al_o per soil depth are shown on the right side of each bar. Significant differences between mean values in ADE versus ADJ per soil depth were tested with orthogonal contrast and are indicated with * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) or n.s. (not significant).

Poorly-crystalline Fe and Al oxides have been suggested as important for the stabilization of SOM via ligand exchange between hydroxyl groups on the surface of soil minerals and negatively charged functional groups of OM compounds (Kleber et al., 2005; Mikutta et al., 2005a). Furthermore, it has been suggested that adsorption of P and SOM to Fe and Al oxides may inhibit their further growth (Eusterhues et al., 2008; Fukushi and Sato, 2005) and are likely to be the cause of nano-sized particles with high surface area in soils (Hiemstra et al., 2010b). In P-rich soils such as ADE, this may lead to a large fraction of nanoparticles with high surface similarly to agricultural top soils as reported by Hiemstra et al. (2010).

The amount of oxalate-extractable P (P_o) (i.e. a proxy for P bound to nano-sized Fe and Al oxides) exhibited a significant (*p* = 0.00) high positive correlation ($R^2 = 0.72$)

with the amount of $(Fe + Al)_o$ in ADE, whereas in ADJ, we did not find a correlation ($R^2 = 0.17$, $p = 0.27$) between these parameters (Fig. 10b). We did not find a correlation ($R^2 = 0.00$, $p = 0.89$) between Total C and the amount of $(Fe \text{ and } Al)_o$ in ADE, whereas in ADJ, these parameters are correlated ($R^2 = 0.62$, $p = 0.01$) (Fig. 10a). Lima et al. (2002) reported a larger content of (Fe_o) in the topsoil in ADE compared to ADJ, which the authors attributed to an effect of higher SOM content in ADE. However, our results indicate that larger $(Fe + Al)_o$ in ADE follows the depth-distribution of P (Fig. 2f). In case of ADE, more $(Fe + Al)_o$ is present at the same C content (Fig.10a), particularly at low levels of OC. It suggests that part of the oxide fraction is not contributing to this association, resulting in the absence of a correlation ($R^2 = 0.00$, $p = 0.89$). It may be due to the competition between P and OC for binding to the oxides. The presence of more adsorbed P (Fig.10b) prevents binding of more OM (Fig.10a).

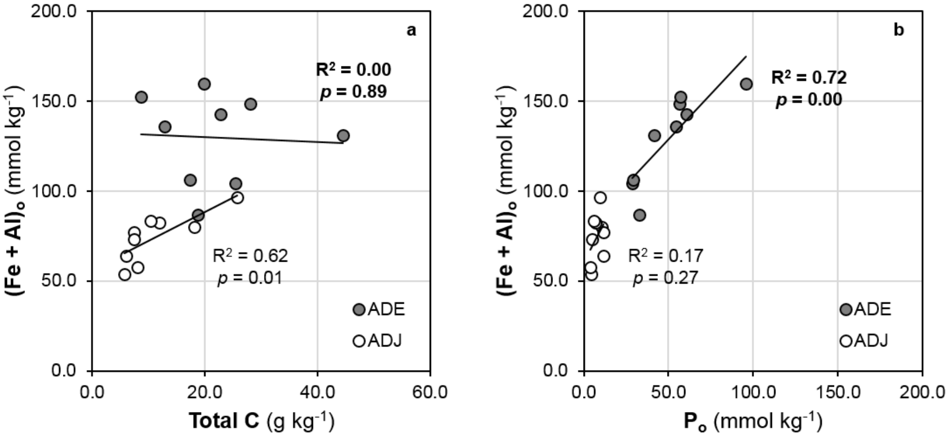

Fig. 10. Relation between Total C (a) and oxalate-extractable P (P_o) (b) and the amount of oxalate-extractable iron and aluminium oxides $(Fe + Al)_o$ in Amazonian Dark Earth (ADE) and the adjacent (ADJ) Ferralsol. For details about the methods, please see text.

These findings are in agreement with our hypothesis that high P input enhances the reactive surface area in ADE, which likely contributed to the overall larger SOM content in these soils by promoting stabilization of SOM. It is surprising that even though studies have highlighted the importance of $(Fe + Al)_o$ in explaining long-term persistence of SOM via organo-mineral associations (Kleber et al., 2005; Mikutta et al., 2005a), this parameter has been largely overlooked in previous studies on ADE, probably because authors relied on the fact that ADE and ADJ share the same parent material.

Moreover, polyvalent cations (mainly Ca^{2+}) are known to form bridges between negatively charged OM compounds and soil minerals (Baldock and Skjemstad, 2000; Muneer and Oades, 1989), further contributing for the stabilization of SOM. The amount of Ca^{2+} showed a trend to be positively correlated with the Total C content in both soil types, but not significantly (p > 0.05) (Fig. 11a). However, the amount of Ca^{2+} exhibited a significant (p = 0.00) very high positive correlation (R2 = 0.92) with the BC content in ADE, whereas in ADJ, we did not find a correlation (R^2 = 0.05, p = 0.57) between these parameters (Fig. 11b). Several studies have shown that BC surface oxidation is a key property in ADE (Jorio et al., 2012; Lehmann et al., 2005; Liang et al., 2013; Mao et al., 2012). BC surface charge can be compensated by the adsorption of Ca^{2+}. Moreover, cation bridging with surrounding organic molecules may promote the stabilization of substantial amount of OC (of non-BC origin) that is associated with highly charged BC in ADE, which likely explains the overall larger non-BC contents in ADE compared to ADJ (Fig. 7).

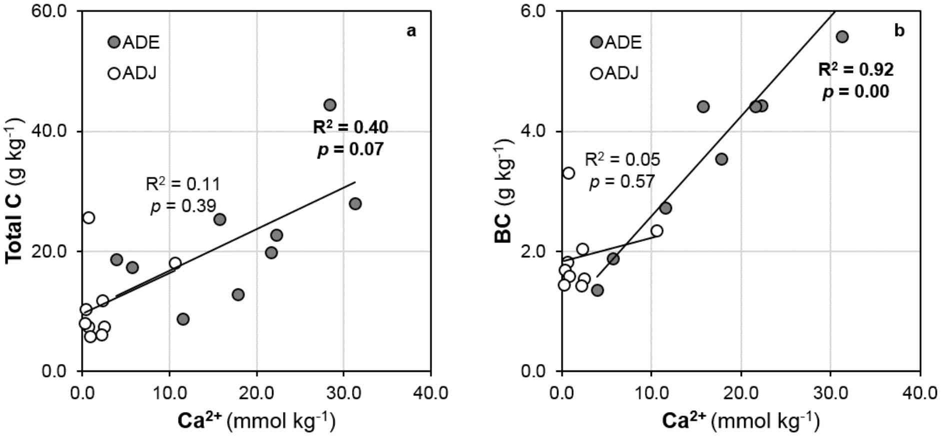

Fig. 11. (a) Relation between exchangeable Ca (Ca^{2+}) and Total C (a) and black carbon (BC) (b) in Amazonian Dark Earth (ADE) and the adjacent (ADJ) Ferralsols. For details about the methods, please see text.

These findings are in agreement with our hypothesis that a high Ca input in ADE likely contributed to the overall larger SOM content in these soils, most likely via Ca bridging (Baldock and Skjemstad, 2000; Muneer and Oades, 1989). Recent studies also suggested that incorporation of Ca into BC structures may occur (Archanjo et al., 2014; Castro et al., 2016). Combined, these findings point to a synergistic effect between Ca and BC in ADE.

Overall, our results suggest that high input of Ca and P in ADE may have played important roles in the long-term persistence of SOM in these soils, rather than BC alone. Our proposal that the interactive effects of BC, Ca and P are crucial for the development of ADE has implications for practices that try to recreate such soils by simply adding BC (e.g. biochar). Future studies could investigate the interactive roles of BC, Ca and P in controlled experiments, where the presence or absence of these elements are manipulated in order to evaluate the added effect of their interactive roles in SOM stabilization.

5.4. Conclusion

Our results showed that the relative contribution of BC in ADE did not differ from the ADJ soil, which contrasts with previous studies on ADE. Moreover, our results raised questions for the isolation of a so-called ‘stable’ OC pool upon chemical oxidation procedures. Our results indicated that besides BC, the nutrients Ca and P are important in further explaining high SOM contents in ADE compared to ADJ. In this study, we suggested possible mechanisms by which Ca and P may contribute to the long-term persistence of SOM in ADE, which brings implications for the sole use of biochar as a strategy to reproduce ADE desirable characteristics in other soils.

5.5. Acknowledgments

This work was done under the scope of the Terra Preta Program and funded by the Interdisciplinary Research and Education Fund (INREF) of Wageningen University and Research and the Brazilian Agricultural Research Corporation (Embrapa).

Supplementary Data

Table S1. Matrices of correlation coefficients between pairs of selected soil parameters in Amazonian Dark Earth (ADE).

ADE	Total OC	NaOCl-resistant OC	HF-soluble OC	BC	Ca^{2+}	P_o	$(Fe + Al)_o$
Total OC	**1.00**						
NaOCl-resistant OC	0.91	**1.00**					
HF-soluble OC	0.89	1.00	**1.00**				
BC	0.75	0.68	0.65	**1.00**			
Ca^{2+}	0.63	0.54	0.51	0.96	**1.00**		
P_o	-0.17	-0.27	-0.28	0.31	0.45	**1.00**	
$(Fe + Al)_o$	-0.05	-0.12	-0.14	0.52	0.65	0.85	**1.00**

Total organic carbon (Total OC), NaOCl-resistant OC (i.e. ‘stable’ OC), HF-soluble OC (i.e. ‘mineral-associated’ OC), black carbon (BC), exchangeable calcium (Ca^{2+}), oxalate-extractable phosphorus (P_o) (i.e. proxy for P bound to soil poorly crystalline oxides) and sum of oxalate-extractable iron (Fe_o) and aluminium (Al_o).

Table S2. Matrices of correlation coefficients between pairs of selected soil parameters in the adjacent (ADJ) Ferralsol.

ADJ	Total OC	NaOCl-resistant OC	HF-soluble OC	BC	Ca^{2+}	P_o	$(Fe + Al)_o$
Total OC	**1.00**						
NaOCl-resistant OC	0.60	**1.00**					
HF-soluble OC	0.57	1.00	**1.00**				
BC	0.97	0.73	0.71	**1.00**			
Ca^{2+}	0.33	0.18	0.16	0.22	**1.00**		
P_o	0.31	0.29	0.30	0.25	0.52	**1.00**	
$(Fe + Al)_o$	0.79	0.57	0.56	0.78	0.17	0.41	**1.00**

Total organic carbon (Total OC), NaOCl-resistant OC (i.e. ‘stable’ OC), HF-soluble OC (i.e. ‘mineral-associated’ OC), black carbon (BC), exchangeable calcium (Ca^{2+}), oxalate-extractable phosphorus (P_o) (i.e. proxy for P bound to soil poorly crystalline oxides) and sum of oxalate-extractable iron (Fe_o) and aluminium (Al_o).

Chapter 6

General Discussion

Carlos Francisco Brazão Vieira Alho

6.1. Prologue

Since first migrants crossed the Bering Strait at the end of the last Ice Age and reached the Amazon (Reich et al., 2012), people have been living and transforming Amazonian forests and soils for more than ten thousand years (Roosevelt, 2013). Current theories suggest that these transformations generally improved the living conditions for these Amerindian people and can therefore be considered as a case of human niche construction (Clement et al., 2015), which contrasts with a previous theory that postulated that adverse conditions would impose limitations for the cultural development in the Amazon (Meggers, 1954). Due to the European conquest in the sixteenth century, the Amerindian population collapsed (Llamas et al., 2016), but their legacy is imprinted in the domesticated landscape nearby ADE sites (Levis et al., 2017) and in the Amazonian Dark Earths (ADE) (Glaser et al., 2001a).

6.2. Main Findings and Conclusions

In my thesis, I used geostatistics (**Chapter 2**), selective chemical extractions (**Chapters 2–5**), soil organic matter (SOM) fractionation (**Chapter 3–5**), spectroscopic techniques (**Chapter 4**) and molecular markers (**Chapter 5**) to investigate the interactive roles of BC, Ca and P on the long-term persistence of SOM in ADE.

In **Chapter 2**, I showed that a combination of spatial modelling techniques with the criteria for a pretic horizon (IUSS Working Group WRB, 2015) was useful to systematically distinguish the pretic area (i.e. proxy for ADE) from the non-pretic area (i.e. proxy for the adjacent (ADJ) Ferralsol) in our study area (Alho et al., 2019). This is important because it takes into consideration quantitative data rather than vague descriptive observations, such as soil colour and the presence of archaeological remains (i.e. ceramic and charcoal fragments). Most importantly, this approach is important to take into account the overall effects of anthropic enrichments in ADE. Since ADE sites are expected to exhibit a high spatial variation (Costa et al., 2013; Costa and Kern, 1999; Kern et al., 2015), comparison of highly contrasting samples (for example, samples selected based on visual inference as aforementioned) likely overemphasize the differences in ADE compared to ADJ. In contrast to previous ADE studies (Fraser et al., 2011; Schmidt et al., 2014), the presented method generates a sharp transition between two areas rather than a smooth continuum. Therefore, I did not find evidences for a subcategory of ADE known as *Terra Mulata*. Furthermore, I showed that the overall Total C stock was 162.9 Mg ha^{-1} m^{-1} in ADE and was on average only 14% larger than ADJ, contrasting with previous studies where Total C stocks of up to 500 Mg ha^{-1} m^{-1} were reported in ADE (Glaser et al., 2002). The Total Ca and Total P stocks were twice as large in ADE and there was a high significant correlation between Total Ca and Total P in ADE, whereas no correlation was found in ADJ, which clearly indicates different sources and reaction pathways for these elements in ADE compared to ADJ.

In **Chapter 3**, I evaluated the use of chemical oxidation with sodium hypochlorite (NaOCl) to isolate a 'stable' pool of SOM, which has been suggested as a promising method for this purpose (Poeplau et al., 2018; von Lützow et al., 2007). Commonly, this procedure is done in three consecutive treatments of 6h each (Bruun et al., 2008; Jagadamma et al., 2010; Mikutta et al., 2006; Spohn and Giani, 2011; Thomsen et al., 2009). However, I showed that chemical oxidation with NaOCl can sharply distinguish a 'labile' (i.e. NaOCl-oxidizable) from a 'stable' (i.e. NaOCl-resistant) pool of organic carbon (OC) in just two consecutive treatments of 2h each, which is about 5 times faster than the procedure commonly applied in the literature. If I interpret results published from long-term bare fallow (LTBF) experiments with a two-pool model in which a 'stable' pool does not contribute to the overall decomposition on the time scale studied, and a 'labile' pool is represented by a first order rate of decomposition (k = 0.03% y^{-1}), the 'stable' OC pool left after ~100 years of biological oxidation in LTBF is similar to the 'stable' OC pool of about ~40–70% of the initial OC content I obtained after 4h of chemical oxidation with NaOCl for soils used in **Chapter 3** (Fig. 1).

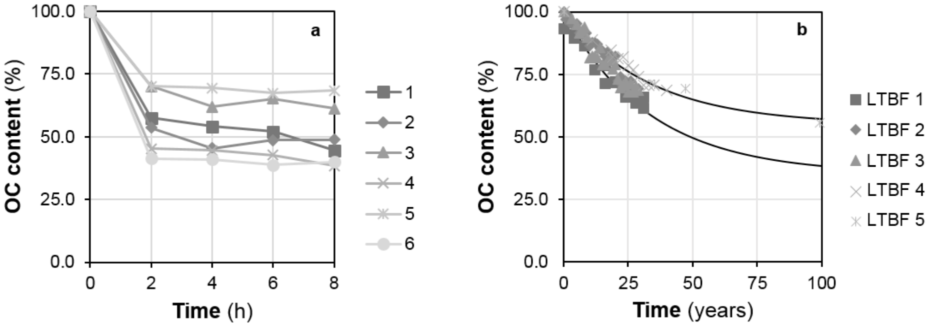

Fig. 1. (a) Organic carbon (OC) content (%) as a function of the reaction time (h) upon chemical oxidation with sodium hypochlorite (NaOCl) and (b) as a function of the reaction time (years) upon biological oxidation in long-term bare fallow (LTBF) experiments. Sample numbering in (a) refer to samples in Table 1 of **Chapter 3**. Sample labelling in (b) refer to data published elsewhere (Barré et al., 2010).

However, the mechanisms by which a certain portion of OC is left after biological oxidation in LTBF experiments or chemical oxidation with NaOCl are still unclear. In other words, it is not clear if mineral association or chemical recalcitrance determines resistance of a certain portion of OC towards biological and chemical oxidation. Contrary to other studies (Kleber et al., 2005; Mikutta et al., 2005a), I did not find a good correlation between mineralogical properties – such as iron (Fe) and (Al) oxides or reactive surface area of soil oxides – and the amount of OC that resists chemical oxidation with NaOCl, which indicates that mineral association is not always a good predictor of 'stable' SOM. In fact, I showed that this NaOCl-resistant OC is closely related with the initial OC ($R^2 = 0.66$, $p = 0.05$), which indicates that besides mineral association, chemical composition of SOM contributes to the amount of 'stable' SOM upon chemical oxidation with NaOCl. Subsequent demineralization with hydrofluoric acid (HF) in NaOCl-treated soil samples has been proposed to further separate 'stable' OC fractions and quantify the relative importance of mechanisms of SOM stabilization (i.e. mineral association and chemical recalcitrance) (Mikutta et al., 2006), which I investigated in **Chapter 4** in ADE and ADJ in combination with spectroscopic techniques to evaluate the chemical composition of SOM pools obtained in the aforementioned procedure.

In **Chapter 4**, I showed that the vast majority (> 80%) of the OC content had to be considered as 'labile', while the vast majority (> 90%) of the 'stable' OC pool had to be considered as 'mineral-associated', with a minimal contribution of 'recalcitrant' OC in both ADE and ADJ. In line with previous studies (Glaser, 2007; Glaser et al., 2001b), I expected ADE to be enriched in 'recalcitrant' OC due to the occurrence of black carbon (BC). High-resolution C 1s X-ray photoelectron spectroscopy (XPS) showed that chemical oxidation with NaOCl increased the relative contribution of aromatic OC (e.g. BC) in the 'stable' OC pool (i.e. NaOCl-resistant OC) in both ADE and ADJ, typically from ~1 to ~5%. However, I did not find this relative increase in aromatic OC in the 'recalcitrant' OC pool (i.e. HF-resistant OC), which contained very little (non-aromatic) OC. For this reason, I concluded that any 'stable' aromatic OC in the NaOCl stable pool of both soils is 'mineral-associated' and not 'chemically-recalcitrant'. It challenges suggestions from previous studies that the chemical recalcitrance of BC determines its persistence in soils (Glaser et al., 2003; Krull et al., 2003) neglecting the role of the mineral matrix on the stabilization of BC in soils (Cusack et al., 2012; Czimczik and Masiello, 2007). Nonetheless, the analysis with XPS it was not possible to determine precisely the relative contribution of aromatic OC (i.e. BC) of SOM pools in a quantitative manner. That, I investigated in **Chapter 5** using molecular markers. Since I found no evidence for a larger contribution of 'recalcitrant' or 'mineral-associated' OC in ADE compared to ADJ – which suggests similar mechanisms of SOM stabilization in both soils despite overall larger OC content in ADE – I concluded that other mechanisms, rather than the occurrence of 'recalcitrant' OC (i.e. BC) likely explain the overall larger OC content in ADE. The results in **Chapter 2** indicated that alongside C, high Ca and P inputs are important characteristics of ADE. Therefore, in **Chapter 5**, I attempted to investigate the possible interactive roles of these other important inputs in ADE (i.e. Ca and P) in further explaining the larger SOM contents in ADE compared to ADJ.

In **Chapter 5**, I showed that the relative contribution of BC to the Total C content did not differ between ADE and ADJ. Moreover, my results show that BC – or biochar as an equivalent term frequently used in the literature – is not a major component of SOM in ADE, which contrast to the conclusions of previous studies on ADE (Glaser, 2007; Glaser et al., 2001a) and brings implications for the sole use of biochar as mean to reproduce the desirable characteristics of ADE in other soils. Previous studies on ADE sites reported impressive BC contents in ADE up to 70 times larger than ADJ (Glaser, 2007; Glaser et al., 2001b). However, it is important to note that the BC content in these studies was determined according to the first version of the BPCA method (Glaser et al., 1998), which used hydrochloric acid (HCl) for the removal of polyvalent cations. Brodowski et al. (2005) reported that the use of HCl can generate BC-like compounds and overestimate the BC content up to 90%. For this reason, Brodowski et al. (2005) recommended the use of trifluoroacetic acid (TFA) for the removal of polyvalent cations in the revised version of the BPCA method, which I applied in my thesis. Despite the fact that earlier estimations on the BC content in ADE proved to be unreliable, the assumption that BC is a major contributor of SOM in ADE persisted in the literature and enabled the development of a biochar hype.

Furthermore, in **Chapter 5**, I showed that chemical oxidation with NaOCl removed BC, since I found no BC via the BPCA method in the ‘stable’ OC pool (i.e. NaOCl-resistant OC). However, it is important to note that in **Chapter 4**, I showed that the NaOCl-resistant OC pool was relatively enriched in aromatic OC (compared to untreated samples) in both ADE and ADJ. Since I did not find aromatic OC in the ‘recalcitrant’ OC pool (HF-resistant), I concluded that this aromatic OC in the ‘stable’ OC pool was ‘mineral-associated’. It could be argued that the BPCA method was unable to detect this ‘mineral-associated’ aromatic OC. One plausible explanation is the occurrence of natural BPCA in soils as the product of BC degradation (Di Rauso Simeone et al., 2018). For instance, assessment of the BC content via the BPCA method involves several wet chemical steps, where natural BPCA are not accounted for. Di Rauso Simeone et al. (2018) reported that naturally occurring BPCA accounted for 3.3% of the BC in ADE and up to 37.8% of the BC in ADJ. Despite the fact that I did not find an impressive contribution of BC to the Total C in ADE compared to ADJ (**Chapter 5**), studies have shown that BC source cannot be exclusively attributed to charred OM (i.e. pyrogenic BC) (Di Rauso Simeone et al., 2018; Glaser and Knorr, 2008). Di Rauso Simeone et al. (2018) reported that naturally occurring BPCA in Ferralsol indicated a biogenic source (e.g. black pigment of the fungus *Aspergillus niger*), whereas in ADE, it was not possible to assign a pyrogenic or a biogenic source of BC based on the distribution pattern of BPCA. In fact, Lucheta et al. (2016) reported that *Aspergillus niger* was more abundant in ADJ than ADE. Biogenic sources of BC have been considered significant (Glaser and Knorr, 2008) or negligible (Brodowski et al., 2005b). Nonetheless, biogenic sources of BC have been largely neglected in the literature. Therefore, more studies are certainly needed in order to better estimate the relative contribution of different sources of BC in soils, including the assessment of naturally occurring BPCA. Combining isotopic analysis with the BPCA method may offer an opportunity to investigate different sources of BC in environmental matrices (Di Rauso Simeone et al., 2018; Glaser and Knorr, 2008).

Moreover, in **Chapter 5**, I showed that exchangeable Ca (Ca^{2+}) was significantly ($p = 0.00$) positively ($R^2 = 0.92$) correlated with the amount of BC in ADE. Several studies have shown that BC surface oxidation is a key property in ADE (Jorio et al., 2012; Lehmann et al., 2005; Liang et al., 2013; Mao et al., 2012). BC surface charge can be compensated by the adsorption of Ca^{2+}. Moreover, cation bridging with surrounding organic molecules may promote the stabilization of substantial amount of OC (of non-BC origin) that is associated with highly charged BC in ADE, which may explain the overall larger non-BC contents in ADE compared to ADJ.

Furthermore, in **Chapter 5**, I also showed that the absolute amounts of oxalate-extractable Fe (Fe_o) and Al (Al_o) oxides (i.e. proxy for poorly crystalline Fe and Al oxides) was larger in ADE compared to ADJ. In addition, I showed that oxalate-extractable P (P_o) (i.e proxy for P bound to Fe and Al oxides) had a significant ($p = 0.01$) positive correlation ($R^2 = 0.66$) with the amount of $(Fe + Al)_o$ in ADE, whereas in ADJ, I did not find a correlation ($R^2 = 0.18$, $p = 0.25$) between these parameters. Therefore, I concluded that high P input in ADE enhanced and maintained a higher reactive surface area of soil oxides in ADE. I propose a hypothetical model that exemplify how high inputs of P in ADE may promote and maintain a large fraction of poorly-crystalline Fe and Al oxides by preventing their growth (Fig. 2).

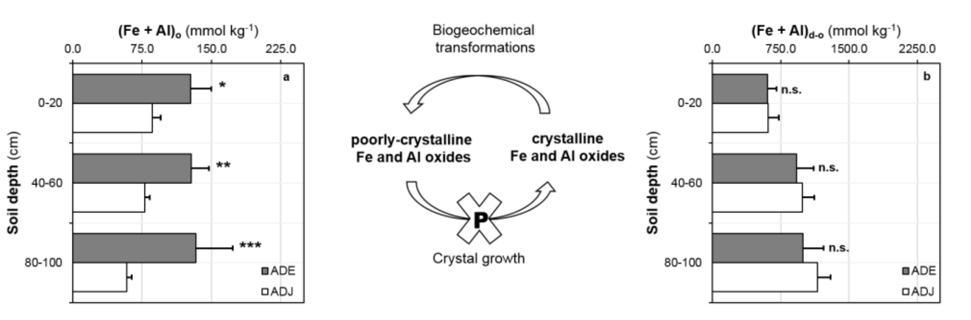

Fig. 2. Schematic representation of the proposed mechanisms by which high input of phosphorus (P) may have led to larger amounts of poorly crystalline iron (Fe_o) and aluminium (Al_o) oxides in Amazonian Dark Earth (ADE) compared to the adjacent (ADJ) Ferralsol (a). Note that the amount of crystalline iron (Fe_{d-o}) and aluminium (Al_{d-o}) did not differ greatly between ADE and ADJ (b). Significant differences between mean values in ADE versus ADJ per soil depth were tested with orthogonal contrast and are indicated with * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) or n.s. (not significant). For details about the methods and further explanations, please see text in **Chapter 5**.

It is surprising that even though studies have highlighted the importance of $(Fe + Al)_o$ in the stabilization of SOM via organo-mineral associations (Kleber et al., 2005; Mikutta et al., 2005a), this parameter has been largely neglected in studies on ADE in the past. This is because the focus was almost exclusively on BC as a source of ‘recalcitrant’ SOM in ADE, while relying on the fact that ADE and ADJ share the same parent material. Therefore, I encourage future studies on ADE to investigate the proposed role of P on larger amount of $(Fe + Al)_o$ that I suggested in **Chapter 5**. Future studies could also investigate the long-term effect of high P input on the amount of $(Fe + Al)_o$ in other anthropic soils, such as the Plaggen soils of northwest Europe by comparison to the adjacent soils in these areas. The suggested mechanisms involving BC, Ca and P in the build-up and maintenance of higher SOM amounts in ADE compared to ADJ are likely to vary spatially given the high spatial variation of these inputs in ADE as shown in (**Chapter 2**). Therefore, future studies could focus on patches with differential enrichment of BC, Ca and P to serve as the basis for future investigations on the interactive roles of BC, Ca an P in ADE. Future experiments under controlled conditions are encouraged to investigate the interactive roles of BC, Ca and P where the presence or absence of these elements are manipulated in order to evaluate the added effect of their interactive roles in SOM stabilization. Studies could also investigate the interactive effects of Ca and P inputs alongside biochar in order to better reproduce ADE desirable characteristics in other soils. For example, future studies could investigate the effects of biochar produced from nutrient-rich sources like animal bones (Zwetsloot et al., 2015), which may also offer an opportunity to fill nutrient gaps in developing countries (Simons et al., 2014) and to increase circularity between production systems (i.e. livestock and agriculture).

6.3. Reflections on the limitations of this work

In this PhD thesis, I focused on one ADE site – the Experimental Research Station of Caldeirão – which is situated in a larger spatial context in Central Amazon, where ADE sites are mostly found (Fig. 3). Several studies have been conducted in Caldeirão to investigate ADE characteristics (Barbosa Lima et al., 2015; Germano et al., 2012; Kern et al., 2019; Nakamura et al., 2014; Schellekens et al., 2017; Schmidt, 2006; Schmidt et al., 2014; Taube et al., 2013), which makes it one of the most extensively studied and represented ADE site. Nonetheless, future studies at other ADE sites are encouraged to use the geostatistical method I propose in **Chapter 2** (Alho et al., 2019) for a better comprehension of the high spatial variation in other ADE sites and to systematically distinguish ADE from ADJ and therefore account for the overall differences between these areas. In fact, future studies at other sites expected to share similarities with ADE, such as the '*African Dark Earths'* (Frausin et al., 2014), the '*Nordic Dark Earths'* (Wiedner et al., 2014) and the '*Australian Dark Earths'* (Downie et al., 2011) are also encouraged to use geostatistical approaches for a better comprehension of the spatial variation in anthropic areas due to the complexity of human settlements in the past.

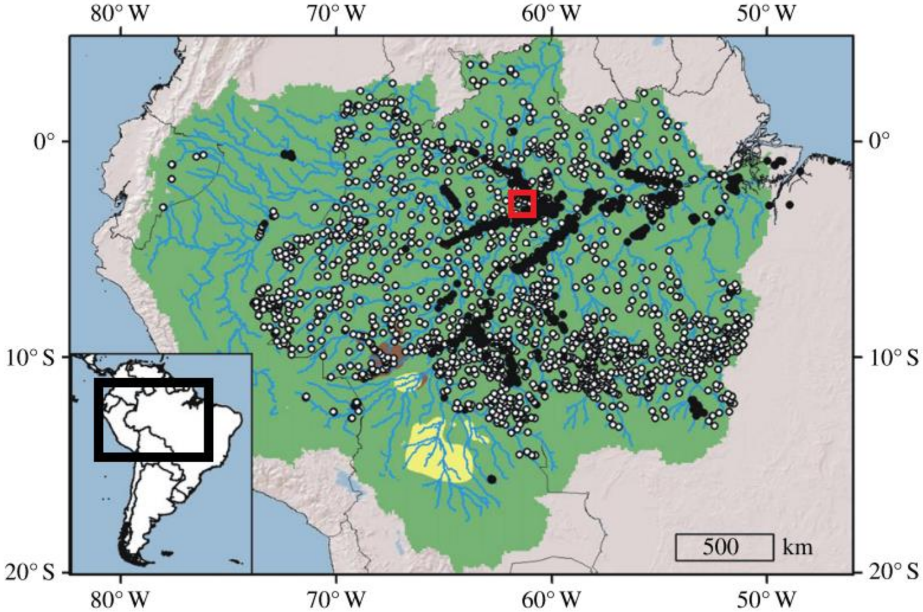

Fig. 3. Location of the Experimental Research Station of Caldeirão (red box) within the spatial context of sites with ADE (filled dots) and without ADE (open dots). Figure adapted from McMichael et al. (2014).

In this PhD thesis, I focused on the SOM fractionation procedure proposed by Mikutta et al. (2006).This method has been chosen among other SOM fractionation procedures because it has been designed to estimate the relative contribution of different mechanisms of SOM stabilization (i.e. mineral association versus inherent chemical recalcitrance), which are at the core of the paradigm shift in theories that attempt to explain the long-term persistence of SOM (Schmidt et al., 2011). The novelty in my study is the combination of the method proposed by Mikutta et al. (2006) with the revised BPCA method as molecular markers for BC (Brodowski et al., 2005b) in an attempt to investigate the relative contribution of mineral association versus inherent chemical recalcitrance in explaining long-term persistence of BC in ADE.

Previous studies assumed that BC could resist chemical oxidation with NaOCl (Bruun et al., 2008; Eusterhues et al., 2003; Leifeld and Zimmermann, 2006; Mikutta et al., 2005b; Simpson and Hatcher, 2004) without specific assessment of the BC content. In **Chapter 5**, I showed that BC did not resist chemical oxidation with NaOCl. For this reason, BC had to be considered part of the 'labile' OC pool within the framework of the method proposed by Mikutta et al. (2006). On one hand, studies have shown that charred SOM (i.e. BC-rich) is more resistant (i.e. is more 'stable') to biological oxidation than uncharred SOM (i.e. BC-poor) (Woo et al., 2016). On the other hand, old and functionalized BC as found in ADE is not more resistant to chemical oxidation (with NaOCl) than other C forms (**Chapter 5**). Therefore, there seems to be a mismatch between BC 'stability' assessed by biological and chemical oxidation procedures. For this reason, my results bring implications for the isolation of a so-called 'stable' OC pool upon chemical oxidation (with NaOCl) and challenges the claims on the 'inherent chemical recalcitrance' of BC as assessed by chemical oxidation procedures, such as the method proposed by Mikutta et al. (2006).

The occurrence of BC in ADE in the context of C sequestration for climate change mitigation has been the flagship of ADE research, which enabled the biochar hype. However, contrary to previous studies (Glaser, 2007; Glaser et al., 2001b), I showed that BC was not a major component of SOM in ADE since the relative contribution of BC to the Total C content did not differ greatly between ADE and ADJ (**Chapter 5**). Nonetheless, future studies at other ADE sites are encouraged to determine the BC content with the revised BPCA method for a more reliable overall picture of the relative contribution of BC to SOM in other ADE sites.

6.4. Reflections on the mechanisms of SOM stabilization

From a hoarding perspective, oxidation of OM compounds (SOM decomposition) is seen as negative because it leads to C loss as CO_2, which makes soils a C source rather than the desired C sink for climate change mitigation. However, in an useful perspective, as SOM decomposes, nutrients are released to the soil, which is certainly desirable for both ecosystem services and agricultural production. This C dilemma regarding SOM decomposition has been already nicely described by Janzen (2006). In the same line of thinking, I propose that there is a similar C dilemma regarding SOM stabilization. For instance, depolymerisation and oxidation of OM compounds through microbial metabolism is likely to (i) decrease their likelihood to undergo further biodegradation since a higher oxidation state yields less energy for microbes (i.e. enhanced chemical recalcitrance) (Kleber, 2010) and to (ii) increase their chemical reactivity through formation of functional groups, thus increasing their likelihood to interact with the soil mineral matrix via organo-mineral associations (i.e. enhanced mineral protection). Therefore, chemical recalcitrance (or lack of biological reactivity) and mineral-protection (as a consequence of chemical reactivity) are not necessarily mutually exclusive mechanisms responsible for SOM stabilization. In many cases (lack) of biological and chemical reactivity are likely to co-occur. This co-occurrence imposes obstacles to operational procedures that aim at separating these mechanisms by assigning them to different SOM pools, such as the method proposed by Mikutta et al. (2006). Therefore, future studies are encouraged to investigate the interactions between chemical and biological reactivity to allow a more explicit focus on changes in the nature and properties of the mineral-associated OC over the soil profile (Kaiser and Kalbitz, 2012).

SOM fractionation procedures combined with spectroscopic techniques can be useful tools to study SOM pools quantitatively and qualitatively. However, the holy grail of proposed methods to mimic the processes by which microbes differentially transform various OM fractions that constitute operationally defined SOM pools, has not been found. Ideally, one should be able to estimate the relative contribution of different mechanisms of SOM stabilization by measuring SOM pools that are linked to the current views on these mechanisms. In order to elucidate the processes controlling SOM decomposition and stabilization in a mechanistic approach, it is fundamental to integrate information on the adaptations of the soil microbial community to decompose OM compounds (Georgiou et al., 2017; Mooshammer et al., 2014; Wieder et al., 2013) with information on the ability of reactive soil minerals in protecting SOM against further biodegradation (Doetterl et al., 2015; Kramer and Chadwick, 2018; Torn et al., 1997). Unravelling the processes controlling SOM decomposition stabilization is crucial for us to develop strategies and technologies to mitigate and adapt to the effects of climate change via soil C sequestration while improving soil fertility to boost plant productivity.

The future of SOM research will most likely rely on advances in non-destructive image-based analytical techniques at the interface of organo-mineral interactions in intact micro-aggregates (Hernandez-Soriano et al., 2018) and undisturbed soil cores (Hobley et al., 2018). These techniques provide information on the elemental composition and speciation of soil constituents, while preserving their natural arrangement. Steffens et al. (2017) combining nanoscale secondary ion mass spectroscopy (nanoSIMS) with digital imaging processing, identified recurring microdomains that the authors propose to be the threshold level for generalization in soil modelling. Studies on artificial soils (Vogel et al., 2014) or in early soil formation after glacial retreat (Schweizer et al., 2018) indicate that OM coverage on mineral surfaces is spatially heterogeneous. This is because organo-mineral associations are more likely to take place in hotspots of favourable conditions for connectivity (e.g. pre-existing OM, water and oxygen availability).

Formation of organo-mineral associations has been suggested to be the main mechanism that explains long-term persistence of SOM (Mikutta et al., 2006; Torn et al., 1997). However, mineral-associated OM is still subject to microbial transformations that change the molecular properties and charge of OM compounds, resulting in competitive interactions between recently added OM and more processed OM and the downward movement of N-enriched but somewhat less charged OM compounds (Kaiser and Kalbitz, 2012). Furthermore, Keiluweit et al.

(2015) reported that mineral-associated SOM can be destabilised by root exudation, counteracting mineral-protection of SOM. Therefore, future studies should certainly include the assessment of these biologically-driven processes that may weaken organo-mineral associations.

Data derived from image-based techniques can also be combined with spatial modelling techniques to build three-dimensional maps of soil properties (Hapca et al., 2015). However, high spatial heterogeneity in soils (Falconer et al., 2015) still impose an obstacle to scale up the processes at the nanoscale into measurable parameters that can be used in new generation of models to investigate SOM dynamics at the global scale (Abramoff et al., 2018; Robertson et al., 2019). Quantification of soil heterogeneity (Wanzek et al., 2018) and the organo-mineral binding (Newcomb et al., 2017) are likely to improve our understanding on the role of mineral protection on the long-term persistence of SOM. Furthermore, Keiluweit et al. (2016) suggested several parameters related to the role of oxygen limitations on SOM (de)stabilization that can be used to improve conceptual and mathematical models of SOM dynamics. Besides consideration on the role of mineral protection and microbial processes in new generation of mathematical models, it is crucial to address processes in plant-microbe-soil systems (Vidal et al., 2018) and atmosphere-soil as a function of depth (Balesdent et al., 2018). In order words, it is important to investigate soils through an integrative biogeochemical perspective in order to better understand soil processes and soil-climate feedbacks.

6.5. Reflections on soil-climate feedbacks

Urgent action is needed to mitigate and adapt to ongoing climate change (Rogelj et al., 2016). Persisting with the burning of fossil fuels will certainly hamper international efforts – such as the Paris Agreement – that aims at limiting the increase in global mean temperature to 1.5 °C. (Tong et al., 2019). Therefore, phasing-out the burning of fossil fuels while transitioning to climate-smart energy sources should be the primary goal. In addition, other human activities (e.g. deforestation and poor soil management) play a major role in GHG emissions (Erb et al., 2018). Therefore, decreasing deforestation rates – most importantly in tropical regions – and improving sustainable use of agricultural soils with climate-smart management practices should be prioritized. In addition, restoration of natural areas is also crucial for better overall ecosystem functioning. Furthermore, global efforts to restore degraded areas through tree restoration (i.e. afforestation) has been suggested as one of the most effective strategies to mitigate climate change (Bastin et al., 2019). In addition to increased aboveground C stocks, afforestation may also offer an opportunity to increase belowground C stocks (Laganière et al., 2010).

Due to climate change, the challenge in agricultural areas will be the need to boost crop yields to feed a growing population in a climate-smart fashion. The 4 per 1000 initiative has been proposed as an inspiring approach to face this challenge by increasing global C stocks in agricultural soils (Minasny et al., 2017). The implementation of this initiative requires aligning agriculture and climate policy (Chabbi et al., 2017). However, the feasibility of this initiative on a global scale is debatable. For example, van Groenigen et al. (2017) pointed out that limitations in N and P availability may hamper increasing C stocks. Furthermore, Baveye et al. (2018) pointed out that soils are not only C sink, but also C source. Therefore, the long-term net effects of climate-smart management practices on C stocks need to be monitored, most importantly because the soil-climate feedbacks are poorly understood.

Crowther et al. (2016) reported that soils in high latitude, which are currently large C sinks, are likely to become large C sources due to global warming, further contributing to climate change. Besides the effects of climate change in high-latitude soils, current climate models also project increase in temperature variability in the tropics, with the Amazon basin being a hotspot of concern (Bathiany et al., 2018). However, data from the tropical region are still scarce compared to the temperate region (van Gestel et al., 2018). Therefore, more studies are certainly needed in order to predict the global effects of climate change more accurately. Furthermore, Walker et al. (2018) suggested that even though decomposition rates are likely to increase due to global warming, substrate depletion could constrain further C loss from the soil. In addition, van Gestel et al. (2018) pointed out that net primary productivity (NPP) is also likely to increase due to global warming, which may offer a possibility to offset C losses from the soil. However, climate change is also likely to increase the occurrence of wildfires (Flannigan et al., 2013), releasing CO_2 back to the atmosphere, which can possibly counteract increased NPP and further contribute to climate change. But at the same time, wildfires are also likely to increase the BC content in soils due to the incomplete combustion of SOM (Santín et al., 2015). Increased BC content in soils may offer a possibility to offset part of the C losses from wildfires (Santín et al., 2017), if BC is to act as a long-term C sink. However, the factors controlling BC production, transformation and residence time on global scale is still poorly understood (Bird et al., 2015) and more research is certainly needed in order to understand the nature of BC and its role to the global C cycle.

6.6. Closing Remarks

The beneficial transformations in Amazonian forests and soils handled by Amerindians has been a source of inspiration for attempts to recreate these soils and landscape to allow sustainable agriculture rather than rampant deforestation to allow unsustainable agricultural practices. Therefore, studies on ADE sites provide fruitful knowledge about the complexity of pre-Columbian indigenous populations in the Amazon (Kern et al., 2017) and may provide insights for future sustainable use of soils (Kern et al., 2019).

Overall, my results (**Chapter 3–5**) have implications for a common SOM methodology proposed to isolate a ‘stable’ pool of SOM upon chemical oxidation with NaOCl and to subsequently differentiate and quantify the relative contribution of two mechanisms of SOM stabilization (i.e. mineral association and chemical recalcitrance) upon soil demineralization with HF. Combining data from **Chapters 4 and 5**, it seems like BC is removed upon chemical oxidation with NaOCl and therefore is part of the operationally defined ‘labile’ rather than the ‘stable’ SOM pool. Removal of BC with NaOCl raises questions about the contrast between low resistance of BC towards chemical oxidation and apparent high resistance of BC against biological oxidation, which certainly deserves more attention in future studies.

Moreover, my results shed light on the importance of high Ca and P inputs (**Chapter 2 and 5**) besides the occurrence of BC in ADE (**Chapter 5**) in explaining long-term persistence of SOM in ADE. Earlier studies have mentioned the large enrichment in Ca and P in ADE (Costa et al., 2013; Glaser, 2007; Glaser et al., 2001b; Kern et al., 2015). However, the causal roles of Ca and P on the stabilization of SOM has been underdeveloped.

In fact, in my thesis, I propose that it is the interaction between OM inputs (including BC inputs), Ca and P that are responsible for the long-term persistence of large SOM contents in ADE, which brings implications for the sole use of charred OM (i.e. biochar) as a strategy to reproduce ADE desirable characteristics in other soils.

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Summary

Amazonian Dark Earths (ADE), highly fertile pre-Columbian anthropic soils found in the Amazon, have intrigued scientists for decades. Despite tropical conditions, these soils exhibit large contents of carbon (C) and nutrients (particularly Ca and P), contrasting with the poor adjacent soils (ADJ) from the Amazon. High carbon (C) content in ADE has been linked with the occurrence of black carbon (BC) in these soils. BC has been claimed to be highly resistant to decomposition due to its polycondensed aromatic structure. However, recent advancements indicate that intrinsic chemical characteristics do not fully explain long-term persistence of soil organic matter (SOM). Soil minerals are known to play an important role on the mechanisms of SOM stabilization mainly through adsorption of SOM onto the surface of reactive soil minerals (i.e. Fe and Al oxides). However, the relative contribution of mineral protection versus chemical recalcitrance in explaining long-term persistence of SOM in ADE is poorly understood. The overall objective of this PhD thesis was to investigate the interactive roles of BC, Ca and P in explaining long-term persistence of SOM in ADE. Overall, my results (**Chapter 3–5**) have major implications for a common SOM methodology proposed to isolate a 'stable' pool of SOM upon chemical oxidation with NaOCl and to subsequently differentiate and quantify the relative contribution of two mechanisms of SOM stabilization (i.e. mineral association and chemical recalcitrance) upon soil demineralization with HF. Combining data from **Chapters 4 and 5**, it seems like BC is removed upon chemical oxidation with NaOCl and therefore is part of the operationally defined 'labile' rather than the 'stable' SOM pool. Removal of BC with NaOCl raises questions about the contrast between low resistance of BC towards chemical oxidation and apparent high resistance of BC against biological oxidation, which certainly deserves more attention in future studies. Furthermore, my results shed light on the importance of high Ca and P inputs (**Chapter 2 and 5**) besides the occurrence of BC in ADE (**Chapter 5**) in explaining long-term persistence of SOM in ADE. In my thesis, I propose that it is the interaction between OM inputs (including BC inputs), Ca and P that are responsible for the long-term persistence of large SOM contents in ADE, which brings major implications for the sole use of charred OM (i.e. biochar) as a strategy to reproduce ADE desirable characteristics in other soils.

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About the author

Carlos Francisco Brazão Vieira Alho was born in Rio de Janeiro (Brazil) on 3 April 1985. He obtained his BSc in Forest Engineering (2010) and his MSc in Environmental and Forest Sciences (2012) at the Federal Rural University of Rio de Janeiro (UFRRJ). It was during his first years as a BSc student while taking courses at the Department of Soils at UFRRJ that he developed a great interest on soil organic matter (SOM). During his BSc studies, he started an internship at the Brazilian Agricultural Research Corporation (Embrapa), where he developed a particular interest on analytical techniques to investigate SOM chemical composition and where he was introduced to a promising strategy to store carbon in soils: biochar. He did his BSc thesis in cooperation with Embrapa Agrobiology, where he studied the effects of biochar on soil nitrous oxide emissions. He did his MSc thesis in cooperation with Embrapa and the United Kingdom Biochar Research Centre (UKBRC), where he studied the effects of pyrolysis final temperature on the stability of biochar produced from soft- and hardwood. He conducted his PhD studies at Wageningen University & Research (WUR) within the framework of the Terra Preta Program and in cooperation with Embrapa, the National Institute of Metrology Standardization and Industrial Quality (Inmetro) and the Martin Luther University of Halle-Wittenberg (MLU). In his PhD thesis, he investigated the long-term persistence of SOM in Amazonian Dark Earth, highly fertile pre-Columbian anthropic soils found in the Amazon that inspired the biochar technology.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

- The interactions between Calcium, Phosphorus and pyrogenic Carbon in Terra Preta soils (2012)

Writing of project proposal (4.5 ECTS)

- Reconstructing the origin of Terra Preta – a mechanistic, stoichiometric nutrient balance approach (2012)

Post-graduate courses (7.2 ECTS)

- Soil, biodiversity and life; PE&RC (2012)
- School of advanced characterization of nanomaterials; Inmetro (2014)
- Applications of synchrotron radiation for environmental and earth sciences; CNPEM (2014)
- Meta-analysis; PE&RC (2016)
- Geostatistics; PE&RC (2018)

Laboratory training and working visits (4.5 ECTS)

- Analytical experiments; Embrapa (2013-2016)
- Analytical experiments; Inmetro (2014-2015)
- Analytical experiments; MLU (2016)

Invited review of (unpublished) journal manuscript (2 ECTS)

- European Journal of Soil Science: soil science (2015)
- Acta Amazonica: environmental science (2018)

Deficiency, refresh, brush-up courses (1.5 ECTS)

- The carbon dilemma; WUR (2013)

Competence strengthening / skills courses (2.1 ECTS)

- Competence assessment; WGS (2012)
- Project & time management; WGS (2013)
- Career assessment; WGS (2018)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.4 ECTS)

- PE&RC First years weekend (2012)
- PE&RC Day extreme life (2012)
- PE&RC Last years weekend (2017)
- PE&RC Day preventing the end of the world (2017)

Discussion groups / local seminars / other scientific meetings (14.5 ECTS)

- 2nd Terra Preta Program Workshop (2012)
- 3rd Terra Preta Program Workshop (2013)
- Embrapa soils discussions (2013-2016)
- 4th Terra Preta Program Workshop (2015)
- 5th Terra Preta Program Workshop (2016)

International symposia, workshops and conferences (7.5 ECTS)

- 10th Meeting of the Brazilian chapter of International Humic Substances Society (2013)
- 11th Meeting of the Brazilian chapter of International Humic Substances Society (2015)
- 5th International Symposium on Soil Organic Matter (2015)

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