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Carbon and nitrogen storage and stability by mineral-organic association in physical fractions of Anthropogenic Dark Earth and of reference soils in Amazonia

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Abstract:	The anthropogenic dark earths (ADEs) are being assumed in recent years as a model representing the result of sustainable soil management practices carried out by Pre-Columbian peoples. However, little is known about the role of mineral-organic associations in organic matter storage in those soils compared to the emphasis generally given to the role of pyrogenic structures. We quantified the changes of carbon and nitrogen and their distribution in physical fractions of ADEs in relation to the reference (adjacent) soil. Four ADEs sites having the different soil textural classes of sandy clay loam, sandy clay, clayey, and very clayey were selected in the Amazon region of Brazil. Soil samples were collected from the 0-10 cm layer and a subset of the sample was separated into large aggregates (>500 µm) and small aggregates (<500 µm). The ADEs stored on average 45% more total organic carbon (TOC) and 44% more total nitrogen (TN) than the reference soils. Of the incremental TOC and TN in ADE relative to the reference soil, the silt size fraction stored on average 92% of this TOC and 37% of this TN and had C:N ratios as high as 25, which may indicate the presence of pyrogenic material. The clay fraction stored a substantial share of 27-46% of the incremental TOC and 27-66% of the incremental TN. The C:N ratio in the clay size fraction of ADEs, on average 10.5, was lower or not different than in the clay fraction of reference soil (average of 11.1), indicating that the organic matter in the clay fraction even of ADEs was predominantly of microbial origin, and not pyrogenic. We therefore conclude that the clay fraction proved to be an important location to the accumulation and stabilization of TOC and TN in these Anthrosols, possibly by mineral-organic association mechanisms.
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Cover letter

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Dear Editor-in-Chief
Catena

Please find enclosed the manuscript "**Carbon and nitrogen storage and stability by mineral-organic association in physical fractions of Anthropogenic Dark Earth and of reference soils in Amazonia**", which is being submitted to be considered for publication in **Catena**. The manuscript has been and approved by all authors and by any person whose aid has been acknowledged. Authors declare no conflict of interest.

It has not been published elsewhere and language, and that it has not been submitted simultaneously for publication elsewhere.

We understand the paper represents a significant contribution in the current questions about the capacity of Anthropogenic Dark Earths (ADEs) to promote soil carbon sequestration and emission mitigation and the role of mineral-organic associations in organic matter storage in those soils compared to the emphasis generally given to the role of pyrogenic structures.

Yours sincerely,

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Highlights

- Anthropogenic dark earth (ADE) has high concentrations of TOC and TN in the soil.
- The TOC in ADE was 45% higher than in references soils of Brazil's humid tropic.
- The clay fraction was responsible for 27-46% of the incremental TOC in the ADE.
- The C:N ratio in the clay size fraction of ADE indicates microbial origin.

Dear Editor,

We really appreciate the time-consuming job that the reviewer and you did at carefully reviewing our paper and critically contributing to improve its quality. All comments were analysed attentively by us. Most of them were integrated as corrections or modifications into the paper; while others were respectfully not, under justifications that we hope are acceptable to you.

We answered each reviewer's point adjacently, in a sort of step-by-step approach of comment-and-answer.

We hope that based on the improvements made in this new version and based on all arguments presented below you can reconsider your decision and accept our paper.

Yours sincerely,

The Authors

COMMENTS FROM EDITORS AND REVIEWERS

Dear Authors,
I've read your replies to the comments made by myself and by the reviewers and I must say that I am not satisfied with them. You have indeed taken into account only the minor comments, discarding the most conceptualy difficult ones. As an example, several comments point to the fact that you speculate about, and do not prove, the importance of clay-organic matter associations. A lot of works have been published about this topic, but the maximum you can say is that your data SUGGEST this is the most important mechanism. In case you cannot improve your analyses, or think it is not necessary, I expect that you take into accounts the comments made by the reviewers and by myself in the discussion and deeply rework your paper, as requested by rev#3. Furthermore, a statistical level of 0.1 is not acceptable for significance in international journals such as Catena.

Answer: Dear Editor and Reviewers, we are grateful for the notes made, and we agree that some points need to be reworked. Respectfully, we would like to reinforce some key messages in the paper:

1. Considering the three stabilization mechanisms of organic matter: chemical recalcitrance, physical occlusion, and mineral-organic association, specifically in the clay size fraction the predominant mechanism is the formation of complexes between the mineral surface and organic functional groups. Therefore, we are assuming with the support and addition of references (Kaiser and Zech, 2000; Wiseman and Puttmann, 2006) (lines 76-77) and Churchman et al. 2020 (lines 71 and 275) that in the clay fraction the mechanism that commands the stabilization of organic matter is the mineral-organic association.

2. It is already known that in anthropogenic soils a large part of the pyrogenic carbon is stabilized by chemical recalcitrance (presence of aromatic structures formed by the thermal decomposition of biomass during the pyrolysis process). Therefore, our study was not focused on evaluating or quantifying this pyrogenic carbon (please see lines 306-307), or even evaluating the most important mechanisms, but understanding the clay fraction contribution to the stabilization of carbon and nitrogen in these soils. In fact, our data only suggest that the mineral-organic association contributed to this pool of organic matter, we assume this given the large amount of carbon stored in the clay fraction (27-46%), that in this fraction occurs mineral-organic association (according to mentioned in item 1. above), and the low C:N ratio (average 10:5) of the clay fraction of ADEs, which indicates that it is NOT pyrogenic carbon that has high C:N ratios. This was reworked in the discussion on the lines 312-315 and 328.

3. Our hypothesis and data suggest that the clay fraction plays a role in total organic carbon accumulation (not the most important), and that this stabilization occurs through mineral-organic interactions. This was reworked in lines 102-106; 286-288 and 310-311.

4. After analysis between authors, statistical level of 0.1 was changed to 0.05. This change did not alter our main results and conclusions of the paper.

Adjusted changes in the text with the level of significance:

- Similar soil C:N ratio also in the clayey texture (12.8 vs 13.8; $p=0.08$; Table 2). That was corrected in the lines 198-199.
- Similar TOC concentration between ADE and reference soil in small aggregates from sites with clayey texture. That was corrected in the lines 219-220.
- Similar C:N ratio to the ADE silt fraction and the reference soil with a sandy clayey loam texture. That was corrected in the lines 224-225.

5. We reworked the text considering a deepening of what had already been done in the previous version below:

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COMMENTS FROM EDITORS:

I personally think that the discussion about pyrogenic carbon in the silt fraction is rather weak and not well supported by the data, as no analysis for pyrogenic carbon was done. Furthermore, in the discussion, you speak about dimensions of pyrogenic carbon to motivate its supposed higher presence in the silt fraction. If it is indeed a question of dimensions, it would have been more suitable to add an additional step in the fractionation method, i.e., to repeat the POM procedure after the sonication to exclude the possibility of having organic matter which is stabilized by different mechanisms.

Answer: Answer: Respectfully, the focus was not on pyrogenic carbon and its accumulation in the other physical fractions of the soil, but that the clay fraction also plays an important role in stabilization of organic matter. We believe that the key messages that the total organic carbon in the clay fraction is stabilized by

mineral-organic association, given the low C:N ratios in this fraction, which do not match the accumulation of pyrogenic material. Therefore, in addition to recalcitrance due to the presence of pyrogenic carbon, part of the organic matter in anthropogenic dark earths is stabilized by mineral-organic association. To clarify our hypothesis and objectives we rework the text on the lines 88-90; 102-106 and 112.

Apparently, you did not use any dispersant, only water, therefore I expect your silt fraction is indeed composed of silt-sized aggregates, i.e., it includes clay. The sonication alone is not enough to avoid reflocculation o dispersed clay particles. In case you are not convinced by this last statement, have at an old paper of mine (Stanchi et al. 2008 SSAJ).

Answer: For total dispersion of silt-size microaggregates a pre-test for determination of the sonication energy was performed. This information was added in lines 160-161. Schmidt et al. (1999) (EJSS) demonstrated in their study that the application of ultrasonic energy can be sufficient for the complete dispersion of samples from a variety of soils and pedogenetic horizons.

Please also have the text checked for language errors, there are several sentences where “de” is written instead of the, as well as some weird words (e.g., lyophilized instead of freeze dried).

Answer: We agree about those English issues and are sorry for that. So, the manuscript was carefully revised, and hopefully the language quality is now improved.

REVIEWER #1: GENERAL COMMENTS

Insert a map of the location of the areas.

Answer: We considered the suggestion and the map was inserted (P25).

REVIEWER #2: GENERAL COMMENTS

The paper presents an interesting data about the increased storage of SOM storage in ADE soils. While it is not surprising that a significant portion of the organic matter change is associated with the clay fraction, I encourage the authors to speculate on processes besides physical protection that might be involved- specifically what about the potential formation of humic substances?

Answer: As answered to the Editor, we understand that the organic carbon in the clay stabilizes by mineral-organic association, and this in turn corresponds to the most stable humic substances in the soil. Therefore, in addition to the already known chemical recalcitrance (aromaticity of pyrogenic structures), the mineral-organic association is also a relevant mechanism in the stabilization of organic carbon in ADEs~~.~~ The word “also” was included in the conclusion, so as not to convey to the reader the idea that the mineral-organic association is the single and the most important mechanism of protection. Please, see lines 329-331 and 360.

REVIEWER #3: GENERAL COMMENTS

This study provides a clear description with higher carbon and nitrogen concentrations in anthropogenic dark earth than those in references soils of Brazil's humid tropic area. However, I wonder whether the authors can draw the conclusion that the clay fraction is an important location to the accumulation and stabilization of TOC and TN in these Anthrosols, possibly by mineral-organic association mechanisms.

Answer: Studies such as Singh et al. (2016) (Geoderma) emphasize that the clay fraction significantly influences the accumulation and stabilization of organic carbon by mineral-organic association. This information and references (Singh et al., 2016; Singh et al., 2018 and Churchman et al., 2020) was added in lines 68-71~~.~~ And our study showed that proportionally of what increased, 27-46% of this organic carbon is in the clay fraction, regardless of the soil.

Major concerns:

1. The hypothesis or research aim in this study (L99-101) did not provide a clear idea how use clay fraction and its mineral-organic to explain the mechanism of higher carbon and nitrogen concentration in anthropogenic dark earth than those in references soils.

Answer: As explained in the previous answer, we understand that the clay fraction plays a fundamental role in the accumulation of organic matter, and that the carbon accumulated in this fraction is stabilized by mineral-organic association as supported in the literature (Singh et al., 2016; Chenu et al., 2009; Kleber et al., 2007; von Lutzow et al., 2006). Please see in the lines (68 - 71) that other studies were inserted to support our hypothesis that the carbon and nitrogen that was accumulated in the ADE and its reference soils is stabilized there by interactions with clay-sized minerals, without discarding or listing the importance of the other fractions in the organic matter pool. Therefore, the clay fraction is only one of the contributors to carbon stocks in ADEs. Please see lines 329-331.

2. The results with higher TOC and TN in the clay fraction of anthropogenic dark earth than those in references soils cannot draw the conclusion that the accumulation and stabilization of TOC and TN in these Anthrosols, possibly by mineral-organic association. In my opinion, the authors need add an incubation experiment of carbon and nitrogen mineralization using the soil different fractions in order to explain the potential mechanisms.

Answer: Here we respectfully disagree because after the samples go through the particle size fractionation, an incubation experiment of carbon and nitrogen mineralization using the soil different fractions in becomes unfeasible.

3. The authors chosen four sites with increment of clay content and measured the carbon and nitrogen in their clay soils. However, the carbon and nitrogen contents in their clay soils did not increased from sandy clay loam, sandy clay, clayey to very clayey soils

(Table 4). In contrast, the carbon in sand-POM significantly increased in soils with increasing clay content (sandy clay loam, sandy clay, clayey to very clayey soils) (Table 4). Meanwhile, we did not find Δ total organic carbon (TOC) and Δ total nitrogen (TN) between anthropogenic dark earth (ADE) and their respective reference soils in sand-POM, silt and clay fractions showed similar patterns in response to the gradient of soil textural classes from sandy clay loam, sandy clay, clayey to very clayey. Therefore, the authors cannot draw the conclusion that clay fraction may contribute more important to carbon and nitrogen accumulation than sand-POM and silt fractions.

Answer: As explained in the previous answer, the objective was not to evaluate pyrogenic carbon that may be retained in the sand-POM. Please, see lines 306-307. Among our main results is that of the total carbon and nitrogen that ADE has in relation to the reference soil, most of it is accumulated in the clay fraction.

Other comments:

L122-128: Two plots were set in each of the four study sites, one plot for the ADE and another for the reference soil. In this situation, how to compare the differences between ADE and reference soil without the replication plots? If the authors used the five sampling points as statistics replications, the experiment design might be a pseudoreplication shortcoming.

Answer: We understand this limitation, but as the study sites are natural environments, it was not possible to perform normal replication plots. Therefore, in this case the five sampling points were considered as statistics replications.

L219-221: The method need be moved to Method (2.4 Statistical analysis).

Answer: This information was moved to Method (L163-165).

Table 1: need add the standard error and significant analysis for the below results.

Answer: This information was included (P19,20).

Figure 1: need add the standard error and significant analysis for the below results.

Answer: This information was included (P26).

I guess the authors may directly compare the differences among the four sites with different soil textural classes from sandy clay loam, sandy clay, clayey to very clayey. Or else, it is not easy to assess the clay fraction role in soils in this study.

Answer: Direct comparison the differences among the four sites with different soil textural classes was performed. As explained in the previous answer proportionally of what increased, 27-46% of this organic carbon is in the clay fraction, regardless of the soil.

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MINOR REVISIONS

This is an interesting study and the authors have collected a good data set using a simple methodology. The paper is generally well written and structured. However, the paper has some shortcomings in data analyses and text. I have provided numerous remarks on the text as it is often vague and long-winded. I also suggest the authors cite more relevant and recent literature to support the discussion of the results.
Answer: Alho *et al*. (2019) (L253) and Schapel *et al*. (2018) (L272) were incorporated. Please, see lines 265 and 284.

Additional suggestions were made for more in-depth analyses of the data. In this paper the highlight is the potential of Anthropogenic Dark Earth for storing Carbon and Nitrogen. In P11, L257 stated: 'Regarding the effect of soil texture, it was possible to notice that the sites with higher clay content also presented higher accumulations of TOC and TN in the soil, regardless of ADE or not.' The authors are attempting to highlight the importance of physical protection by clays in storing C and N. While mineral organic associations do contribute to organic carbon stabilization, they are not the only mechanism by which organic matter becomes stabilized. Include inorganic C data to have a complete understanding of the chemistry of the soils.
Answer: We respectfully disagree about include inorganic C data, because we know that these other mechanisms contribute to the stabilization of organic matter, which was evidenced by the high C:N ratios in the silt fraction by the presence of aromatic structures. Our focus was not on pyrogenic carbon and physical protection, but rather on showing that the mineral-organic association also plays an important role in stabilizing organic matter in these Anthroposols.

I suggest that you redo the statistical analysis and redraw the tables and figures.
Answer: We understand the limitation in relation to the statistical analysis, but since it is natural environments without a regular experimental design, we chose the Student's t-test and standard error to compare the results. Given this, we could not see other way if not keeping its length for the sake of providing a comprehensive understanding of results to readers.

Correct use of language but needs improvement
Answer: As answered to the Editor, there were really problems in English language, and we corrected them.

Title needs to be reconstructed to reflect the content of the article. The study focus is on total soil organic carbon and total organic nitrogen and this should be reflected in the title.
Answer: We considered the suggestion and changed the title of the paper to "Carbon and nitrogen storage and stability by mineral-organic association in physical fractions of Anthropogenic Dark Earth and of reference soils in Amazonia".

P1, L21 word 'increment' is inappropriate in the context. I rather include 'changes'
Answer: That was corrected (L23).

P2, L29 wording 'some' pyrogenic material is inappropriate
Answer: That was corrected (L32).

P2, L33 what is the average C:N of the reference soil?
Answer: This information was included (L36).

P2, L37 Your study does not reflect evidence for the mineral-organic associations
Answer: We respectfully disagree, as it was possible to note that up to 46% of the incremental TOC of these soils were accumulated in the clay fraction, considered a reactive mineral phase for forming polar covalent bonds with the organic functional groups, according to the information entered in the lines 68-71~~.~~

P4, L97 It is total 'organic' carbon
Answer: That was corrected (L100).

P4, L100 How was the mineral-organic associations assessed?
Answer: We assume by the C:N ratio of the clay fraction (average of 9.7) that it indicates an organic material of microbial origin (approximately 10:1), and no presence of pyrogenic material. Therefore, in addition to recalcitrance due to the presence of pyrogenic carbon, commonly found in these soils due to its genesis, part of the organic matter in anthropogenic dark earths is also stabilized by mineral-organic association. Please, see lines 310-315, the text was reworked.

P5, L107 Include a map of your sampling sites
Answer: We considered the suggestion and the map was inserted (P25).

P5, L112-L116 Sentence is not clear
Answer: We considered the suggestion and the sentence was rewritten (L118-120).

P5, L129 State a logical reason to select >500 µm to separate the two size classes
Answer: We agree with the concern and what is established in the literature, but we established a separation criterion of about 50% of the soil mass of the samples for separation into two size classes.

P6, L134 Was a soil moisture correction done?
Answer: Soil moisture correction was not performed.

P7, L165 Is this a paired or unpaired t-test? Need to be clear on why the statistical test was chosen.
Answer: Was an unpaired t-test: two samples assuming different variances. This information was inserted in L172.

P7, L165 Mention the confidence level that was chosen. In Table 2 it is stated as $p \leq 0.1$. Why was 0.1 chosen over 0.05?

Answer: Our research group has been using p ≤0.1 for field research as we consider it a good level of significance. The significance level was changed to $p < 0.05$. This change was adjusted in the footnotes of the tables and in Figure 2 (please, see pages 20, 21, 22, 23, 24, 25 and 28).

P7, L166 Which software was used to perform the test? Please elaborate more on the statistical analysis.
Answer: This information was included (L174, 175).

P8, L188 It is not appropriate to use the word physical treatment.
Answer: That was corrected (L198).

P8, L189 Better to use TOC content.
Answer: That was corrected (L198).

P8, L193 Use of 'similar trends' is not clear in the context.
Answer: That was corrected (L202).

Suggest that the results section be re-written. Break the results into sections and write clear, concise results.
Answer: We respectfully disagree, we could not see other way if not keeping its length for the sake of providing a comprehensive understanding of results to readers. In order to facilitate the discussion and understand the contribution of the mineral-organic association, we chose to separate the carbon and nitrogen results from the whole soil and from the physical fractions.

P10, L245 'some authors? cite the papers clearly
Answer: Some authors correspond to Moreira *et al.* (2009) (line 268) and Chagas *et al.* (2017) (line 269)~~.~~

P10, L250 'release of this element? Please re-phrase
Answer: The sentence was rewritten (L257-258).

P10, L254 Is there support in the literature that 'ADEs do not impact C:N ratios?
Answer: Chagas et al., 2017 and Teixeira et al., 2009 with studies in Anthropogenic Dark Earth in in the Brazilian Amazon also report little or no effect of ADE on C:N ratio. As well as Asare et al. (2021) who found a C:N ratio minor in Archaeological Dark Earth in the Czech Republic compared with the control soil, this information and reference was inserted in the lines 275-276~~.~~

P12, L294 'some pyrogenic material' …rephrase the sentence
Answer: That was corrected (L306).

Do you have any explanations for not observing pyrogenic carbon in the clayey fractions?
Answer: It was not physically observed in clay fraction (only in whole soil samples)~~,~~ but rather by the C:N ratio of the clay fraction (average of 9.7) that it indicates an organic material of microbial origin (approximately 10:1), and no presence of pyrogenic material that has higher C:N ratios~~.~~ The text was reworked in lines 306-307 and 312-315.

As mentioned in the discussion P13, L309, the mesh size selected is not logical
Answer: As explained in the previous answer, we agree with the concern and what is established in the literature, but we established a separation criterion of about 50% of the soil mass of the samples for separation into two size classes.

Suggest that all graphs be re-constructed Need clear labelling. Where are the error bars? Statistical significance not shown in your graphs.
Answer: Error bars was inserted in the figure (P26), as well as the significance level information in the figure caption (P27, L588-589).

P23, Table 5 replace comma with a decimal point Suggest to include additional figures for the comparisons.
Answer: That was corrected (P24).

I do not see clear evidence from your research to state mineral organic associations are important contributors for carbon storage and stabilization
Answer: We believe that the key messages that the total organic carbon in the clay fraction is stabilized by mineral-organic association, given the low C:N ratios in this fraction, which do not match the accumulation of pyrogenic material (chemical recalcitrance), or physical protection. Therefore, we know that these other mechanisms contribute to the stabilization of organic matter, but from the results obtained, part of the organic matter in anthropogenic dark earths is stabilized by mineral-organic association.

Revision, changes marked (including Abstract & Keywords & Title page with author details)

Carbon and nitrogen storage and stability by mineral-organic association in physical fractions of Anthropogenic Dark Earth and of reference soils in Amazonia

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Abstract

The anthropogenic dark earths (ADEs) are being assumed in recent years as a model representing the result of sustainable soil management practices carried out by Pre-Columbian peoples. However, little is known about the role of mineral-organic associations in organic matter storage in those soils compared to the emphasis generally given to the role of pyrogenic structures. We quantified the changes of carbon and nitrogen and their distribution in physical fractions of ADEs in relation to the reference (adjacent) soil. Four ADEs sites having the different soil textural classes of sandy clay loam, sandy clay, clayey, and very clayey were selected in the Amazon region of Brazil. Soil samples were collected from the 0-10 cm layer and a subset of the sample was separated into large aggregates (>500 μm) and small aggregates (<500 μm). The ADEs stored on average 45% more total organic carbon (TOC) and 44% more total nitrogen (TN) than the reference soils. Of the incremental TOC and TN in ADE relative to the

reference soil, the silt size fraction stored on average 92% of this TOC and 37% of this TN and had C:N ratios as high as 25, which may indicate the presence of pyrogenic material. The clay fraction stored a substantial share of 27-46% of the incremental TOC and 27-66% of the incremental TN. The C:N ratio in the clay size fraction of ADEs, on average 10.5, was lower or not different than in the clay fraction of reference soil (average of 11.1), indicating that the organic matter in the clay fraction even of ADEs was predominantly of microbial origin, and not pyrogenic. We therefore conclude that the clay fraction proved to be an important location to the accumulation and stabilization of TOC and TN in these Anthrosols, possibly by mineral-organic association mechanisms.

Keywords: Anthrosols, organic matter, physical fractionation, clay fraction, protection mechanisms

1. Introduction

Land use systems beneficial to soil organic matter stabilization are vital for maintaining terrestrial carbon stocks, especially in the Amazonian region where tropical conditions accelerate the decomposition of residues (Craswell and Lefroy, 2001). The anthropogenic dark earths (ADEs) comprise Anthrosols that have an anthropic A horizon with high fertility and organic matter content that resulted from human-induced deposition of organic material, some of pyrogenic origin (Clement et al., 2015; Kern et al., 2017; Smith, 1980; Soares et al., 2021; Watling et al., 2018). These soils cover about 3% (6.~~,~~000-18.~~,~~000 km^2) of the Amazon (McMichael et al., 2014; Sombroek et al., 2004), and are being assumed in recent years as a model derived from a sustainable agriculture (Glaser, 2007; Glaser and Birk, 2012).

The ADEs have shown high capacity of storing carbon and nitrogen (Batistao et al., 2020; Glaser et al., 2000; Woods and McCann, 1999) and therefore of having high soil quality level and potential to mitigate greenhouse gases (Novotny et al., 2009). These anthropogenic soils differ from the other soil orders found in the Amazon (identified as reference soils in the present study) due to their dark color, presence of artifacts (e.g., ceramics and bones) and large amounts of pyrogenic materials (black carbon), formed by the slow carbonization of these residues under low temperatures with little or no presence of oxygen (Liu et al., 2013; Shafizadeh, 1982). The pyrogenic carbon represents a stable fraction of organic carbon (Gerke, 2019) and due to its highly aromatic characteristic (chemically recalcitrant structures) (Schellekens et al., 2017) it has been considered as the key factor in the permanence of organic matter in ADEs. Yet, it is well-~~-~~known that physiochemical carbon stabilization mechanisms such as physical protection and mineral-organic association also slows the carbon return to the atmosphere as carbon dioxide (Six et al., 2002).

The adsorption and stabilization of organic carbon is directly influenced by soil clay minerals (Singh et al., 2016; Singh et al., 2018), considered the most reactive fraction for forming interactions with organic matter

(Churchman et al., 2020). Mineral-organic complexes are characterized by polar covalent bonds between the organic functional groups and the pedogenic mineral surfaces in the clay size fraction which reduces the susceptibility of organic matter to oxidative attacks (Six et al., 2002; Sollins et al., 1996). The reactive mineral phase of this mechanism is represented by the minerals with large specific surfaces areas, usually nanometer-sized Fe and Al oxides and the mainly phyllosilicate clay minerals (Kaiser and Zech, 2000; Kleber et al., 2015; Wiseman and Puttmann, 2006). ~~-~~Therefore, studies have used the soil texture for predictions of accumulation and distribution of ~~carbon~~ organic carbon pool (Han et al., 2014; Kogel-Knabner et al., 2008). The capacity of clayey soils to store more carbon and nitrogen by surface interactions and physical protection provided by stable aggregates is widely accepted, compared to coarse-textured~~r~~ soils.

Dalal and Mayer (1986) showed in their study that the soil carbon loss is inversely proportional to the clay content; consistent with results found in Brazilian tropical Ferralsol and Acrisol (Dieckow et al., 2009) and A~~a~~mazon ADEs (Chagas et al., 2017; de Souza et al., 2009).

The carbon storage can be up to three times greater in ADEs compared to their reference soils (Glaser, 2007; Glaser et al., 2001) and further optimized in soils with higher clay contents (von Lutzow et al., 2006). The millenary permanence of this pyrogenic organic matter formed by anthropic fires and stabilized by chemical recalcitrance of aromatic structures in ADEs is already well-known (Glaser and Birk, 2012; Pandey et al., 2020; Solomon et al., 2007). However, ~~P~~particularly in soils with old pyrogenic materials that have undergone physi~~c~~o~~-~~chemical changes due to the oxidation of particles and the formation of functional groups at the edges of the aromatic rings (Lehmann et al., 2005), several studies (Burgeon et al., 2021; Kleber et al., 2011) indicate the contribution of the mineral-organic association as a stabilizer of this portion of carbon; pyrogenic and non-pyrogenic. ~~-~~The use of physical fractionation techniques allows us to quantify the contribution of fractions associated with minerals (clay + silt) in this carbon storage as well as qualitative aspects through the C:N ratios of these fractions that can reflect the origin of the organic phase and be used for soil quality assessments. ~~Given this, there are still gaps to be answered about the importance of physical and chemical processes, and not only of recalcitrant structures, in the millenary permanence of carbon in ADE.~~ Taking part in this debate, we hypothesize in this study ~~our hypothesis is~~ that in ADE, in addition to the incorporation in the soil matrix of carbon in the form of pyrogenic charcoal, the clay size fraction of these soils and consequently the mineral-organic associations that predominate in this fraction play an important role in carbon and nitrogen storage and stability.~~the mineral-organic association, in addition to the already~~

~~known aromaticity of pyrogenic structures, is also a relevant mechanism to increase the mean residence of soil organic carbon in ADEs.~~

Our study aimed to evaluate (i) the accumulation of total organic carbon and total nitrogen in the ADEs in comparison with their respective reference soils (adjacent, without anthropic horizon); and (ii) the distribution of total organic carbon and total nitrogen in the physical fractions of ADE and reference soils, as well as the contribution of the clay fraction and its mineral-organic association to the storage of organic matter in these anthropic soils.

2. Material and methods

2.1. Study sites

Four ADE sites in the Amazon region of Brazil, in the states of Amazonas, Pará and Rondônia were selected for this study (Figure 1). For each of the four sites, the ADE was paired with an adjacent reference soil, which has no anthropic horizon and was 150-1300 m distant from the ADE sampling points. This difference in sampling distance was due to the choice of sites with similar vegetation, soil group and land use between the ADE and reference soil. The four sites allowed us to arrange a gradient of soil textural classes, with increments of clay content, from (i) sandy clay loam, (ii) sandy clay, (iii) clayey, and to (iv) very clayey. All ADEs were classified as Anthrosols, while the reference soils with sandy clay loam or sandy clay texture were classified as Acrisols, and the sites with clayey or very clayey textures as Ferralsols (IUSS, 2015). The climate in the four sites was tropical monsoon, Am (Köppen). Detailed information on soil, climate, location, use and other aspects of the sites are presented in Table 1.

2.2. Soil sampling and total carbon and nitrogen determination

Two plots measuring 1 hectare each were set in each of the four study sites, one plot for the ADE and another for the reference soil. Within each plot, soil samples from the 0-10 cm layer were collected with a hoe, at three sampling points which were about 60 m from each other. Sampling was in January and February 2016. Samples of approximately 1 kg were oven dried at 60 ºC until constant weight, passed through a 2-mm sieve and stored in plastic pots; separately for each of the five sampling points. A portion of approximately 500 g of soil was separated from the bulk sample and fractionated by sieving into two size-classes of aggregates: large, >500 µm; and small, <500 µm.

A sample subset was taken from the bulk soil sample, from the small aggregates class and from the large aggregates class to be ground to < 200 µm and analyzed for total organic carbon (TOC) and total nitrogen (TN) concentrations by dry combustion in a Vario EL III elemental analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany).

2.3. Soil physical fractionation

Samples of small aggregates and large aggregates were subjected to particle size fractionation using a method adapted from Christensen (1992) and Ramalho et al. (2020). Initially, 20 g of sample, 80 mL~~l~~ of water and seven polyacetal beads (Ø 1.1 cm) were added into a 600 mL flask and then shaken for 16 hours. After this period, the suspension was poured through a 53 µm sieve to recover the sand plus particulate organic matter fraction (sand-POM), which was washed with a gentle waterjet and oven dried at 45 ºC. The passing suspension (<53 µm) had its solid concentrated by centrifugation and then sonicated at 200 J mL^{-1}, in a 200 mL water suspension (a pre-test for determination of the sonication energy for the complete dispersion of clay and silt particles was performed). Then, the sonicated suspension was transferred to a 1000 mL measuring cylinder and subjected to 10-12 cycles of gravitational sedimentation with the time calculated according to Stokes' Law (Christensen, 1992). At the end of each cycle, the supernatant suspension containing the clay particles was siphoned and temporally stored in a 20 L plastic container. After the last sedimentation cycle, the bulk siphoned volume in the container went through flocculation with 200 mL of 0.5 mol L^{-1} $CaCl_2$ and remained during 12 hours at rest. The flocculated clay fraction at the bottom of the container and the silt fraction sedimented at the bottom of the measuring cylinder after the last sedimentation cycle were transferred to smaller containers and freeze dried over 3-4 days. At the end of the physical granulometric fractionation the sand-POM (> 53 µm), silt (53-2 µm) and clay (<2 µm) fractions were ground to <200 µm to determine the concentrations of TOC and TN on the same Vario EL III elemental analyzer. The TOC and TN concentrations in the physical fractions were normalized into a single whole soil basis (g kg^{-1} soil), considering their respective concentrations and the proportions of each aggregate size in the bulk soil.

2.4. Statistical analysis

Normality tests (Shapiro-Wilk) and homogeneity of variance (Bartlett's test) were performed, without the need for data transformation. Due to the fact that the study does not have a regular experimental design (without randomization), the results were analyzed using a parametric method using the Student's t-test (assuming different variances) to compare ADE vs reference soil and interpreted by the p-value and the standard error of the sample. The statistical tests were performed in Microsoft Excel® and graphs in SigmaPlot 14.0 software.

3. Results

3.1. Total soil carbon and nitrogen concentrations

The total organic carbon (TOC) and total nitrogen (TN) concentrations in the 0-10 cm soil layer, in all four textural classes, were higher in ADEs compared to their respective reference soils; so that on average across the four sites the TOC was 45% higher (46.8 g C kg^{-1} vs. 32.3 g C kg^{-1}; Table 2) and TN was 44% higher in ADEs (3.60 g N kg^{-1} vs. 2.50 g kg^{-1}; Table 2). Regarding the soil C:N ratio, it was higher in ADE than in reference soil in the sandy clay texture (15.0 vs. 12.7; p=0.02) but was similar for ADE and reference in the sandy clay loam (12.7 vs. 13.2; p=0.26), ~~and~~ in the very clayey ~~textures~~ (12.2 vs. 12.2 p=0.44) and in the ~~was lower for ADE in the~~ clayey textures (12.8 vs. 13.8; p=0.08; Table 2).

Among the four sites, either in the ADE or in the reference soil, the concentrations of TOC and TN in the 0-10 cm soil layer increased according to increments in the clay content, in the order sandy clay loam < sandy clay < clayey < very clayey (Table 2).

3.2. Total carbon and nitrogen in physical fractions

As for the whole soil (original soil samples without physical fractionation), the TOC content in the clay fraction was higher in the ADEs than in the reference soils, in almost all the textural classes and aggregate size classes (Tables 3 and 4). Although there was no difference in the sandy clay loam texture (p=0.13), the increment of TOC in the clay fraction was numerically 15-20% higher in ADE relative to the reference soil (Tables 3 and 4). Similar results were observed for the TN concentration, which was higher in ADE than in reference soil in all textural classes and aggregate classes; 15-21% higher in sandy clay texture to 95-107% higher in clayey texture (Tables 3 and 4). The C:N ratio of the clay fraction~~,~~ showed a pattern of being lower or not being significantly different in ADE than in reference soil (Tables 3 and 4).

In the silt fraction, the TOC and TN concentrations were also higher in ADE than in reference soils, regardless of texture and ~~class of~~ aggregate~~s~~ class (except for the clayey texture in small aggregates). For TOC, it was 19-21% higher, as in the ADE of the very clayey textural class, to 57-91% higher, as in the ADE of the sandy clay (Tables 3 and 4). For TN, it was 3-18% higher, as in sandy clay, to 67-87% higher, as in clayey texture (Tables 3 and 4). The C:N ratio of the silt fraction, in general, was higher in ADE than in a sandy clay reference soil,~~ in the sandy clay loam and sandy clay textures,~~ while it was similar or lower in ADE with~~in~~ sandy clay loam, clayey or very clayey textures (Tables 3 and 4).

In the sand-POM fraction, the TOC and TN concentrations did not follow a consistent pattern between ADE and the reference soil (Tables 3 and 4). TOC in the sand-

POM fraction of small aggregates was higher ($p < 0.05$~~10~~) for ADE relative to the reference soil in sites of sandy clay loam (11.4 g kg^{-1} vs. 7.9 g kg^{-1}; p=0.03) or sandy clay textures (13.0 g kg^{-1} vs. 12.0 g kg^{-1}; p=0.02), and similar in clayey (50.7 g kg^{-1} vs. 53.5 g kg^{-1}; p=0.40) or very clayey textures (70.3 g kg^{-1} vs. 65.9 g kg^{-1}; p=0.37) (Table 3). And this trend was observed somewhat in large aggregates (Table 4). Accordingly, the C:N ratio also did not show a coherent pattern in the comparison between ADE and the reference soil, in both ~~classes of~~ aggregates and textural classes (Tables 3 and 4).

In view of the similar results between the two ~~aggregate size classes~~ size-classes of aggregates (Tables 3 and 4), we normalized the TOC and TN concentrations in the physical fractions of ADE and reference soil into a single whole soil basis (g kg^{-1} soil, Table 5), considering their respective concentrations and the proportions of each aggregate size in the bulk soil, presented in Tables 3 and 4. In this case, we could compute the incremental carbon (ΔTOC) and the incremental nitrogen (ΔTN) stored in each physical fraction of ADE relative to the same fractions in the reference soil. The highest ΔTOCs in ADE occurred in the very clayey and in the clayey textural classes (8.4 and 7.4 g C kg^{-1} soil, Figure 2a), and the lowest in the sandy clay and sandy clay loam classes (4.1 and 5.0 g C kg^{-1} soil, Figure 2a). A similar trend occurred for ΔTN (Figure 2b). Overall, of the total incremental TOC and TN stored in ADE relative to the reference soil across the four sites, 42-129% of this TOC and 30-119% of this TN was stored in the silt size fraction, with C:N ratios as high as 25, which that may indicate the presence of some pyrogenic material (Figure 2c, calculated from ΔTOC and ΔTN in Table 5). Meanwhile, the clay fraction in ADE stored a substantial share of 27-46% of the incremental TOC and 27-66% of the incremental TN, and had a C:N that was lower or not significantly different than in reference soil (Figure 2c).

4. Discussion

4.1. Carbon and nitrogen in anthropogenic dark earth

The fact that the ADE had a higher TOC and TN concentrations (on average 45% and 44% higher, respectively) than the reference soil confirms the potential for organic matter accumulation in the ADE (Correa et al., 2013; Glaser et al., 2000; Kern et al., 2019; Maezumi et al., 2018), which is generally justified by the presence of highly stable organic material (Costa et al., 2004; Glaser, 2007; Glaser et al., 2000). Studies in the Central Amazon have reported carbon stocks up to 80% higher in ADE relative to its adjacent soil (Alho et al., 2019; Chagas et al., 2017; Schellekens et al., 2017).

The lack of a consistent pattern to conclude about the effects of ADE in soil C:N ratios (Table 2) has also been reported by some authors, with C:N ratios only 20% higher when compared to the reference soil (Moreira et al., 2009), or even without statistical differences (Chagas et al., 2017). The fact that ADE does not interfere in the soil C:N ratio can be justified by lost and deplete the soil nitrogen during the pyrolysis process in the formation and genesis of ADE (Teixeira et al., 2009), being immobilized by microbial biomass, and thus, equating the C:N ratios with those of the reference soil. Although the small effect of ADE in the C:N ratios of the whole soil, its values between 12.2 - 15.0, found in the present study are within the range reported by other studies also conducted in the Amazon region (Chagas et al., 2017; Teixeira et al., 2009) and in the Czech Republic (Asare et al., 2021). Although ADEs do not impact soil C:N ratios, its importance in the TOC and TN accumulation is indisputable compared to the other classes of adjacent soils found in the Amazon, and its potential in the sequestration of these elements.

Regarding the effect of soil texture, it was possible to notice that the sites with higher clay content also presented higher accumulations of TOC and TN in the soil, regardless of ADE or not. This positive relationship between clay content and carbon accumulation was also observed by others authors (Dieckow et al., 2009; Feller and Beare, 1997; Schapel et al., 2018), and indicates the role of clay in protection mechanisms of soil organic matter (Blanco-Canqui and Lal, 2004; Churchman et al., 2020; von Lutzow et al., 2006), even in ADEs. According to Churchman et al. (2020), soils with higher clay contents can provide new surfaces for the retention of carbon, consequently increasing the stocks of this element in the soil.

4.2. Distribution of total organic carbon and total nitrogen in physical fractions

As in the whole soil, the physical fractions of ADE had higher concentrations of TOC and TN compared to the reference soil (without anthropic horizon). Our results are supported by others also obtained through ~~via~~ fractionation techniques in ADE (Chagas et al., 2017; Glaser et al., 2001), which reinforces the contribution of these anthropic soils to organic matter storage. The largest incremental TOC accumulations in the clay fraction of clayey ADE (7.4 g kg^{-1} soil) and very clayey ADE (8.4 g kg^{-1} soil) are related to the well-known ability of clay-rich soils to accumulate more carbon and nitrogen compared to soils with a sandy texture (Churchman et al., 2020; Dalal and Mayer, 1986; Dieckow et al., 2009; Korschens, 1980). However, even in the sandy clay loam and sandy clay sites, our results showed that the clay fraction played an important role in the accumulations of TOC and TN. In relative terms, of the total incremental carbon (ΔTOC) accumulated in the ADE, the clay fraction was responsible for 34-48%; and 50-72% of the TN (ΔTN) (Figures 2a and 2b).

Although the presence of ~~the~~ pyrogenic carbon in ~~present in~~ ADEs (not evaluated in this study, but physically observed in whole soil samples) could~~may~~ play a significant role at stabilizing organic matter, our results showed that the clay fraction proved to be also an important factor to the accumulation of TOC and TN in the ADEs, possibly by mineral-organic association mechanisms; since, in the clay fraction~~.~~ the mechanism that commands the stabilization of organic matter is the mineral-organic association (Kaiser and Zech, 2000; Wiseman and Puttmann, 2006). In addition, the low C:N ratio in the clay fraction (average 10.5; Figure 2c) clearly indicates that there is no pyrogenic material; for which a larger C:N ratio would be expected (Bruun et al., 2012), generally greater than 16 when charcoal is present (Hassink, 1994).~~ The low C:N ratio in the clay fraction (average 10.5; Figure 2) clearly indicate that its organic material is of microbial origin (Clevelend and Liptzin, 2007), which then associates to the mineral surfaces; and not of pyrogenic origin, to which a much higher C:N ratio would be expected~~ (Bruun et al., 2012; Hassink, 1994)~~.~~ When computing ΔTOC/ΔTN, which means de C:N ratio of the incremental organic matter in ADE relative to the reference soil, ~~a~~an average~~mean~~ value of 9.7 was obtained, and that also indicates an organic material of microbial origin (approximately 10:1) (Clevelend and Liptzin, 2007). ~~-~~The importance of the mineral-organic association in carbon stabilization in soil has been discussed in several studies (Coward et al., 2017; Eusterhues et al., 2005; Kleber et al., 2007), and is considered a key mechanism in the permanence and longevity of soil organic matter (Churchman et al., 2020; Xiao et al., 2016).

Regarding the silt fraction, the high concentrations of TOC and TN in ADE associated with higher C:N ratios in this fraction (average of 18.4), especially in the sandy sites, possibly~~might~~ indicate ~~the presence of pyrogenic material, and therefore, also~~ the contribution of high~~these~~ chemical recalcitrance structures in parallel with mineral-organic association in the stabilization of this organic matter. ~~–~~Although fragments of pyrogenic carbon can have variable sizes (Carvalho et al., 2018; Jenkins et al., 2014), specifically particles of pyrogenic charcoal that are similar in size to silt particles, can accumulate predominantly in this intermediate fraction (2 - 53 μm). This situation was also observed in Acrisol by Dieckow et al. (2005) and Brazilian Ferralsol by Ramalho et al. (2020).

The lack of a clear pattern between TOC and TN results in the sand-POM fraction of ADE and reference soil, may be associated with the fact that this fraction has the lowest proportions of TOC and TN, and has been directly affected and masked by the current land use. The particulate fraction of the soil organic matter is considered highly responsive to changes in soil land use and management practices, and in the present study, both the ADE and the reference soil of all evaluated sites are under modern anthropic effect, therefore, far from their native condition. The separation of aggregate classes in a 0.5 mm (500 μm) mesh has shown to have no effect on distinguishing between TOC and NT accumulations in physical fractions. Possibly a separation on smaller scales is necessary to observe the preferential accumulations and distributions of organic matter. Additional studies are needed to better elucidate the dynamics of TOC and TN stocks in physical fractions of ADE with different size-classes of aggregates ~~classes of aggregates~~.

5. Conclusions

The anthropogenic dark earths accumulated 44-45% more carbon and nitrogen than their respective reference soils (on average across de four sites); what confirms the capacity of anthropogenic soils to store organic matter and serve as a model for optimizing carbon sequestration and soil quality.

The accumulation of 34-48% of the incremental carbon and 50-72% of the incremental nitrogen, relative to the reference soil, in the clay fraction of anthropogenic dark earths and the C:N ratio of this accumulated material suggesting microbial and non-pyrogenic origin, demonstrated that the clay fraction and its mineral-organic association are also important contributors to the storage and stabilization of organic matter in these Anthrosols.

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Table 1

Geographic and soil characteristics of the four study sites of anthropogenic dark earth (ADE) and its respective reference soil (adjacent) in the Brazilian Amazon. The four sites comprise a gradient of soil textural classes, from sandy clay loam, sandy clay, clayey to very clayey.

	Sandy clay loam			Sandy clay			Clayey			Very clayey	
	ADE	Reference	p^b	ADE	Reference	*p*	ADE	Reference	*p*	ADE	Reference
Site name	Caldeirão			Lago Grande			Santa Paula			Maguari	
Municipality, State	Iranduba, Amazonas			Iranduba, Amazonas			Porto Velho, Rondônia			Belterra, Pará	
Coordinates	3°13’47’’S 60°16’06’’W	3°13’30’’S 60°16’28’’W		3°15’08’’S 60°13’45’’W	3°14’49’’S 60°13’29’’ W		8°51’57’’S 64°03’39’’W	8°52’36’’S 64°03’57’’W		2°46’59’’S 55°00’46’’W	2°47’02’’S 54°59’54’’W
Altitude (m)	32			52			72			80	
Climate	Tropical monsoon (Am)			Tropical monsoon (Am)			Tropical monsoon (Am)			Tropical monsoon (Am)	
Mean temperature	26.7 °C			26.7 °C			25.6 °C			24.9 °C	
Annual precipitation	2300 mm			2300 mm			2255 mm			1946 mm	
Parent material	Sandstone/claystone			Sandstone/claystone			Granite/syenite/monzonite			Sandstone/claystone	
Land use	Native forest			Agriculture			Native forest			Native forest	
Soil											
Soil group WRB	Anthrosol	Acrisol		Anthrosol	Acrisol		Anthrosol	Ferralsol		Anthrosol	Ferralsol
Sand (g kg^{-1})	535	657	0.00	355	419	0.00	199	134	0.00	127	61
Silt (g kg^{-1})	94	51	0.03	155	149	0.34	272	213	0.10	198	159
Clay (g kg^{-1})	371	293	0.03	490	433	0.02	529	653	0.03	675	780
Total Fe (g kg^{-1})[a]	39.2	36.5	0.31	42.9	45.2	0.26	42.9	37.2	0.17	37.7	37.5
pH $CaCl_2$	4.7	3.6	0.00	4.5	3.9	0.00	4.9	3.9	0.00	4.8	4.3
Al ($cmol_c$ dm^{-3})	0.4	2.6	0.00	0.1	2,0	0.01	0.2	2.9	0.00	1.6	3.2
H+Al ($cmol_c$ dm^{-3})	7.1	7.9	0.12	10.5	8.9	0.17	7.3	11.3	0.00	11.2	10.4
Ca ($cmol_c$ dm^{-3})	8.2	0.1	0.00	9.0	0.9	0.01	8.4	0.1	0.02	10.5	3.7
Mg ($cmol_c$ dm^{-3})	0.6	0.0	0.02	0.9	0.02	0.01	1.0	0.0	0.02	1.2	0.7
K ($cmol_c$ dm^{-3})	0.08	0.05	0.19	0.14	0.07	0.04	0.17	0.11	0.00	0.15	0.11

P (mg dm^{-3})	255.6	17.3	0.01	641.6	12.0	0.06	274.6	60.7	0.00	19.2	9.2
CEC (mg dm^{-3})	14.5	8.5	0.09	19.0	11.2	0.00	17.1	13.3	0.06	19.4	14.0
Base saturation (%)	71	2	0.02	53	9	0.00	55	2	0.006	57	29
Al saturation (%)	3	94	0.00	2	92	0.00	2	92	0.00	10	33

[a] SW 846-3051A method (USEPA, 2007); [b] Student’s t test ($p \leq 0.\cancel{1}05$).

Table 2

Total organic carbon (TOC), total nitrogen (TN) and C:N ratio of the top 10 cm layer of anthropogenic dark earth (ADE) and their respective reference soil (adjacent) in the Brazilian Amazon, across a gradient of soil textural classes from sandy clay loam, sandy clay, clayey to very clayey.

	Sandy clay loam			Sandy clay			Clayey			Very clayey		
	ADE	Reference	*p* [b]	ADE	Reference	*p*	ADE	Reference	*p*	ADE	Reference	*p*
TOC (*g kg*$^{-1}$)	32.0 ±0.6[a]	22.0 ±0.7	*0.002*	40.3 ±0.6	29.0 ±0.7	*0.001*	54.2 ±4.8	35.4 ±1.6	*0.03*	60.5 ±4.6	42.7 ±1.2	*0.03*
TN (*g kg*$^{-1}$)	2.51 ±0.05	1.67 ±0.05	*0.002*	2.69 ±0.05	2.28 ±0.00	*0.01*	4.25 ±0.40	2.57 ±0.06	*0.04*	4.94 ±0.31	3.49 ±0.12	*0.02*
C:N ratio	12.7 ±0.5	13.2 ±0.1	*0.26*	15.0 ±0.6	12.7 ±0.3	*0.02*	12.8 ±0.4	13.8 ±0.2	*0.08*	12.2 ±0.0	12.2 ±0.2	*0.44*

[a] ± Standard error; [b] Student's t test ($p \leq 0.$~~1~~05).

Table 3

Total organic carbon (TOC) and total nitrogen (TN) concentrations, and C:N ratio of physical fractions in small aggregates (< 500 μm) of the top 10 cm layer of anthropogenic dark earth (ADE) and their respective reference soil (adjacent) in the Brazilian Amazon, across a gradient of soil textural classes from sandy clay loam, sandy clay, clayey to very clayey.

Fraction [a]	Sandy clay loam			Sandy clay			Clayey			Very clayey		
	ADE	Reference	*p* [c]	ADE	Reference	*p*	ADE	Reference	*p*	ADE	Reference	*p*
TOC (g kg^{-1} fraction)												
Sand-POM	11.4 ±1.0 [b]	7.9 ±1.0	*0.03*	13.0 ±0.3	12.0 ±0.1	*0.02*	50.7 ±7.1	53.5 ±7.2	*0.40*	70.3 ±6.0	65.9 ±10.5	*0.37*
Silt	69.5 ±3.7	54.2 ±2.3	*0.02*	97.0 ±1.4	50.9 ±1.1	*0.00*	54.1 ±2.8	38.8 ±6.3	*0.06*	108.8 ±2.0	89.9 ±7.8	*0.11*
Clay	47.1 ±2.7	41.0 ±3.9	*0.13*	38.6 ±0.5	33.4 ±0.6	*0.00*	53.8 ±7.1	28.2 ±1.2	*0.04*	42.7 ±2.4	27.6 ±1.1	*0.01*
Aggregate	29.7 ±0.5	20.8 ±0.9	*0.00*	38.4 ±0.4	27.2 ±1.1	*0.00*	56.5 ±3.3	38.4 ±2.9	*0.01*	63.4 ±6.5	44.8 ±2.7	*0.04*
TN (g kg^{-1} fraction)												
Sand-POM	0.75 ±0.01	0.55 ±0.06	*0.05*	0.73 ±0.00	0.87 ±0.03	*0.03*	2.96 ±0.41	2.58 ±0.23	*0.28*	4.73 ±0.13	3.60 ±0.41	*0.08*
Silt	3.81 ±0.07	3.54 ±0.20	*0.18*	3.93 ±0.22	3.32 ±0.13	*0.07*	3.25 ±0.26	1.95 ±0.14	*0.02*	6.63 ±0.08	5.38 ±0.56	*0.11*
Clay	4.03 ±0.21	2.86 ±0.10	*0.01*	3.39 ±0.02	2.81 ±0.00	*0.00*	4.80 ±0.48	2.32 ±0.08	*0.03*	3.83 ±0.20	2.48 ±0.04	*0.02*
Aggregate	2.36 ±0.05	1.64 ±0.06	*0.00*	2.56 ±0.08	2.22 ±0.01	*0.04*	4.35 ±0.34	2.74 ±0.11	*0.03*	5.19 ±0.43	3.61 ±0.26	*0.04*
C:N ratio												
Sand-POM	15.2 ±1.3	14.4 ±0.2	*0.31*	17.8 ±0.4	13.8 ±0.5	*0.00*	17.1 ±0.8	20.7 ±0.02	*0.02*	14.9 ±1.2	18.3 ±0.5	*0.04*
Silt	18.2 ±1.2	15.3 ±0.4	*0.07*	24.7 ±1.9	15.3 ±0.4	*0.02*	16.6 ±1.0	19.9 ±1.6	*0.12*	16.4 ±0.1	16.7 ±1.1	*0.34*
Clay	11.7 ±0.1	14.3 ±0.9	*0.05*	11.4 ±0.1	11.9 ±0.2	*0.06*	11.2 ±0.1	12.2 ±0.2	*0.01*	11.1 ±0.1	11.1 ±0.3	*0.47*
Aggregate	12.6 ±0.5	12.7 ±0.0	*0.46*	15.0 ±0.6	12.3 ±0.4	*0.01*	13.0 ±0.6	14.0 ±0.4	*0.13*	12.2 ±0.1	12.4 ±0.4	*0.28*
Recovery of mass after fractionation (g fraction g^{-1} small aggregate) [d]												
Sand-POM	562 ±22	661 ±24	*0.03*	380 ±5	460 ±8	*0.001*	227 ±12	134 ±5	*0.01*	176 ±31	115 ±5	*0.12*
Silt	111 ±14	61 ±5	*0.08*	154 ±6	134 ±4	*0.06*	297 ±10	300 ±6	*0.42*	178 ±19	146 ±6	*0.17*
Clay	327 ±14	278 ±23	*0.07*	466 ±6	406 ±12	*0.02*	476 ±17	566 ±6	*0.03*	646 ±19	739 ±5	*0.13*

[a] Sand-POM, sand plus particulate organic matter (>53 μm); silt (2 - 53 μm); clay (< 2 μm); [b] Standard error; [c] Student's t test ($p \leq$ 0.~~1~~05); [d] Recovery of physical fractions was normalized to obtain a sum of 1000 g fraction kg^{-1} small aggregate.

Table 4

Total organic carbon (TOC) and total nitrogen (TN) concentrations, and C:N ratio of physical fractions in large aggregates (> 500 µm) of the top 10 cm layer of anthropogenic dark earth (ADE) and their respective reference soil (adjacent) in the Brazilian Amazon, across a gradient of soil textural classes from sandy clay loam, sandy clay, clayey to very clayey.

Fraction [a]	Sandy clay loam			Sandy clay			Clayey			Very clayey		
	ADE	Reference	*p* [c]	ADE	Reference	*p*	ADE	Reference	*p*	ADE	Reference	*p*
TOC (g kg^{-1} fraction)												
Sand-POM	10.3 ±0.9	12.0 ±1.7	*0.22*	12.0 ±1.1	11.8 ±0.5	*0.44*	30.2 ±0.6	30.9 ±6.3	*0.46*	70.9 ±7.3	70.4 ±2.1	*0.47*
Silt	79.6 ±1.6	66.2 ±1.6	*0.01*	91.9 ±3.9	58.6 ±1.0	*0.01*	54.3 ±4.9	35.1 ±3.8	*0.02*	94.8 ±8.9	80.0 ±9.4	*0.16*
Clay	41.0 ±4.1	34.2 ±1.1	*0.13*	35.3 ±0.0	29.8 ±0.4	*0.00*	51.6 ±3.3	30.6 ±2.7	*0.00*	40.1 ±2.3	28.3 ±3.5	*0.03*
Aggregate	34.8 ±0.4	23.5 ±0.3	*0.00*	41.4 ±1.1	31.0 ±0.7	*0.00*	52.8 ±5.8	32.1 ±1.8	*0.04*	59.0 ±3.7	41.8 ±2.3	*0.01*
TN (g kg^{-1} fraction)												
Sand-POM	0.65 ±0.06	0.68 ±0.07	*0.40*	0.60 ±0.05	0.66 ±0.03	*0.24*	1.49 ±0.10	1.29 ±0.14	*0.20*	3.90 ±0.25	3.18 ±0.20	*0.07*
Silt	4.18 ±0.11	3.63 ±0.19	*0.07*	3.64 ±0.33	3.52 ±0.08	*0.40*	3.05 ±0.23	1.63 ±0.11	*0.01*	5.49 ±0.26	4.54 ±0.33	*0.10*
Clay	3.87 ±0.29	2.82 ±0.18	*0.04*	3.19 ±0.04	2.77 ±0.04	*0.00*	4.46 ±0.42	2.29 ±0.03	*0.03*	3.67 ±0.20	2.64 ±0.10	*0.02*
Aggregate	2.70 ±0.06	1.71 ±0.04	*0.00*	2.77 ±0.03	2.35 ±0.03	*0.00*	4.18 ±0.46	2.38 ±0.06	*0.04*	4.80 ±0.25	3.43 ±0.17	*0.02*
C:N ratio												
Sand-POM	15.8 ±0.4	17.6 ±0.4	*0.02*	20.0 ±1.1	17.9 ±0.8	*0.09*	20.3 ±1.5	24.0 ±1.7	*0.13*	18.2 ±0.9	22.1 ±1.1	*0.02*
Silt	19.0 ±0.9	18.2 ±0.5	*0.25*	25.2 ±1.8	16.6 ±0.5	*0.02*	17.8 ±1.1	21.5 ±0.7	*0.03*	17.3 ±0.8	17.6 ±0.7	*0.36*
Clay	10.6 ±0.2	12.1 ±0.6	*0.06*	11.1 ±0.2	10.8 ±0.3	*0.24*	11.6 ±0.8	13.4 ±1.0	*0.14*	10.9 ±0.2	10.7 ±0.8	*0.38*
Aggregate	12.9 ±0.5	13.7 ±0.2	*0.13*	14.9 ±0.6	13.2 ±0.2	*0.05*	12.6 ±0.3	13.5 ±0.4	*0.01*	12.3 ±0.0	12.2 ±0.2	*0.33*
Recovery of mass after fractionation (g fraction g^{-1} large aggregate) [d]												
Sand-POM	534 ±21	666 ±4	*0.02*	357 ±7	413 ±11	*0.02*	164 ±2	146 ±4	*0.02*	105 ±17	67 ±1	*0.10*
Silt	128 ±3	71 ±3	*0.01*	152 ±3	120 ±5	*0.01*	378 ±17	220 ±7	*0.01*	164 ±20	146 ±12	*0.27*
Clay	338 ±19	263 ±6	*0.04*	491 ±10	467 ±13	*0.15*	458 ±18	634 ±6	*0.01*	731 ±37	787 ±13	*0.17*

[a] Sand-POM, sand plus particulate organic matter (>53 µm); silt (2 - 53 µm); clay (< 2 µm); [b] Standard error; [c] Student's t test ($p \leq$ 0.~~1~~05); [d] Recovery of physical fractions was normalized to obtain a sum of 1000 g fraction kg^{-1} large aggregate.

Table 5

Proportion of small and large aggregates in whole soil samples, and total organic carbon (TOC), total nitrogen (TN) and C:N ratio in physical fractions of the top 10 cm layer of anthropogenic dark earth (ADE) and their respective reference soil (adjacent) in the Brazilian Amazon, across a gradient of soil textural classes from sandy clay loam, sandy clay, clayey to very clayey.

	Sandy clay loam			Sandy clay			Clayey			Very clayey		
	ADE	Ref	*p*	ADE	Ref	*p*	ADE	Ref	*p*	ADE	Ref	*p*
Proportion of soil aggregates classes (g aggregate g^{-1} soil)												
Small aggregate	545.2 ±18.3[b]	532.3 ±35.8	*0.41*[c]	373.6 ±26.5	521.9 ±71.0	*0.11*	384.2 ±33.2	533.2 ±13.6	*0.02*	348.3 ±26.2	349.1 ±12.6	*0.49*
Large aggregate	454.8 ±18.3	467.7 ±35.8	*0.41*	626.4 ±26.5	478.1 ±71.0	*0.11*	615.8 ±33.2	466.8 ±13.6	*0.02*	651.7 ±26.2	650.9 ±12.6	*0.49*
Total	1000	1000		1000	1000		1000	1000		1000	1000	
TOC (g kg^{-1} solo)												
Sand-POM[a]	6.5 ±0.4	6.9 ±0.6	*0.27*	5.0 ±0.2	5.8 ±0.0	*0.03*	8.0 ±0.8	6.3 ±0.6	*0.11*	10.1 ±1.6	6.2 ±0.1	*0.10*
Silt	9.6 ±0.8	4.2 ±0.5	*0.00*	15.8 ±0.6	7.8 ±0.4	*0.00*	20.1 ±0.8	10.4 ±1.3	*0.22*	18.7 ±2.7	13.2 ±1.3	*0.10*
Clay	15.9 ±0.3	10.9 ±0.5	*0.01*	19.5 ±0.4	15.4 ±0.8	*0.02*	26.1 ±2.3	18.7 ±0.6	*0.02*	31.7 ±0.4	23.5 ±0.5	*0.00*
TN (g kg^{-1} solo)												
Sand-POM	0.45 ±0.0	0.48 ±0.0	*0.25*	0.27 ±0.0	0.39 ±0.0	*0.01*	0.48 ±0.1	0.33 ±0.0	*0.15*	0.66 ±0.1	0.33 ±0.0	*0.08*
Silt	0.55 ±0.0	0.28 ±0.0	*0.00*	0.65 ±0.1	0.50 ±0.0	*0.16*	1.27 ±0.1	0.58 ±0.1	*0.18*	1.18 ±0.2	0.83 ±0.1	*0.10*
Clay	1.51 ±0.0	0.91 ±0.1	*0.01*	1.77 ±0.0	1.39 ±0.1	*0.02*	2.50 ±0.2	1.66 ±0.0	*0.02*	3.10 ±0.2	2.33 ±0.2	*0.04*

[a] Sand-POM. sand plus particulate organic matter (>53 µm); silt (2 - 53 µm); clay (< 2 µm); [b] Standard error; [c] Student's t test (p ≤ 0.~~1~~05); [d] TOC and TN of physical fractions for the whole soil were calculated by considering the respective concentrations in Tables 3 and 4. and the proportion of small and large aggregates in this Table.

Figure 1

Map of sampling sites in Iranduba (state of Amazonas), Belterra (state of Pará) and Porto Velho (state of Rondônia) in the Brazilian Amazon.

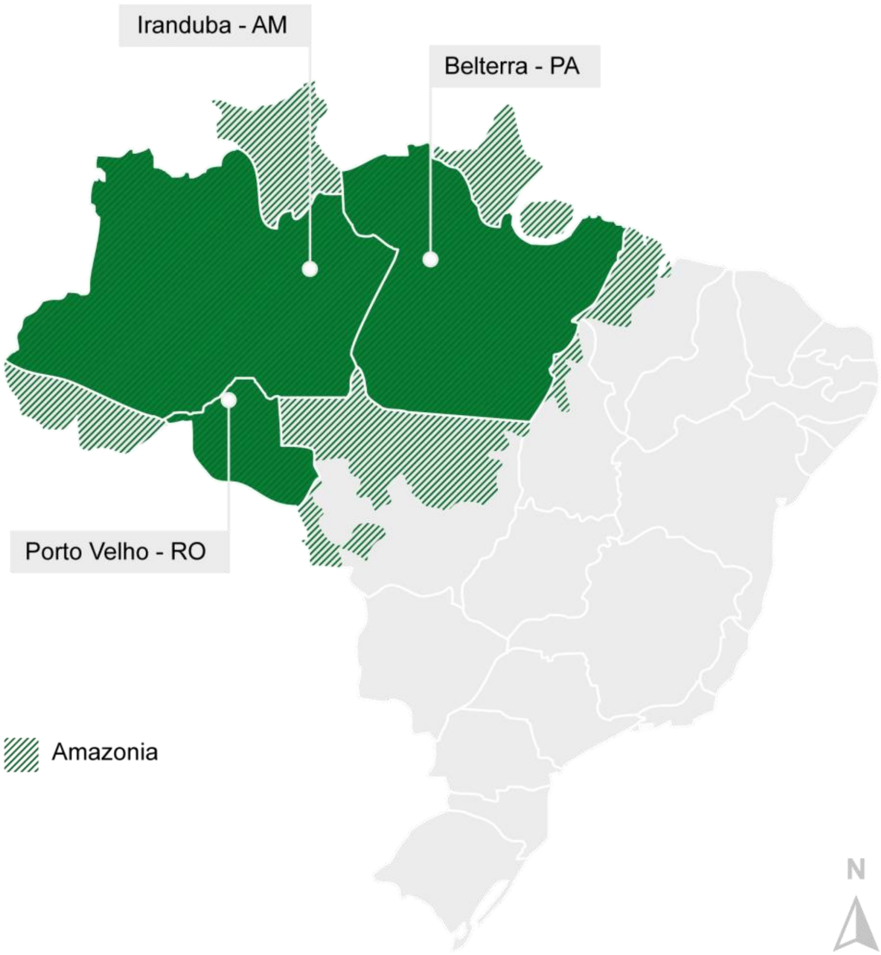
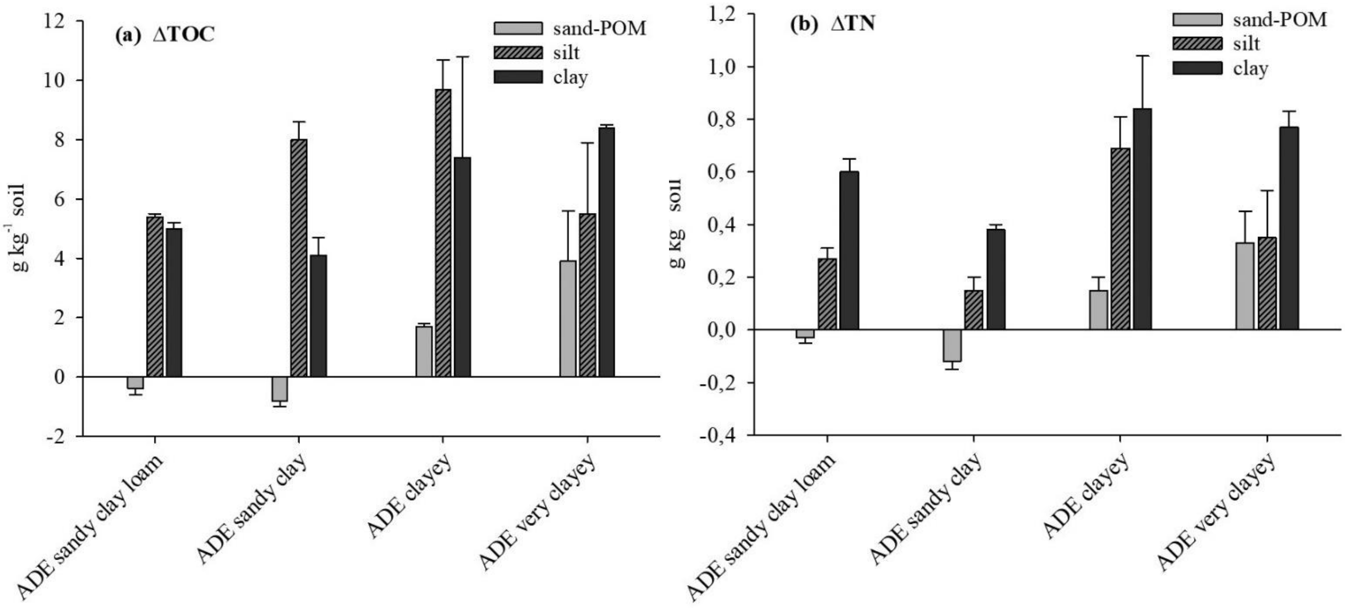
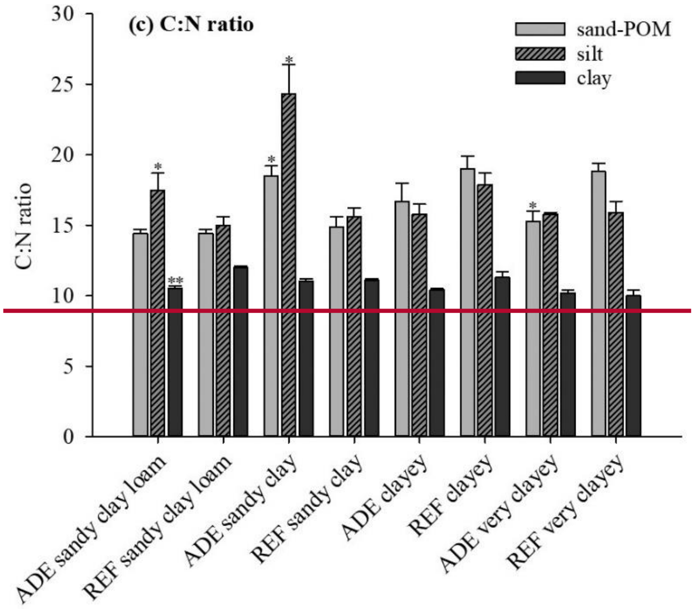
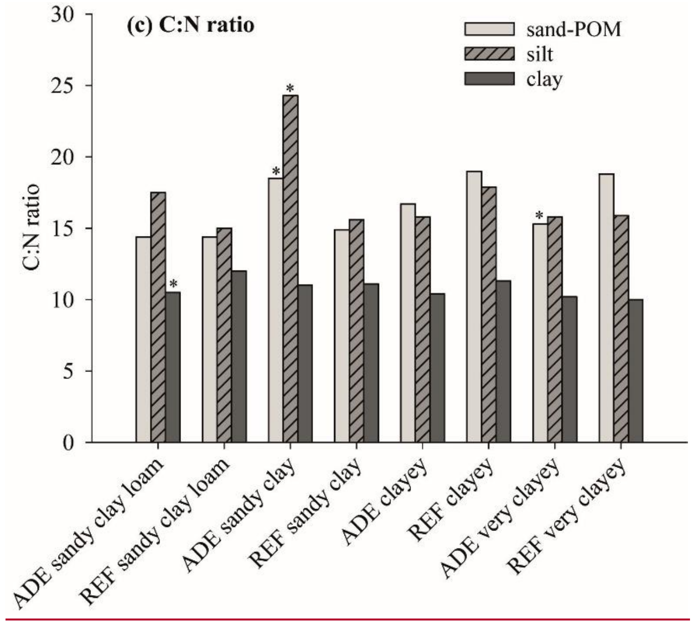


Figure 2

Δ total organic carbon (TOC) (a), Δ total nitrogen (TN) (b) and C:N ratio (c) in physical fractions of the top 10 cm layer of anthropogenic dark earth (ADE) and their respective reference soils (adjacent) in the Brazilian Amazon, across a gradient of soil textural classes from sandy clay loam, sandy clay, clayey to very clayey.







Δ calculated by difference in TOC or TN between dark earth and reference soil; Student's t test (* p ≤ 0.~~1~~05~~; ** p ≤ 0.001~~) ± standard error.

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Carbon and nitrogen storage and stability by mineral-organic association in physical fractions of Anthropogenic Dark Earth and of reference soils in Amazonia

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Abstract

The anthropogenic dark earths (ADEs) are being assumed in recent years as a model representing the result of sustainable soil management practices carried out by Pre-Columbian peoples. However, little is known about the role of mineral-organic associations in organic matter storage in those soils compared to the emphasis generally given to the role of pyrogenic structures. We quantified the changes of carbon and nitrogen and their distribution in physical fractions of ADEs in relation to the reference (adjacent) soil. Four ADEs sites having the different soil textural classes of sandy clay loam, sandy clay, clayey, and very clayey were selected in the Amazon region of Brazil. Soil samples were collected from the 0-10 cm layer and a subset of the sample was separated into large aggregates (>500 μm) and small aggregates (<500 μm). The ADEs stored on average 45% more total organic carbon (TOC) and 44% more total nitrogen (TN) than the reference soils. Of the incremental TOC and TN in ADE relative to the reference soil, the silt size fraction stored on average 92% of this TOC and 37% of this TN and had C:N ratios as high as 25, which may indicate the presence of pyrogenic material. The clay fraction stored a substantial share of 27-46% of the incremental TOC and 27-66% of the incremental TN. The C:N ratio in the clay size fraction of ADEs, on average 10.5, was lower or not different than in the clay fraction of reference soil (average of 11.1), indicating that the organic matter in the clay fraction even of ADEs was predominantly of microbial origin, and not pyrogenic. We therefore conclude that the clay fraction proved to be an important location to the accumulation and stabilization of TOC and TN in these Anthrosols, possibly by mineral-organic association mechanisms.

Keywords: Anthrosols, organic matter, physical fractionation, clay fraction, protection mechanisms

1. Introduction

Land use systems beneficial to soil organic matter stabilization are vital for maintaining terrestrial carbon stocks, especially in the Amazonian region where tropical conditions accelerate the decomposition of residues (Craswell and Lefroy, 2001). The anthropogenic dark earths (ADEs) comprise Anthrosols that have an anthropic A horizon with high fertility and organic matter content that resulted from human-induced deposition of organic material, some of pyrogenic origin (Clement et al., 2015; Kern et al., 2017; Smith, 1980; Soares et al., 2021; Watling et al., 2018). These soils cover about 3% (6.000-18.000 km^2) of the Amazon (McMichael et al., 2014; Sombroek et al., 2004), and are being assumed in recent years as a model derived from a sustainable agriculture (Glaser, 2007; Glaser and Birk, 2012).

The ADEs have shown high capacity of storing carbon and nitrogen (Batistao et al., 2020; Glaser et al., 2000; Woods and McCann, 1999) and therefore of having high soil quality level and potential to mitigate greenhouse gases (Novotny et al., 2009). These anthropogenic soils differ from the other soil orders found in the Amazon (identified as reference soils in the present study) due to their dark color, presence of artifacts (e.g., ceramics and bones) and large amounts of pyrogenic materials (black carbon), formed by the slow carbonization of these residues under low temperatures with little or no presence of oxygen (Liu et al., 2013; Shafizadeh, 1982). The pyrogenic carbon represents a stable fraction of organic carbon (Gerke, 2019) and due to its highly aromatic characteristic (chemically recalcitrant structures) (Schellekens et al., 2017) it has been considered as the key factor in the permanence of organic matter in ADEs. Yet, it is well-known that physiochemical carbon stabilization mechanisms such as physical protection and mineral-organic association also slows the carbon return to the atmosphere as carbon dioxide (Six et al., 2002).

The adsorption and stabilization of organic carbon is directly influenced by soil clay minerals (Singh et al., 2016; Singh et al., 2018), considered the most reactive fraction for forming interactions with organic matter (Churchman et al., 2020). Mineral-organic complexes are characterized by polar covalent bonds between the organic functional groups and the pedogenic mineral surfaces in the clay size fraction which reduces the susceptibility of organic matter to oxidative attacks (Six et al., 2002; Sollins et al., 1996). The reactive mineral phase of this mechanism is represented by the minerals with large specific surfaces areas, usually nanometer-sized Fe and Al oxides and the mainly phyllosilicate clay minerals (Kaiser and Zech, 2000; Kleber et al., 2015; Wiseman and Puttmann, 2006). Therefore, studies have used the soil texture for predictions of accumulation and distribution of organic carbon pool (Han et al., 2014; Kogel-Knabner et al., 2008). The capacity of clayey soils to store more carbon and nitrogen by surface interactions and physical protection provided by stable aggregates is widely accepted, compared to coarse-textured soils. Dalal and Mayer (1986) showed in their study that the soil carbon loss is inversely proportional to the clay content; consistent with results found in Brazilian tropical Ferralsol and Acrisol (Dieckow et al., 2009) and Amazon ADEs (Chagas et al., 2017; de Souza et al., 2009).

The carbon storage can be up to three times greater in ADEs compared to their reference soils (Glaser, 2007; Glaser et al., 2001) and further optimized in soils with higher clay contents (von Lutzow et al., 2006). The millenary permanence of this pyrogenic organic matter formed by anthropic fires and stabilized by chemical recalcitrance of aromatic structures in ADEs is already well-known (Glaser and Birk, 2012; Pandey et al., 2020; Solomon et al., 2007). However, particularly in soils with old pyrogenic materials that have undergone physiochemical changes due to the oxidation of particles and the formation of functional groups at the edges of the aromatic rings (Lehmann et al., 2005), several studies (Burgeon et al., 2021; Kleber et al., 2011) indicate the contribution of the mineral-organic association as a stabilizer of this portion of carbon; pyrogenic and non-pyrogenic. The use of physical fractionation techniques allows us to quantify the contribution of fractions associated with minerals (clay + silt) in this carbon storage as well as qualitative aspects through the C:N ratios of these fractions that can reflect the origin of the organic phase and be used for soil quality assessments. Taking part in this debate, we hypothesize in this study that in ADE, in addition to the incorporation in the soil matrix of carbon in the form of pyrogenic charcoal, the clay size fraction of these soils and consequently the mineral-organic associations that predominate in this fraction play an important role in carbon and nitrogen storage and stability.

Our study aimed to evaluate (i) the accumulation of total organic carbon and total nitrogen in the ADEs in comparison with their respective reference soils (adjacent, without anthropic horizon); and (ii) the distribution of total organic carbon and total nitrogen in the physical fractions of ADE and reference soils, as well as the contribution of the clay fraction and its mineral-organic association to the storage of organic matter in these anthropic soils.

2. Material and methods

2.1. Study sites

Four ADE sites in the Amazon region of Brazil, in the states of Amazonas, Pará and Rondônia were selected for this study (Figure 1). For each of the four sites, the ADE was paired with an adjacent reference soil, which has no anthropic horizon and was 150-1300 m distant from the ADE sampling points. This difference in sampling distance was due to the choice of sites with similar vegetation, soil group and land use between the ADE and reference soil. The four sites allowed us to arrange a gradient of soil textural classes, with increments of clay content, from (i) sandy clay loam, (ii) sandy clay, (iii) clayey, and to (iv) very clayey. All ADEs were classified as Anthrosols, while the reference soils with sandy clay loam or sandy clay texture were classified as Acrisols, and the sites with clayey or very clayey textures as Ferralsols (IUSS, 2015). The climate in the four sites was tropical monsoon, Am (Köppen). Detailed information on soil, climate, location, use and other aspects of the sites are presented in Table 1.

2.2. Soil sampling and total carbon and nitrogen determination

Two plots measuring 1 hectare each were set in each of the four study sites, one plot for the ADE and another for the reference soil. Within each plot, soil samples from the 0-10 cm layer were collected with a hoe, at three sampling points which were about

60 m from each other. Sampling was in January and February 2016. Samples of approximately 1 kg were oven dried at 60 ºC until constant weight, passed through a 2-mm sieve and stored in plastic pots; separately for each of the five sampling points. A portion of approximately 500 g of soil was separated from the bulk sample and fractionated by sieving into two size-classes of aggregates: large, >500 μm; and small, <500 μm.

A sample subset was taken from the bulk soil sample, from the small aggregates class and from the large aggregates class to be ground to < 200 µm and analyzed for total organic carbon (TOC) and total nitrogen (TN) concentrations by dry combustion in a Vario EL III elemental analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany).

2.3. Soil physical fractionation

Samples of small aggregates and large aggregates were subjected to particle size fractionation using a method adapted from Christensen (1992) and Ramalho et al. (2020). Initially, 20 g of sample, 80 mL of water and seven polyacetal beads (Ø 1.1 cm) were added into a 600 mL flask and then shaken for 16 hours. After this period, the suspension was poured through a 53 µm sieve to recover the sand plus particulate organic matter fraction (sand-POM), which was washed with a gentle waterjet and oven dried at 45 ºC. The passing suspension (<53 µm) had its solid concentrated by centrifugation and then sonicated at 200 J mL^{-1}, in a 200 mL water suspension (a pre-test for determination of the sonication energy for the complete dispersion of clay and silt particles was performed). Then, the sonicated suspension was transferred to a 1000 mL measuring cylinder and subjected to 10-12 cycles of gravitational sedimentation with the time calculated according to Stokes' Law (Christensen, 1992). At the end of each cycle, the supernatant suspension containing the clay particles was siphoned and temporally stored in a 20 L plastic container. After the last sedimentation cycle, the bulk siphoned volume in the container went through flocculation with 200 mL of 0.5 mol L^{-1} $CaCl_2$ and remained during 12 hours at rest. The flocculated clay fraction at the bottom of the container and the silt fraction sedimented at the bottom of the measuring cylinder after the last sedimentation cycle were transferred to smaller containers and freeze dried over 3-4 days. At the end of the physical granulometric fractionation the sand-POM (> 53 µm), silt (53-2 µm) and clay (<2 µm) fractions were ground to <200 µm to determine the concentrations of TOC and TN on the same Vario EL III elemental analyzer. The TOC and TN concentrations in the physical fractions were normalized into a single whole soil basis (g kg^{-1} soil), considering their respective concentrations and the proportions of each aggregate size in the bulk soil.

2.4. Statistical analysis

Normality tests (Shapiro-Wilk) and homogeneity of variance (Bartlett's test) were performed, without the need for data transformation. Due to the fact that the study does not have a regular experimental design (without randomization), the results were analyzed using a parametric method using the Student's t-test (assuming different variances) to compare ADE vs reference soil and interpreted by the p-value and the standard error of the sample. The statistical tests were performed in Microsoft Excel® and graphs in SigmaPlot 14.0 software.

3. Results

3.1. Total soil carbon and nitrogen concentrations

The total organic carbon (TOC) and total nitrogen (TN) concentrations in the 0-10 cm soil layer, in all four textural classes, were higher in ADEs compared to their respective reference soils; so that on average across the four sites the TOC was 45% higher (46.8 g C kg^{-1} vs. 32.3 g C kg^{-1}; Table 2) and TN was 44% higher in ADEs (3.60 g N kg^{-1} vs. 2.50 g kg^{-1}; Table 2). Regarding the soil C:N ratio, it was higher in ADE than in reference soil in the sandy clay texture (15.0 vs. 12.7; p=0.02) but was similar for ADE and reference in the sandy clay loam (12.7 vs. 13.2; p=0.26), in the very clayey (12.2 vs. 12.2 p=0.44) and in the clayey textures (12.8 vs. 13.8; p=0.08; Table 2).

Among the four sites, either in the ADE or in the reference soil, the concentrations of TOC and TN in the 0-10 cm soil layer increased according to increments in the clay content, in the order sandy clay loam < sandy clay < clayey < very clayey (Table 2).

3.2. Total carbon and nitrogen in physical fractions

As for the whole soil (original soil samples without physical fractionation), the TOC content in the clay fraction was higher in the ADEs than in the reference soils, in almost all the textural classes and aggregate size classes (Tables 3 and 4). Although there was no difference in the sandy clay loam texture (p=0.13), the increment of TOC in the clay fraction was numerically 15-20% higher in ADE relative to the reference soil (Tables 3 and 4). Similar results were observed for the TN concentration, which was higher in ADE than in reference soil in all textural classes and aggregate classes; 15-21% higher in sandy clay texture to 95-107% higher in clayey texture (Tables 3 and 4). The C:N ratio of the clay fraction showed a pattern of being lower or not being significantly different in ADE than in reference soil (Tables 3 and 4).

In the silt fraction, the TOC and TN concentrations were also higher in ADE than in reference soils, regardless of texture and aggregate class (except for the clayey texture in small aggregates). For TOC, it was 19-21% higher, as in the ADE of the very clayey textural class, to 57-91% higher, as in the ADE of the sandy clay (Tables 3 and 4). For TN, it was 3-18% higher, as in sandy clay, to 67-87% higher, as in clayey texture (Tables 3 and 4). The C:N ratio of the silt fraction, in general, was higher in ADE than in a sandy clay reference soil, while it was similar or lower in ADE with sandy clay loam, clayey or very clayey textures (Tables 3 and 4).

In the sand-POM fraction, the TOC and TN concentrations did not follow a consistent pattern between ADE and the reference soil (Tables 3 and 4). TOC in the sand-POM fraction of small aggregates was higher ($p < 0.05$) for ADE relative to the reference soil in sites of sandy clay loam (11.4 g kg^{-1} vs. 7.9 g kg^{-1}; $p=0.03$) or sandy clay textures (13.0 g kg^{-1} vs. 12.0 g kg^{-1}; $p=0.02$), and similar in clayey (50.7 g kg^{-1} vs. 53.5 g kg^{-1}; $p=0.40$) or very clayey textures (70.3 g kg^{-1} vs. 65.9 g kg^{-1}; $p=0.37$) (Table 3). And this trend was observed somewhat in large aggregates (Table 4). Accordingly, the C:N ratio also did not show a coherent pattern in the comparison between ADE and the reference soil, in both aggregates and textural classes (Tables 3 and 4).

In view of the similar results between the two size-classes of aggregates (Tables 3 and 4), we normalized the TOC and TN concentrations in the physical fractions of ADE and reference soil into a single whole soil basis (g kg^{-1} soil, Table 5), considering their respective concentrations and the proportions of each aggregate size in the bulk soil, presented in Tables 3 and 4. In this case, we could compute the incremental carbon

(ΔTOC) and the incremental nitrogen (ΔTN) stored in each physical fraction of ADE relative to the same fractions in the reference soil. The highest ΔTOCs in ADE occurred in the very clayey and in the clayey textural classes (8.4 and 7.4 g C kg^{-1} soil, Figure 2a), and the lowest in the sandy clay and sandy clay loam classes (4.1 and 5.0 g C kg^{-1} soil, Figure 2a). A similar trend occurred for ΔTN (Figure 2b). Overall, of the total incremental TOC and TN stored in ADE relative to the reference soil across the four sites, 42-129% of this TOC and 30-119% of this TN was stored in the silt size fraction, with C:N ratios as high as 25, which that may indicate the presence of some pyrogenic material (Figure 2c, calculated from ΔTOC and ΔTN in Table 5). Meanwhile, the clay fraction in ADE stored a substantial share of 27-46% of the incremental TOC and 27-66% of the incremental TN, and had a C:N that was lower or not significantly different than in reference soil (Figure 2c).

4. Discussion

4.1. Carbon and nitrogen in anthropogenic dark earth

The fact that the ADE had a higher TOC and TN concentrations (on average 45% and 44% higher, respectively) than the reference soil confirms the potential for organic matter accumulation in the ADE (Correa et al., 2013; Glaser et al., 2000; Kern et al., 2019; Maezumi et al., 2018), which is generally justified by the presence of highly stable organic material (Costa et al., 2004; Glaser, 2007; Glaser et al., 2000). Studies in the Central Amazon have reported carbon stocks up to 80% higher in ADE relative to its adjacent soil (Alho et al., 2019; Chagas et al., 2017; Schellekens et al., 2017).

The lack of a consistent pattern to conclude about the effects of ADE in soil C:N ratios (Table 2) has also been reported by some authors, with C:N ratios only 20% higher when compared to the reference soil (Moreira et al., 2009), or even without statistical differences (Chagas et al., 2017). The fact that ADE does not interfere in the soil C:N ratio can be justified by lost and deplete the soil nitrogen during the pyrolysis process in the formation and genesis of ADE (Teixeira et al., 2009), being immobilized by microbial biomass, and thus, equating the C:N ratios with those of the reference soil. Although the small effect of ADE in the C:N ratios of the whole soil, its values between 12.2 - 15.0, found in the present study are within the range reported by other studies also conducted in the Amazon region (Chagas et al., 2017; Teixeira et al., 2009) and in the Czech Republic (Asare et al., 2021). Although ADEs do not impact soil C:N ratios, its importance in the TOC and TN accumulation is indisputable compared to the other classes of adjacent soils found in the Amazon, and its potential in the sequestration of these elements.

Regarding the effect of soil texture, it was possible to notice that the sites with higher clay content also presented higher accumulations of TOC and TN in the soil, regardless of ADE or not. This positive relationship between clay content and carbon accumulation was also observed by others authors (Dieckow et al., 2009; Feller and Beare, 1997; Schapel et al., 2018), and indicates the role of clay in protection mechanisms of soil organic matter (Blanco-Canqui and Lal, 2004; Churchman et al., 2020; von Lutzow et al., 2006), even in ADEs. According to Churchman et al. (2020), soils with higher clay contents can provide new surfaces for the retention of carbon, consequently increasing the stocks of this element in the soil.

4.2. Distribution of total organic carbon and total nitrogen in physical fractions

As in the whole soil, the physical fractions of ADE had higher concentrations of TOC and TN compared to the reference soil (without anthropic horizon). Our results are supported by others also obtained through fractionation techniques in ADE (Chagas et al., 2017; Glaser et al., 2001), which reinforces the contribution of these anthropic soils to organic matter storage. The largest incremental TOC accumulations in the clay fraction of clayey ADE (7.4 g kg^{-1} soil) and very clayey ADE (8.4 g kg^{-1} soil) are related to the well-known ability of clay-rich soils to accumulate more carbon and nitrogen compared to soils with a sandy texture (Churchman et al., 2020; Dalal and Mayer, 1986; Dieckow et al., 2009; Korschens, 1980). However, even in the sandy clay loam and sandy clay sites, our results showed that the clay fraction played an important role in the accumulations of TOC and TN. In relative terms, of the total incremental carbon (ΔTOC) accumulated in the ADE, the clay fraction was responsible for 34-48%; and 50-72% of the TN (ΔTN) (Figures 2a and 2b).

Although the presence of pyrogenic carbon in ADEs (not evaluated in this study, but physically observed in whole soil samples) could play a significant role at stabilizing organic matter, our results showed that the clay fraction proved to be also an important factor to the accumulation of TOC and TN in the ADEs, possibly by mineral-organic association mechanisms; since, in the clay fraction the mechanism that commands the stabilization of organic matter is the mineral-organic association (Kaiser and Zech, 2000; Wiseman and Puttmann, 2006). In addition, the low C:N ratio in the clay fraction (average 10.5; Figure 2c) clearly indicates that there is no pyrogenic material; for which a larger C:N ratio would be expected (Bruun et al., 2012), generally greater than 16 when charcoal is present (Hassink, 1994). When computing ΔTOC/ΔTN, which means de C:N ratio of the incremental organic matter in ADE relative to the reference soil, an average value of

9.7 was obtained, and that also indicates an organic material of microbial origin (approximately 10:1) (Clevelend and Liptzin, 2007). The importance of the mineral-organic association in carbon stabilization in soil has been discussed in several studies (Coward et al., 2017; Eusterhues et al., 2005; Kleber et al., 2007), and is considered a key mechanism in the permanence and longevity of soil organic matter (Churchman et al., 2020; Xiao et al., 2016).

Regarding the silt fraction, the high concentrations of TOC and TN in ADE associated with higher C:N ratios in this fraction (average of 18.4), especially in the sandy sites, possibly indicate the contribution of high chemical recalcitrance structures in parallel with mineral-organic association in the stabilization of this organic matter. Although fragments of pyrogenic carbon can have variable sizes (Carvalho et al., 2018; Jenkins et al., 2014), specifically particles of pyrogenic charcoal that are similar in size to silt particles, can accumulate predominantly in this intermediate fraction (2 - 53 μm). This situation was also observed in Acrisol by Dieckow et al. (2005) and Brazilian Ferralsol by Ramalho et al. (2020).

The lack of a clear pattern between TOC and TN results in the sand-POM fraction of ADE and reference soil, may be associated with the fact that this fraction has the lowest proportions of TOC and TN, and has been directly affected and masked by the current land use. The particulate fraction of the soil organic matter is considered highly responsive to changes in soil land use and management practices, and in the present study, both the ADE and the reference soil of all evaluated sites are under modern anthropic effect, therefore, far from their native condition. The separation of aggregate classes in a 0.5 mm (500 μm) mesh has shown to have no effect on distinguishing between TOC and NT accumulations in physical fractions. Possibly a separation on smaller scales is necessary to observe the preferential accumulations and distributions of organic matter.

Additional studies are needed to better elucidate the dynamics of TOC and TN stocks in physical fractions of ADE with different size-classes of aggregates .

5. Conclusions

The anthropogenic dark earths accumulated 44-45% more carbon and nitrogen than their respective reference soils (on average across de four sites); what confirms the capacity of anthropogenic soils to store organic matter and serve as a model for optimizing carbon sequestration and soil quality.

The accumulation of 34-48% of the incremental carbon and 50-72% of the incremental nitrogen, relative to the reference soil, in the clay fraction of anthropogenic dark earths and the C:N ratio of this accumulated material suggesting microbial and non-pyrogenic origin, demonstrated that the clay fraction and its mineral-organic association are also important contributors to the storage and stabilization of organic matter in these Anthrosols.

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Table 1

Geographic and soil characteristics of the four study sites of anthropogenic dark earth (ADE) and its respective reference soil (adjacent) in the Brazilian Amazon. The four sites comprise a gradient of soil textural classes, from sandy clay loam, sandy clay, clayey to very clayey.

	Sandy clay loam			Sandy clay			Clayey			Very clayey	
	ADE	Reference	*p*[b]	ADE	Reference	*p*	ADE	Reference	*p*	ADE	Reference
Site name	Caldeirão			Lago Grande			Santa Paula			Maguari	
Municipality, State	Iranduba, Amazonas			Iranduba, Amazonas			Porto Velho, Rondônia			Belterra, Pará	
Coordinates	3°13’47’’S 60°16’06’’W	3°13’30’’S 60°16’28’’W		3°15’08’’S 60°13’45’’W	3°14’49’’S 60°13’29’’ W		8°51’57’’S 64°03’39’’W	8°52’36’’S 64°03’57’’W		2°46’59’’S 55°00’46’’W	2°47’02’’S 54°59’54’’W
Altitude (m)	32			52			72			80	
Climate	Tropical monsoon (Am)			Tropical monsoon (Am)			Tropical monsoon (Am)			Tropical monsoon (Am)	
Mean temperature	26.7 °C			26.7 °C			25.6 °C			24.9 °C	
Annual precipitation	2300 mm			2300 mm			2255 mm			1946 mm	
Parent material	Sandstone/claystone			Sandstone/claystone			Granite/syenite/monzonite			Sandstone/claystone	
Land use	Native forest			Agriculture			Native forest			Native forest	
Soil											
Soil group WRB	Anthrosol	Acrisol		Anthrosol	Acrisol		Anthrosol	Ferralsol		Anthrosol	Ferralsol
Sand ($g\ kg^{-1}$)	535	657	0.00	355	419	0.00	199	134	0.00	127	61
Silt ($g\ kg^{-1}$)	94	51	0.03	155	149	0.34	272	213	0.10	198	159
Clay ($g\ kg^{-1}$)	371	293	0.03	490	433	0.02	529	653	0.03	675	780
Total Fe ($g\ kg^{-1}$)[a]	39.2	36.5	0.31	42.9	45.2	0.26	42.9	37.2	0.17	37.7	37.5
pH $CaCl_2$	4.7	3.6	0.00	4.5	3.9	0.00	4.9	3.9	0.00	4.8	4.3
Al ($cmol_c\ dm^{-3}$)	0.4	2.6	0.00	0.1	2,0	0.01	0.2	2.9	0.00	1.6	3.2
H+Al ($cmol_c\ dm^{-3}$)	7.1	7.9	0.12	10.5	8.9	0.17	7.3	11.3	0.00	11.2	10.4
Ca ($cmol_c\ dm^{-3}$)	8.2	0.1	0.00	9.0	0.9	0.01	8.4	0.1	0.02	10.5	3.7
Mg ($cmol_c\ dm^{-3}$)	0.6	0.0	0.02	0.9	0.02	0.01	1.0	0.0	0.02	1.2	0.7
K ($cmol_c\ dm^{-3}$)	0.08	0.05	0.19	0.14	0.07	0.04	0.17	0.11	0.00	0.15	0.11

P (mg dm^{-3})	255.6	17.3	0.01	641.6	12.0	0.06	274.6	60.7	0.00	19.2	9.2
CEC (mg dm^{-3})	14.5	8.5	0.09	19.0	11.2	0.00	17.1	13.3	0.06	19.4	14.0
Base saturation (%)	71	2	0.02	53	9	0.00	55	2	0.006	57	29
Al saturation (%)	3	94	0.00	2	92	0.00	2	92	0.00	10	33

[a] SW 846-3051A method (USEPA, 2007); [b] Student's t test ($p \leq 0.05$).

Table 2

Total organic carbon (TOC), total nitrogen (TN) and C:N ratio of the top 10 cm layer of anthropogenic dark earth (ADE) and their respective reference soil (adjacent) in the Brazilian Amazon, across a gradient of soil textural classes from sandy clay loam, sandy clay, clayey to very clayey.

	Sandy clay loam			Sandy clay			Clayey			Very clayey		
	ADE	Reference	*p* [b]	ADE	Reference	*p*	ADE	Reference	*p*	ADE	Reference	*p*
TOC ($g\ kg^{-1}$)	32.0 ±0.6[a]	22.0 ±0.7	*0.002*	40.3 ±0.6	29.0 ±0.7	*0.001*	54.2 ±4.8	35.4 ±1.6	*0.03*	60.5 ±4.6	42.7 ±1.2	*0.03*
TN ($g\ kg^{-1}$)	2.51 ±0.05	1.67 ±0.05	*0.002*	2.69 ±0.05	2.28 ±0.00	*0.01*	4.25 ±0.40	2.57 ±0.06	*0.04*	4.94 ±0.31	3.49 ±0.12	*0.02*
C:N ratio	12.7 ±0.5	13.2 ±0.1	*0.26*	15.0 ±0.6	12.7 ±0.3	*0.02*	12.8 ±0.4	13.8 ±0.2	*0.08*	12.2 ±0.0	12.2 ±0.2	*0.44*

[a] ± Standard error; [b] Student's t test ($p \leq 0.05$).

Table 3

Total organic carbon (TOC) and total nitrogen (TN) concentrations, and C:N ratio of physical fractions in small aggregates (< 500 μm) of the top 10 cm layer of anthropogenic dark earth (ADE) and their respective reference soil (adjacent) in the Brazilian Amazon, across a gradient of soil textural classes from sandy clay loam, sandy clay, clayey to very clayey.

Fraction [a]	Sandy clay loam			Sandy clay			Clayey			Very clayey		
	ADE	Reference	*p* [c]	ADE	Reference	*p*	ADE	Reference	*p*	ADE	Reference	*p*
TOC (g kg^{-1} fraction)												
Sand-POM	11.4 ±1.0 [b]	7.9 ±1.0	*0.03*	13.0 ±0.3	12.0 ±0.1	*0.02*	50.7 ±7.1	53.5 ±7.2	*0.40*	70.3 ±6.0	65.9 ±10.5	*0.37*
Silt	69.5 ±3.7	54.2 ±2.3	*0.02*	97.0 ±1.4	50.9 ±1.1	*0.00*	54.1 ±2.8	38.8 ±6.3	*0.06*	108.8 ±2.0	89.9 ±7.8	*0.11*
Clay	47.1 ±2.7	41.0 ±3.9	*0.13*	38.6 ±0.5	33.4 ±0.6	*0.00*	53.8 ±7.1	28.2 ±1.2	*0.04*	42.7 ±2.4	27.6 ±1.1	*0.01*
Aggregate	29.7 ±0.5	20.8 ±0.9	*0.00*	38.4 ±0.4	27.2 ±1.1	*0.00*	56.5 ±3.3	38.4 ±2.9	*0.01*	63.4 ±6.5	44.8 ±2.7	*0.04*
TN (g kg^{-1} fraction)												
Sand-POM	0.75 ±0.01	0.55 ±0.06	*0.05*	0.73 ±0.00	0.87 ±0.03	*0.03*	2.96 ±0.41	2.58 ±0.23	*0.28*	4.73 ±0.13	3.60 ±0.41	*0.08*
Silt	3.81 ±0.07	3.54 ±0.20	*0.18*	3.93 ±0.22	3.32 ±0.13	*0.07*	3.25 ±0.26	1.95 ±0.14	*0.02*	6.63 ±0.08	5.38 ±0.56	*0.11*
Clay	4.03 ±0.21	2.86 ±0.10	*0.01*	3.39 ±0.02	2.81 ±0.00	*0.00*	4.80 ±0.48	2.32 ±0.08	*0.03*	3.83 ±0.20	2.48 ±0.04	*0.02*
Aggregate	2.36 ±0.05	1.64 ±0.06	*0.00*	2.56 ±0.08	2.22 ±0.01	*0.04*	4.35 ±0.34	2.74 ±0.11	*0.03*	5.19 ±0.43	3.61 ±0.26	*0.04*
C:N ratio												
Sand-POM	15.2 ±1.3	14.4 ±0.2	*0.31*	17.8 ±0.4	13.8 ±0.5	*0.00*	17.1 ±0.8	20.7 ±0.02	*0.02*	14.9 ±1.2	18.3 ±0.5	*0.04*
Silt	18.2 ±1.2	15.3 ±0.4	*0.07*	24.7 ±1.9	15.3 ±0.4	*0.02*	16.6 ±1.0	19.9 ±1.6	*0.12*	16.4 ±0.1	16.7 ±1.1	*0.34*
Clay	11.7 ±0.1	14.3 ±0.9	*0.05*	11.4 ±0.1	11.9 ±0.2	*0.06*	11.2 ±0.1	12.2 ±0.2	*0.01*	11.1 ±0.1	11.1 ±0.3	*0.47*
Aggregate	12.6 ±0.5	12.7 ±0.0	*0.46*	15.0 ±0.6	12.3 ±0.4	*0.01*	13.0 ±0.6	14.0 ±0.4	*0.13*	12.2 ±0.1	12.4 ±0.4	*0.28*
Recovery of mass after fractionation (g fraction g^{-1} small aggregate) [d]												
Sand-POM	562 ±22	661 ±24	*0.03*	380 ±5	460 ±8	*0.001*	227 ±12	134 ±5	*0.01*	176 ±31	115 ±5	*0.12*
Silt	111 ±14	61 ±5	*0.08*	154 ±6	134 ±4	*0.06*	297 ±10	300 ±6	*0.42*	178 ±19	146 ±6	*0.17*
Clay	327 ±14	278 ±23	*0.07*	466 ±6	406 ±12	*0.02*	476 ±17	566 ±6	*0.03*	646 ±19	739 ±5	*0.13*

[a] Sand-POM, sand plus particulate organic matter (>53 μm); silt (2 - 53 μm); clay (< 2 μm); [b] Standard error; [c] Student's t test ($p \leq 0.05$); [d] Recovery of physical fractions was normalized to obtain a sum of 1000 g fraction kg^{-1} small aggregate.

Table 4

Total organic carbon (TOC) and total nitrogen (TN) concentrations, and C:N ratio of physical fractions in large aggregates (> 500 μm) of the top 10 cm layer of anthropogenic dark earth (ADE) and their respective reference soil (adjacent) in the Brazilian Amazon, across a gradient of soil textural classes from sandy clay loam, sandy clay, clayey to very clayey.

Fraction [a]	Sandy clay loam			Sandy clay			Clayey			Very clayey		
	ADE	Reference	*p* [c]	ADE	Reference	*p*	ADE	Reference	*p*	ADE	Reference	*p*
TOC ($g\ kg^{-1}$ fraction)												
Sand-POM	10.3 ±0.9	12.0 ±1.7	*0.22*	12.0 ±1.1	11.8 ±0.5	*0.44*	30.2 ±0.6	30.9 ±6.3	*0.46*	70.9 ±7.3	70.4 ±2.1	*0.47*
Silt	79.6 ±1.6	66.2 ±1.6	*0.01*	91.9 ±3.9	58.6 ±1.0	*0.01*	54.3 ±4.9	35.1 ±3.8	*0.02*	94.8 ±8.9	80.0 ±9.4	*0.16*
Clay	41.0 ±4.1	34.2 ±1.1	*0.13*	35.3 ±0.0	29.8 ±0.4	*0.00*	51.6 ±3.3	30.6 ±2.7	*0.00*	40.1 ±2.3	28.3 ±3.5	*0.03*
Aggregate	34.8 ±0.4	23.5 ±0.3	*0.00*	41.4 ±1.1	31.0 ±0.7	*0.00*	52.8 ±5.8	32.1 ±1.8	*0.04*	59.0 ±3.7	41.8 ±2.3	*0.01*
TN ($g\ kg^{-1}$ fraction)												
Sand-POM	0.65 ±0.06	0.68 ±0.07	*0.40*	0.60 ±0.05	0.66 ±0.03	*0.24*	1.49 ±0.10	1.29 ±0.14	*0.20*	3.90 ±0.25	3.18 ±0.20	*0.07*
Silt	4.18 ±0.11	3.63 ±0.19	*0.07*	3.64 ±0.33	3.52 ±0.08	*0.40*	3.05 ±0.23	1.63 ±0.11	*0.01*	5.49 ±0.26	4.54 ±0.33	*0.10*
Clay	3.87 ±0.29	2.82 ±0.18	*0.04*	3.19 ±0.04	2.77 ±0.04	*0.00*	4.46 ±0.42	2.29 ±0.03	*0.03*	3.67 ±0.20	2.64 ±0.10	*0.02*
Aggregate	2.70 ±0.06	1.71 ±0.04	*0.00*	2.77 ±0.03	2.35 ±0.03	*0.00*	4.18 ±0.46	2.38 ±0.06	*0.04*	4.80 ±0.25	3.43 ±0.17	*0.02*
C:N ratio												
Sand-POM	15.8 ±0.4	17.6 ±0.4	*0.02*	20.0 ±1.1	17.9 ±0.8	*0.09*	20.3 ±1.5	24.0 ±1.7	*0.13*	18.2 ±0.9	22.1 ±1.1	*0.02*
Silt	19.0 ±0.9	18.2 ±0.5	*0.25*	25.2 ±1.8	16.6 ±0.5	*0.02*	17.8 ±1.1	21.5 ±0.7	*0.03*	17.3 ±0.8	17.6 ±0.7	*0.36*
Clay	10.6 ±0.2	12.1 ±0.6	*0.06*	11.1 ±0.2	10.8 ±0.3	*0.24*	11.6 ±0.8	13.4 ±1.0	*0.14*	10.9 ±0.2	10.7 ±0.8	*0.38*
Aggregate	12.9 ±0.5	13.7 ±0.2	*0.13*	14.9 ±0.6	13.2 ±0.2	*0.05*	12.6 ±0.3	13.5 ±0.4	*0.01*	12.3 ±0.0	12.2 ±0.2	*0.33*
Recovery of mass after fractionation (g fraction g^{-1} large aggregate) [d]												
Sand-POM	534 ±21	666 ±4	*0.02*	357 ±7	413 ±11	*0.02*	164 ±2	146 ±4	*0.02*	105 ±17	67 ±1	*0.10*
Silt	128 ±3	71 ±3	*0.01*	152 ±3	120 ±5	*0.01*	378 ±17	220 ±7	*0.01*	164 ±20	146 ±12	*0.27*
Clay	338 ±19	263 ±6	*0.04*	491 ±10	467 ±13	*0.15*	458 ±18	634 ±6	*0.01*	731 ±37	787 ±13	*0.17*

[a] Sand-POM, sand plus particulate organic matter (>53 µm); silt (2 - 53 µm); clay (< 2 µm); [b] Standard error; [c] Student's t test ($p \leq 0.05$); [d] Recovery of physical fractions was normalized to obtain a sum of 1000 g fraction kg^{-1} large aggregate.

Table 5

Proportion of small and large aggregates in whole soil samples, and total organic carbon (TOC), total nitrogen (TN) and C:N ratio in physical fractions of the top 10 cm layer of anthropogenic dark earth (ADE) and their respective reference soil (adjacent) in the Brazilian Amazon, across a gradient of soil textural classes from sandy clay loam, sandy clay, clayey to very clayey.

	Sandy clay loam			Sandy clay			Clayey			Very clayey		
	ADE	Ref	*p*	ADE	Ref	*p*	ADE	Ref	*p*	ADE	Ref	*p*
Proportion of soil aggregates classes (g aggregate g^{-1} soil)												
Small aggregate	545.2 ±18.3[b]	532.3 ±35.8	*0.41*[c]	373.6 ±26.5	521.9 ±71.0	*0.11*	384.2 ±33.2	533.2 ±13.6	*0.02*	348.3 ±26.2	349.1 ±12.6	*0.49*
Large aggregate	454.8 ±18.3	467.7 ±35.8	*0.41*	626.4 ±26.5	478.1 ±71.0	*0.11*	615.8 ±33.2	466.8 ±13.6	*0.02*	651.7 ±26.2	650.9 ±12.6	*0.49*
Total	1000	1000		1000	1000		1000	1000		1000	1000	
TOC (g kg^{-1} solo)												
Sand-POM[a]	6.5 ±0.4	6.9 ±0.6	*0.27*	5.0 ±0.2	5.8 ±0.0	*0.03*	8.0 ±0.8	6.3 ±0.6	*0.11*	10.1 ±1.6	6.2 ±0.1	*0.10*
Silt	9.6 ±0.8	4.2 ±0.5	*0.00*	15.8 ±0.6	7.8 ±0.4	*0.00*	20.1 ±0.8	10.4 ±1.3	*0.22*	18.7 ±2.7	13.2 ±1.3	*0.10*
Clay	15.9 ±0.3	10.9 ±0.5	*0.01*	19.5 ±0.4	15.4 ±0.8	*0.02*	26.1 ±2.3	18.7 ±0.6	*0.02*	31.7 ±0.4	23.5 ±0.5	*0.00*
TN (g kg^{-1} solo)												
Sand-POM	0.45 ±0.0	0.48 ±0.0	*0.25*	0.27 ±0.0	0.39 ±0.0	*0.01*	0.48 ±0.1	0.33 ±0.0	*0.15*	0.66 ±0.1	0.33 ±0.0	*0.08*
Silt	0.55 ±0.0	0.28 ±0.0	*0.00*	0.65 ±0.1	0.50 ±0.0	*0.16*	1.27 ±0.1	0.58 ±0.1	*0.18*	1.18 ±0.2	0.83 ±0.1	*0.10*
Clay	1.51 ±0.0	0.91 ±0.1	*0.01*	1.77 ±0.0	1.39 ±0.1	*0.02*	2.50 ±0.2	1.66 ±0.0	*0.02*	3.10 ±0.2	2.33 ±0.2	*0.04*

[a] Sand-POM. sand plus particulate organic matter (>53 μm); silt (2 - 53 μm); clay (< 2 μm); [b] Standard error; [c] Student's t test ($p \leq 0.05$); [d] TOC and TN of physical fractions for the whole soil were calculated by considering the respective concentrations in Tables 3 and 4. and the proportion of small and large aggregates in this Table.

Figure 1

Map of sampling sites in Iranduba (state of Amazonas), Belterra (state of Pará) and Porto Velho (state of Rondônia) in the Brazilian Amazon.

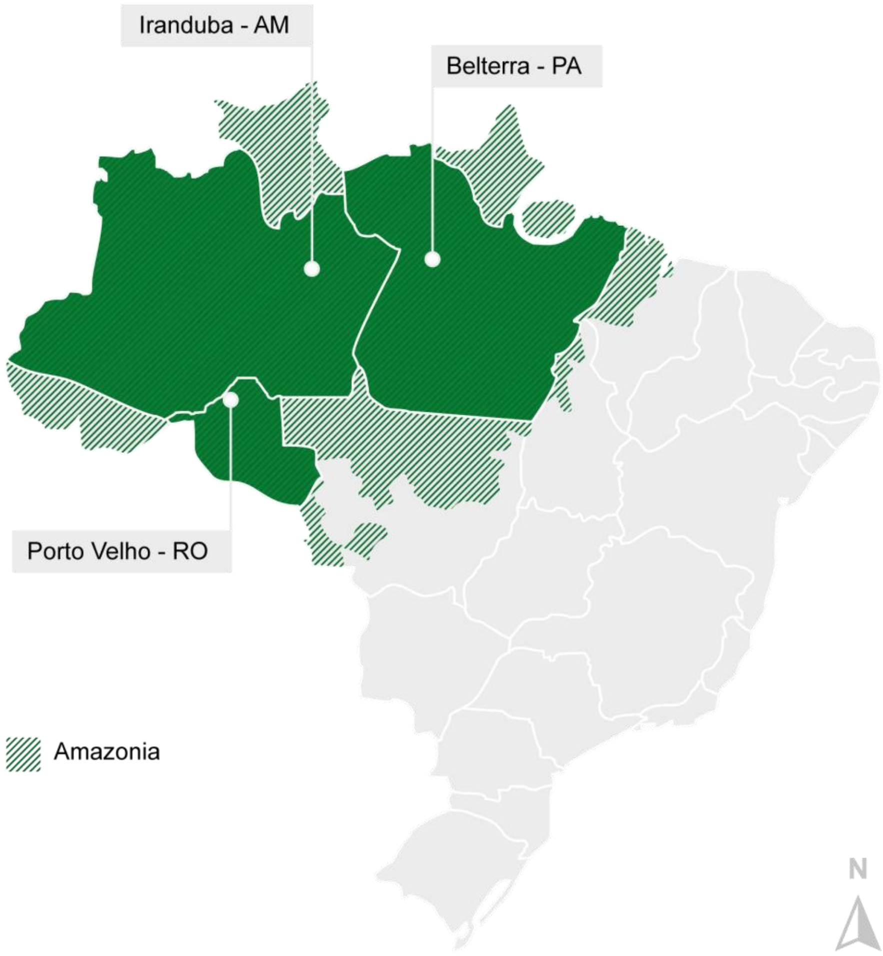
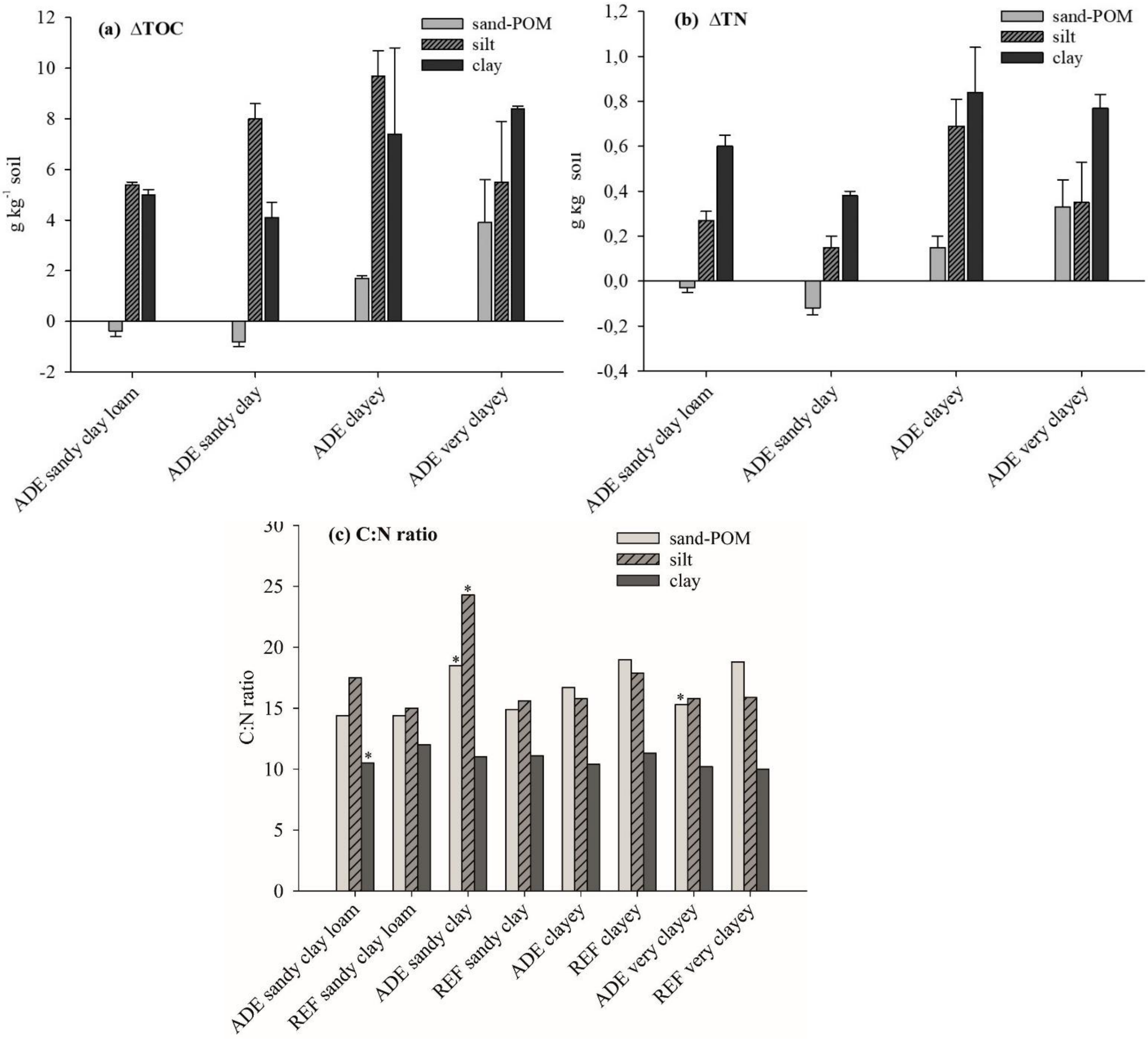


Figure 2

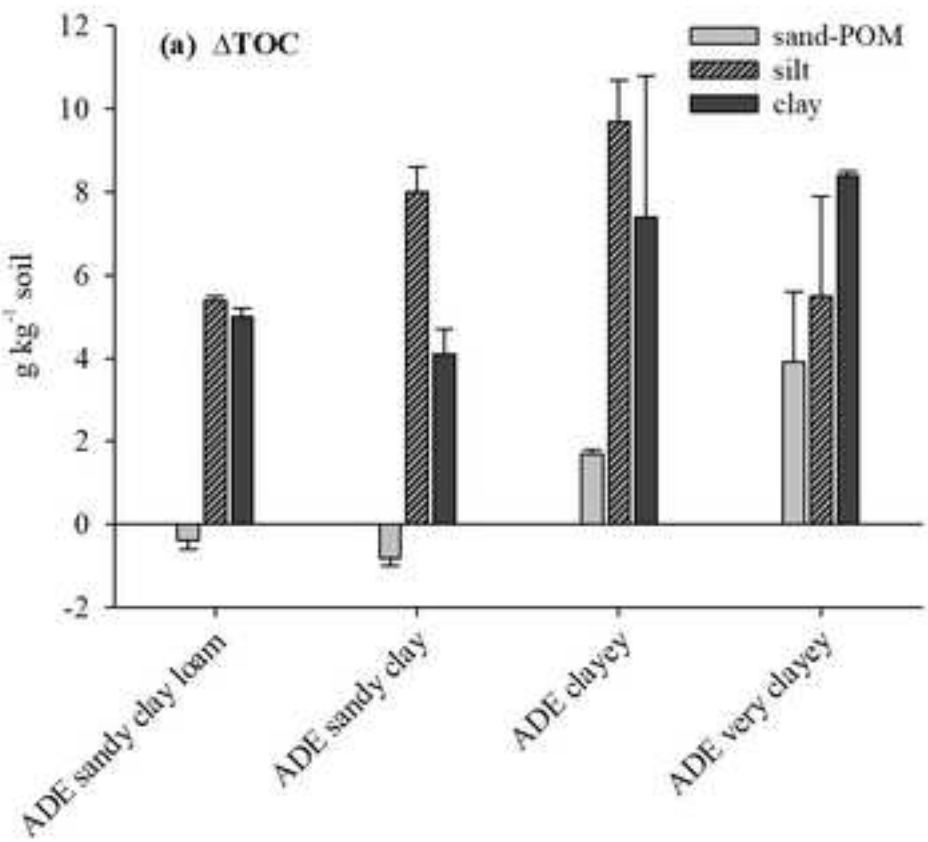
Δ total organic carbon (TOC) (a), Δ total nitrogen (TN) (b) and C:N ratio (c) in physical fractions of the top 10 cm layer of anthropogenic dark earth (ADE) and their respective reference soils (adjacent) in the Brazilian Amazon, across a gradient of soil textural classes from sandy clay loam, sandy clay, clayey to very clayey.



Δ calculated by difference in TOC or TN between dark earth and reference soil; Student's t test (* $p \leq 0.05$) ± standard error.

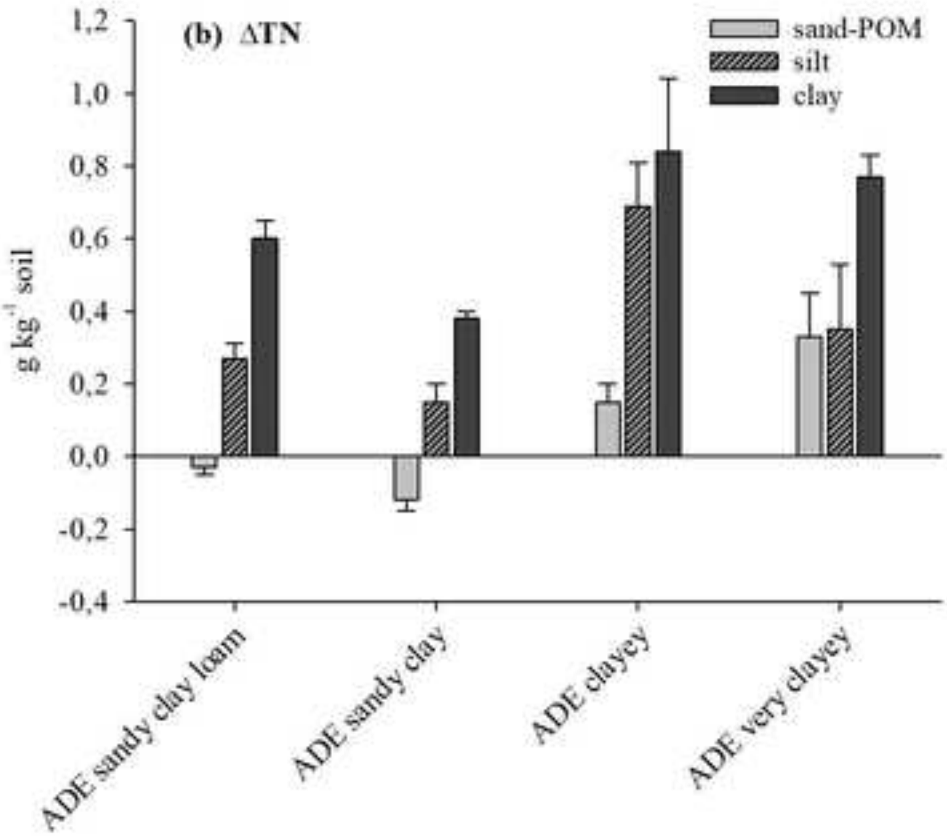
Figure 1

Figure 2a



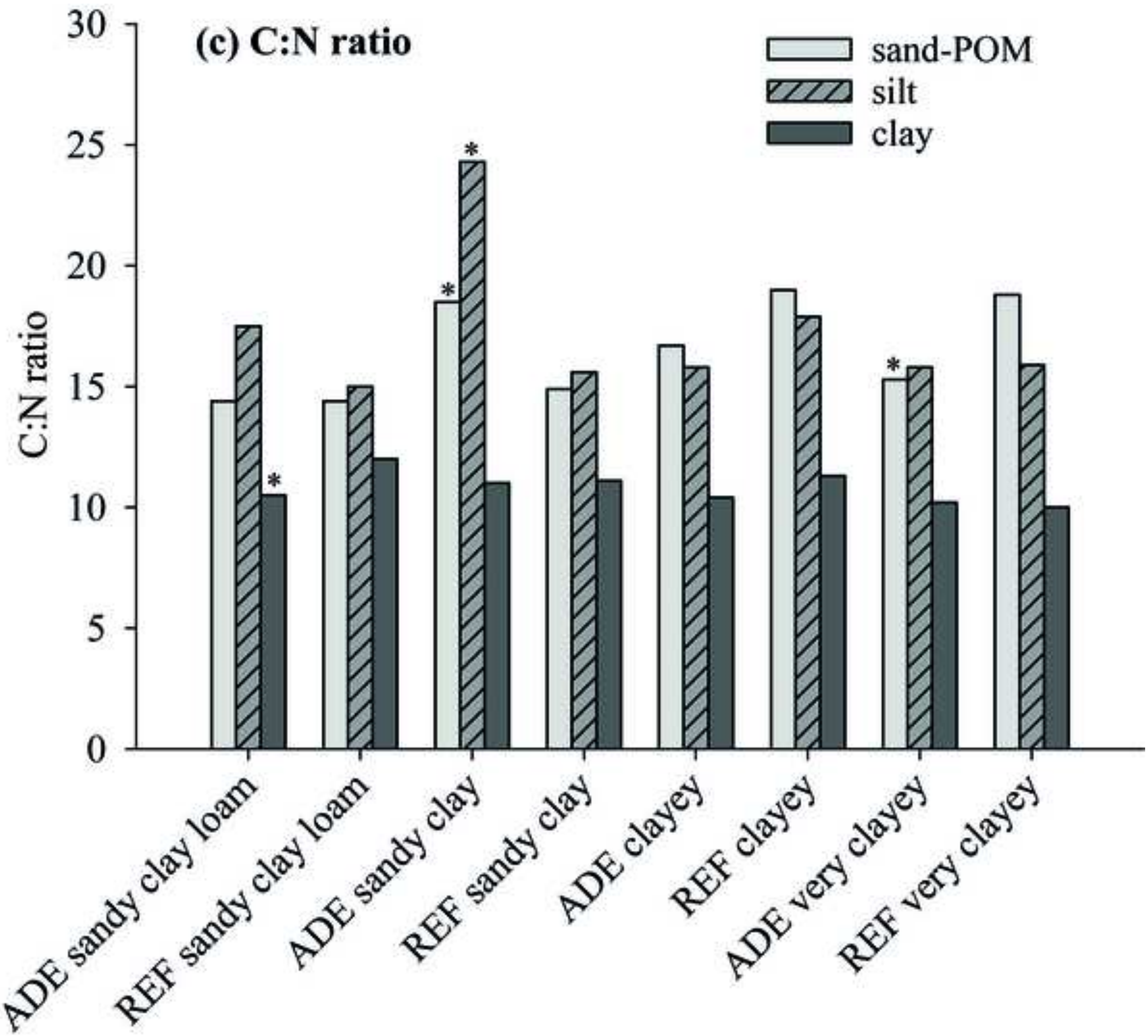
(a) ΔTOC
sand-POM
silt
clay
g kg-1 soil
12
10
8
6
4
2
0
-2
ADE sandy clay loam
ADE sandy clay
ADE clayey
ADE very clayey

Figure 2b



(b) ΔTN
sand-POM
silt
clay
g kg⁻¹ soil
1,2
1,0
0,8
0,6
0,4
0,2
0,0
-0,2
-0,4
ADE sandy clay loam
ADE sandy clay
ADE clayey
ADE very clayey

Figure 2c



(c) C:N ratio
sand-POM
silt
clay
C:N ratio
30
25
20
15
10
5
0
*
*
*
*
ADE sandy clay loam
REF sandy clay loam
ADE sandy clay
REF sandy clay
ADE clayey
REF clayey
ADE very clayey
REF very clayey

Tables

Table 1

Geographic and soil characteristics of the four study sites of anthropogenic dark earth (ADE) and its respective reference soil (adjacent) in the Brazilian Amazon. The four sites comprise a gradient of soil textural classes, from sandy clay loam, sandy clay, clayey to very clayey.

	Sandy clay loam			Sandy clay			Clayey			Very clayey	
	ADE	Reference	p[b]	ADE	Reference	p	ADE	Reference	p	ADE	Reference
Site name	Caldeirão			Lago Grande			Santa Paula			Maguari	
Municipality, State	Iranduba, Amazonas			Iranduba, Amazonas			Porto Velho, Rondônia			Belterra, Pará	
Coordinates	3°13’47’’S 60°16’06’’W	3°13’30’’S 60°16’28’’W		3°15’08’’S 60°13’45’’W	3°14’49’’S 60°13’29’’ W		8°51’57’’S 64°03’39’’W	8°52’36’’S 64°03’57’’W		2°46’59’’S 55°00’46’’W	2°47’02’’S 54°59’54’’W
Altitude (m)	32			52			72			80	
Climate	Tropical monsoon (Am)			Tropical monsoon (Am)			Tropical monsoon (Am)			Tropical monsoon (Am)	
Mean temperature	26.7 °C			26.7 °C			25.6 °C			24.9 °C	
Annual precipitation	2300 mm			2300 mm			2255 mm			1946 mm	
Parent material	Sandstone/claystone			Sandstone/claystone			Granite/syenite/monzonite			Sandstone/claystone	
Land use	Native forest			Agriculture			Native forest			Native forest	
Soil											
Soil group WRB	Anthrosol	Acrisol		Anthrosol	Acrisol		Anthrosol	Ferralsol		Anthrosol	Ferralsol
Sand (g kg^{-1})	535	657	0.00	355	419	0.00	199	134	0.00	127	61
Silt (g kg^{-1})	94	51	0.03	155	149	0.34	272	213	0.10	198	159
Clay (g kg^{-1})	371	293	0.03	490	433	0.02	529	653	0.03	675	780
Total Fe (g kg^{-1})[a]	39.2	36.5	0.31	42.9	45.2	0.26	42.9	37.2	0.17	37.7	37.5
pH $CaCl_2$	4.7	3.6	0.00	4.5	3.9	0.00	4.9	3.9	0.00	4.8	4.3

Al ($cmol_c\ dm^{-3}$)	0.4	2.6	0.00	0.1	2,0	0.01	0.2	2.9	0.00	1.6	3.2
H+Al ($cmol_c\ dm^{-3}$)	7.1	7.9	0.12	10.5	8.9	0.17	7.3	11.3	0.00	11.2	10.4
Ca ($cmol_c\ dm^{-3}$)	8.2	0.1	0.00	9.0	0.9	0.01	8.4	0.1	0.02	10.5	3.7
Mg ($cmol_c\ dm^{-3}$)	0.6	0.0	0.02	0.9	0.02	0.01	1.0	0.0	0.02	1.2	0.7
K ($cmol_c\ dm^{-3}$)	0.08	0.05	0.19	0.14	0.07	0.04	0.17	0.11	0.00	0.15	0.11
P ($mg\ dm^{-3}$)	255.6	17.3	0.01	641.6	12.0	0.06	274.6	60.7	0.00	19.2	9.2
CEC ($mg\ dm^{-3}$)	14.5	8.5	0.09	19.0	11.2	0.00	17.1	13.3	0.06	19.4	14.0
Base saturation (%)	71	2	0.02	53	9	0.00	55	2	0.006	57	29
Al saturation (%)	3	94	0.00	2	92	0.00	2	92	0.00	10	33

[a] SW 846-3051A method (USEPA, 2007); [b] Student's t test ($p \leq 0.05$).

Table 2

Total organic carbon (TOC), total nitrogen (TN) and C:N ratio of the top 10 cm layer of anthropogenic dark earth (ADE) and their respective reference soil (adjacent) in the Brazilian Amazon, across a gradient of soil textural classes from sandy clay loam, sandy clay, clayey to very clayey.

	Sandy clay loam			Sandy clay			Clayey			Very clayey		
	ADE	Reference	*p* [b]	ADE	Reference	*p*	ADE	Reference	*p*	ADE	Reference	*p*
TOC ($g\ kg^{-1}$)	32.0 ±0.6[a]	22.0 ±0.7	*0.002*	40.3 ±0.6	29.0 ±0.7	*0.001*	54.2 ±4.8	35.4 ±1.6	*0.03*	60.5 ±4.6	42.7 ±1.2	*0.03*
TN ($g\ kg^{-1}$)	2.51 ±0.05	1.67 ±0.05	*0.002*	2.69 ±0.05	2.28 ±0.00	*0.01*	4.25 ±0.40	2.57 ±0.06	*0.04*	4.94 ±0.31	3.49 ±0.12	*0.02*
C:N ratio	12.7 ±0.5	13.2 ±0.1	*0.26*	15.0 ±0.6	12.7 ±0.3	*0.02*	12.8 ±0.4	13.8 ±0.2	*0.08*	12.2 ±0.0	12.2 ±0.2	*0.44*

[a] ± Standard error; [b] Student's t test ($p \leq 0.05$).

Table 3

Total organic carbon (TOC) and total nitrogen (TN) concentrations, and C:N ratio of physical fractions in small aggregates (< 500 μm) of the top 10 cm layer of anthropogenic dark earth (ADE) and their respective reference soil (adjacent) in the Brazilian Amazon, across a gradient of soil textural classes from sandy clay loam, sandy clay, clayey to very clayey.

Fraction [a]	Sandy clay loam			Sandy clay			Clayey			Very clayey		
	ADE	Reference	*p* [c]	ADE	Reference	*p*	ADE	Reference	*p*	ADE	Reference	*p*
TOC (g kg^{-1} fraction)												
Sand-POM	11.4 ±1.0 [b]	7.9 ±1.0	*0.03*	13.0 ±0.3	12.0 ±0.1	*0.02*	50.7 ±7.1	53.5 ±7.2	*0.40*	70.3 ±6.0	65.9 ±10.5	*0.37*
Silt	69.5 ±3.7	54.2 ±2.3	*0.02*	97.0 ±1.4	50.9 ±1.1	*0.00*	54.1 ±2.8	38.8 ±6.3	*0.06*	108.8 ±2.0	89.9 ±7.8	*0.11*
Clay	47.1 ±2.7	41.0 ±3.9	*0.13*	38.6 ±0.5	33.4 ±0.6	*0.00*	53.8 ±7.1	28.2 ±1.2	*0.04*	42.7 ±2.4	27.6 ±1.1	*0.01*
Aggregate	29.7 ±0.5	20.8 ±0.9	*0.00*	38.4 ±0.4	27.2 ±1.1	*0.00*	56.5 ±3.3	38.4 ±2.9	*0.01*	63.4 ±6.5	44.8 ±2.7	*0.04*
TN (g kg^{-1} fraction)												
Sand-POM	0.75 ±0.01	0.55 ±0.06	*0.05*	0.73 ±0.00	0.87 ±0.03	*0.03*	2.96 ±0.41	2.58 ±0.23	*0.28*	4.73 ±0.13	3.60 ±0.41	*0.08*
Silt	3.81 ±0.07	3.54 ±0.20	*0.18*	3.93 ±0.22	3.32 ±0.13	*0.07*	3.25 ±0.26	1.95 ±0.14	*0.02*	6.63 ±0.08	5.38 ±0.56	*0.11*
Clay	4.03 ±0.21	2.86 ±0.10	*0.01*	3.39 ±0.02	2.81 ±0.00	*0.00*	4.80 ±0.48	2.32 ±0.08	*0.03*	3.83 ±0.20	2.48 ±0.04	*0.02*
Aggregate	2.36 ±0.05	1.64 ±0.06	*0.00*	2.56 ±0.08	2.22 ±0.01	*0.04*	4.35 ±0.34	2.74 ±0.11	*0.03*	5.19 ±0.43	3.61 ±0.26	*0.04*
C:N ratio												
Sand-POM	15.2 ±1.3	14.4 ±0.2	*0.31*	17.8 ±0.4	13.8 ±0.5	*0.00*	17.1 ±0.8	20.7 ±0.02	*0.02*	14.9 ±1.2	18.3 ±0.5	*0.04*
Silt	18.2 ±1.2	15.3 ±0.4	*0.07*	24.7 ±1.9	15.3 ±0.4	*0.02*	16.6 ±1.0	19.9 ±1.6	*0.12*	16.4 ±0.1	16.7 ±1.1	*0.34*
Clay	11.7 ±0.1	14.3 ±0.9	*0.05*	11.4 ±0.1	11.9 ±0.2	*0.06*	11.2 ±0.1	12.2 ±0.2	*0.01*	11.1 ±0.1	11.1 ±0.3	*0.47*

Aggregate	12.6 ±0.5	12.7 ±0.0	*0.46*	15.0 ±0.6	12.3 ±0.4	*0.01*	13.0 ±0.6	14.0 ±0.4	*0.13*	12.2 ±0.1	12.4 ±0.4	*0.28*
Recovery of mass after fractionation (g fraction g^{-1} small aggregate) [d]												
Sand-POM	562 ±22	661 ±24	*0.03*	380 ±5	460 ±8	*0.001*	227 ±12	134 ±5	*0.01*	176 ±31	115 ±5	*0.12*
Silt	111 ±14	61 ±5	*0.08*	154 ±6	134 ±4	*0.06*	297 ±10	300 ±6	*0.42*	178 ±19	146 ±6	*0.17*
Clay	327 ±14	278 ±23	*0.07*	466 ±6	406 ±12	*0.02*	476 ±17	566 ±6	*0.03*	646 ±19	739 ±5	*0.13*

[a] Sand-POM, sand plus particulate organic matter (>53 µm); silt (2 - 53 µm); clay (< 2 µm); [b] Standard error; [c] Student's t test ($p \leq 0.05$); [d] Recovery of physical fractions was normalized to obtain a sum of 1000 g fraction kg^{-1} small aggregate.

Table 4

Total organic carbon (TOC) and total nitrogen (TN) concentrations, and C:N ratio of physical fractions in large aggregates (> 500 µm) of the top 10 cm layer of anthropogenic dark earth (ADE) and their respective reference soil (adjacent) in the Brazilian Amazon, across a gradient of soil textural classes from sandy clay loam, sandy clay, clayey to very clayey.

Fraction [a]	Sandy clay loam			Sandy clay			Clayey			Very clayey		
	ADE	Reference	*p* [c]	ADE	Reference	*p*	ADE	Reference	*p*	ADE	Reference	*p*
TOC (g kg^{-1} fraction)												
Sand-POM	10.3 ±0.9	12.0 ±1.7	*0.22*	12.0 ±1.1	11.8 ±0.5	*0.44*	30.2 ±0.6	30.9 ±6.3	*0.46*	70.9 ±7.3	70.4 ±2.1	*0.47*
Silt	79.6 ±1.6	66.2 ±1.6	*0.01*	91.9 ±3.9	58.6 ±1.0	*0.01*	54.3 ±4.9	35.1 ±3.8	*0.02*	94.8 ±8.9	80.0 ±9.4	*0.16*
Clay	41.0 ±4.1	34.2 ±1.1	*0.13*	35.3 ±0.0	29.8 ±0.4	*0.00*	51.6 ±3.3	30.6 ±2.7	*0.00*	40.1 ±2.3	28.3 ±3.5	*0.03*
Aggregate	34.8 ±0.4	23.5 ±0.3	*0.00*	41.4 ±1.1	31.0 ±0.7	*0.00*	52.8 ±5.8	32.1 ±1.8	*0.04*	59.0 ±3.7	41.8 ±2.3	*0.01*
TN (g kg^{-1} fraction)												
Sand-POM	0.65 ±0.06	0.68 ±0.07	*0.40*	0.60 ±0.05	0.66 ±0.03	*0.24*	1.49 ±0.10	1.29 ±0.14	*0.20*	3.90 ±0.25	3.18 ±0.20	*0.07*
Silt	4.18 ±0.11	3.63 ±0.19	*0.07*	3.64 ±0.33	3.52 ±0.08	*0.40*	3.05 ±0.23	1.63 ±0.11	*0.01*	5.49 ±0.26	4.54 ±0.33	*0.10*
Clay	3.87 ±0.29	2.82 ±0.18	*0.04*	3.19 ±0.04	2.77 ±0.04	*0.00*	4.46 ±0.42	2.29 ±0.03	*0.03*	3.67 ±0.20	2.64 ±0.10	*0.02*
Aggregate	2.70 ±0.06	1.71 ±0.04	*0.00*	2.77 ±0.03	2.35 ±0.03	*0.00*	4.18 ±0.46	2.38 ±0.06	*0.04*	4.80 ±0.25	3.43 ±0.17	*0.02*
C:N ratio												
Sand-POM	15.8 ±0.4	17.6 ±0.4	*0.02*	20.0 ±1.1	17.9 ±0.8	*0.09*	20.3 ±1.5	24.0 ±1.7	*0.13*	18.2 ±0.9	22.1 ±1.1	*0.02*
Silt	19.0 ±0.9	18.2 ±0.5	*0.25*	25.2 ±1.8	16.6 ±0.5	*0.02*	17.8 ±1.1	21.5 ±0.7	*0.03*	17.3 ±0.8	17.6 ±0.7	*0.36*
Clay	10.6 ±0.2	12.1 ±0.6	*0.06*	11.1 ±0.2	10.8 ±0.3	*0.24*	11.6 ±0.8	13.4 ±1.0	*0.14*	10.9 ±0.2	10.7 ±0.8	*0.38*
Aggregate	12.9 ±0.5	13.7 ±0.2	*0.13*	14.9 ±0.6	13.2 ±0.2	*0.05*	12.6 ±0.3	13.5 ±0.4	*0.01*	12.3 ±0.0	12.2 ±0.2	*0.33*
Recovery of mass after fractionation (g fraction g^{-1} large aggregate) [d]												
Sand-POM	534 ±21	666 ±4	*0.02*	357 ±7	413 ±11	*0.02*	164 ±2	146 ±4	*0.02*	105 ±17	67 ±1	*0.10*
Silt	128 ±3	71 ±3	*0.01*	152 ±3	120 ±5	*0.01*	378 ±17	220 ±7	*0.01*	164 ±20	146 ±12	*0.27*

Clay	338 ±19	263 ±6	*0.04*	491 ±10	467 ±13	*0.15*	458 ±18	634 ±6	*0.01*	731 ±37	787 ±13	*0.17*

[a] Sand-POM, sand plus particulate organic matter (>53 µm); silt (2 - 53 µm); clay (< 2 µm); [b] Standard error; [c] Student's t test ($p \leq 0.05$); [d] Recovery of physical fractions was normalized to obtain a sum of 1000 g fraction kg^{-1} large aggregate.

Table 5

Proportion of small and large aggregates in whole soil samples, and total organic carbon (TOC), total nitrogen (TN) and C:N ratio in physical fractions of the top 10 cm layer of anthropogenic dark earth (ADE) and their respective reference soil (adjacent) in the Brazilian Amazon, across a gradient of soil textural classes from sandy clay loam, sandy clay, clayey to very clayey.

	Sandy clay loam					Sandy clay					Clayey					Very clayey				
	ADE		Ref		*p*	ADE		Ref		*p*	ADE		Ref		*p*	ADE		Ref		*p*
Proportion of soil aggregates classes (g aggregate g^{-1} soil)																				
Small aggregate	545.2	±18.3[b]	532.3	±35.8	*0.41*[c]	373.6	±26.5	521.9	±71.0	*0.11*	384.2	±33.2	533.2	±13.6	*0.02*	348.3	±26.2	349.1	±12.6	*0.49*
Large aggregate	454.8	±18.3	467.7	±35.8	*0.41*	626.4	±26.5	478.1	±71.0	*0.11*	615.8	±33.2	466.8	±13.6	*0.02*	651.7	±26.2	650.9	±12.6	*0.49*
Total	1000		1000			1000		1000			1000		1000			1000		1000		
TOC (g kg^{-1} solo)																				
Sand-POM[a]	6.5	±0.4	6.9	±0.6	*0.27*	5.0	±0.2	5.8	±0.0	*0.03*	8.0	±0.8	6.3	±0.6	*0.11*	10.1	±1.6	6.2	±0.1	*0.10*
Silt	9.6	±0.8	4.2	±0.5	*0.00*	15.8	±0.6	7.8	±0.4	*0.00*	20.1	±0.8	10.4	±1.3	*0.22*	18.7	±2.7	13.2	±1.3	*0.10*
Clay	15.9	±0.3	10.9	±0.5	*0.01*	19.5	±0.4	15.4	±0.8	*0.02*	26.1	±2.3	18.7	±0.6	*0.02*	31.7	±0.4	23.5	±0.5	*0.00*
TN (g kg^{-1} solo)																				
Sand-POM	0.45	±0.0	0.48	±0.0	*0.25*	0.27	±0.0	0.39	±0.0	*0.01*	0.48	±0.1	0.33	±0.0	*0.15*	0.66	±0.1	0.33	±0.0	*0.08*
Silt	0.55	±0.0	0.28	±0.0	*0.00*	0.65	±0.1	0.50	±0.0	*0.16*	1.27	±0.1	0.58	±0.1	*0.18*	1.18	±0.2	0.83	±0.1	*0.10*
Clay	1.51	±0.0	0.91	±0.1	*0.01*	1.77	±0.0	1.39	±0.1	*0.02*	2.50	±0.2	1.66	±0.0	*0.02*	3.10	±0.2	2.33	±0.2	*0.04*

[a] Sand-POM. sand plus particulate organic matter (>53 µm); silt (2 - 53 µm); clay (< 2 µm); [b] Standard error; [c] Student's t test ($p \leq 0.05$); [d] TOC and TN of physical fractions for the whole soil were calculated by considering the respective concentrations in Tables 3 and 4. and the proportion of small and large aggregates in this Table.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: