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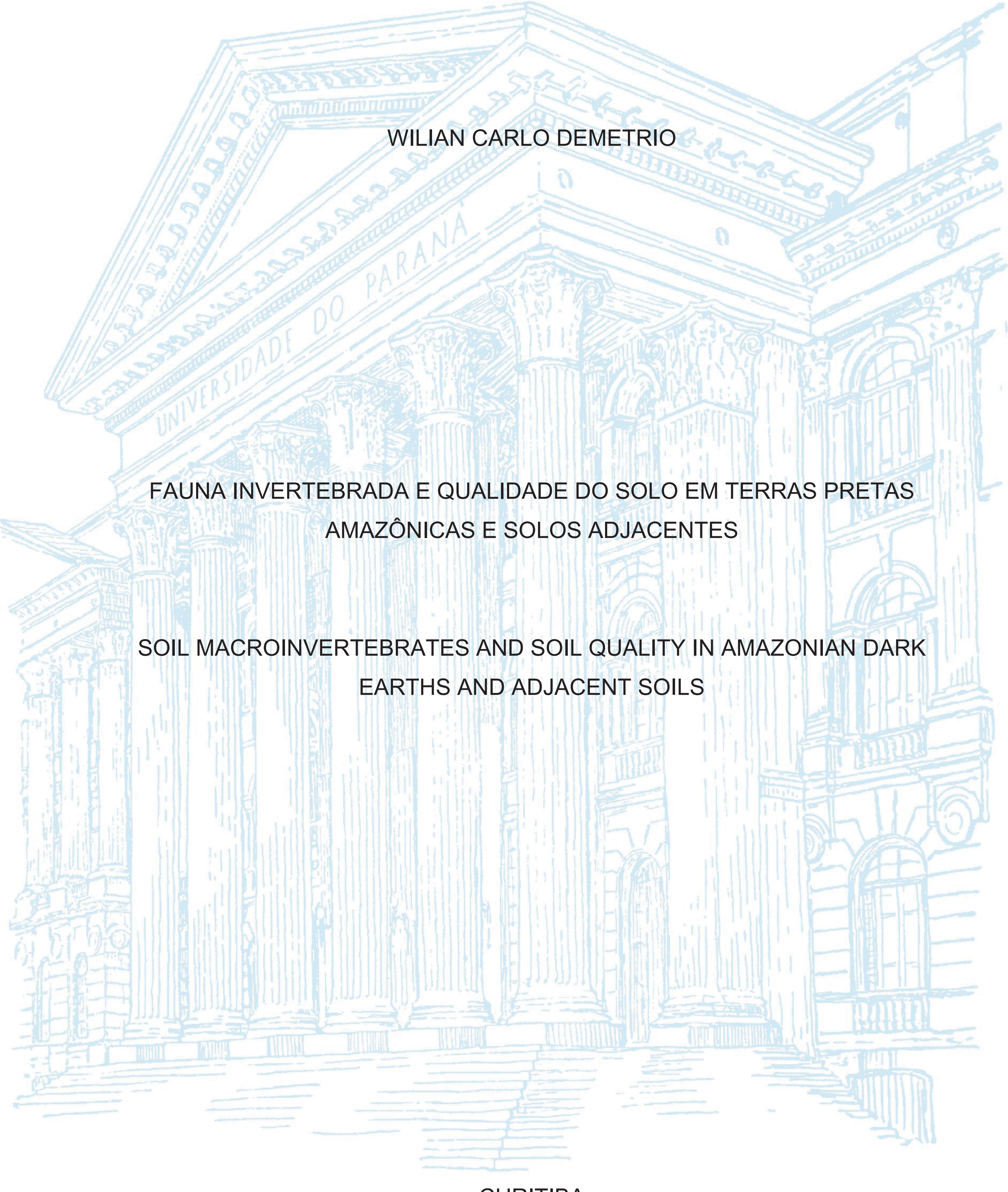

WILIAN CARLO DEMETRIO

FAUNA INVERTEBRADA E QUALIDADE DO SOLO EM TERRAS PRETAS AMAZÔNICAS E SOLOS ADJACENTES

SOIL MACROINVERTEBRATES AND SOIL QUALITY IN AMAZONIAN DARK EARTHS AND ADJACENT SOILS

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Tese apresentada ao curso de Pós-Graduação em Ciência do Solo, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Ciência do Solo.

Orientador: Prof. Dr. George Gardner Brown

Coorientador: Prof. Dr. Luís Cunha

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIAS DO SOLO da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **WILIAN CARLO DEMETRIO** intitulada: **Soil macroinvertebrates and soil quality in Amazonian Dark Earths and adjacent soils**, após terem inquirido o aluno e realizado a avaliação do trabalho, são de parecer pela sua aprovação no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 17 de Abril de 2019.

GEORGE GARDNER BROWN
Presidente da Banca Examinadora ()

RODRIGO DOS SANTOS MACHADO FEITOSA
Avaliador Externo (DZ - UFPR)

PATRICK MARIE LAVELLE
Avaliador Externo (null)

JEFERSON DIECKOW
Avaliador Interno (UFPR)

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“Se cheguei até aqui foi porque me apoiei nos ombros de gigantes”

Isaac Newton

RESUMO

Por pelo menos 10 mil anos, as atividades humanas vêm modificando a floresta amazônica. Os povos pré-Colombianos alteraram profundamente a paisagem Amazônica, construindo um novo habitat neste local com características contrastes aos solos naturais (REF), conhecido como Terra Preta de Índio (TPI). Durante muitos anos estes solos têm captado a atenção da comunidade científica e atualmente diversas características das TPIs, tais como fertilidade, mineralogia e propriedade microbiológicas do solo já foram estudadas, entretanto até o momento estes locais carecem de estudos relacionados a fauna invertebrada do solo que são importantes provedores de serviços ecossistêmicos, fundamentais para o correto funcionamento dos ecossistemas terrestres. O objetivo deste estudo foi avaliar a pegada ecológica dos povos pré-Colombianos nas comunidades de macroinvertebrados em TPIs e os efeitos das alterações antrópicas nas comunidades de invertebrados e na qualidade do solo em TPIs e REF. Foram avaliados 18 locais pareados (9 TPI e 9 REF) em três níveis de perturbação humana: florestas antigas (OF) florestas secundárias em estágio avançado de regeneração (> 20 anos); florestas jovens (YF) florestas secundárias em estágio inicial de regeneração (<20 anos); e sistemas agrícolas (AS), em três estados da Amazônia Central. Foram utilizados métodos padronizados ou bem conhecidos para amostragem de macroinvertebrados de solo, e para análises de atributos químicos e físicos e da macromorfologia do solo. Foram coletados mais de 9.000 macroinvertebrados do solo pertencentes a 667 morfoespécies, principalmente de formigas, besouros e aranhas, mas também uma alta riqueza de cupins, milipéias, hemípteros, baratas e minhocas. A riqueza total de espécies não diferiu entre as TPIs e os solos REF, mas as comunidades eram bem diferentes, havendo uma clara pegada ecológica dos povos pré-Colombianos, onde 43% das espécies foram encontradas exclusivamente em TPIs. Observamos também que a atividade biológica de invertebrados do solo é maior em TPIs quando comparado aos solos REF, indicando mudanças significativas nos serviços ecossistêmicos nos solos antropogênicos. Além disso, alguns invertebrados, como as minhocas, foram mais abundantes em TPIs, indicando que as comunidades destes animais são mais adaptadas à perturbação humana, pois apresentam populações mais elevadas mesmo em campos agrícolas, em comparação com os solos REF, principalmente devido ao maior teor de nutrientes de matéria orgânica nas TPIs. A qualidade do solo nas TPIs foi maior que nos solos REF, e nas OF que nas YF e AS. Adicionalmente, a qualidade do solo nas TPIs foi mais resiliente à mudança no sistema de uso que os solos REF. A agricultura moderna reduziu a biodiversidade do solo tanto nas TPIs quanto nos solos REF, com menor riqueza específica em AS, e maior em OF. Portanto, as TPIs representam um habitat distinto e importante para a biodiversidade do solo na Amazônia, especialmente em OF, e podem servir como refúgios para um alto número de espécies raras/exclusivas, que estão ausentes ou apresentam baixa população nos solos REF. Além disso, a alta qualidade desses solos, e o efeito negative de usos mais intensivos, atenta para a necessidade de manejo adequado e maiores esforços de conservação nas TPIs da Amazônia.

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Palavras-chave: Biologia do solo. TPIs. Macrofauna do solo. Serviços ecossistêmicos. Floresta tropical. Mudança do uso do solo.

ABSTRACT

For at least 10,000 years human activities has been modifying the Amazonian rainforest. Pre-Columbian settlements strongly altered the landscape, building a new habitat in the natural forest contrasting with that of natural soils (REF), known as Amazonia dark earths (ADEs). These soils have captured the attention of the scientific community, and currently several characteristics of ADEs such as it's chemical, mineralogical and microbiological properties are well-known, but little is known of it's soil invertebrate communities, that include important ecosystem service providers, essential to the functioning of soil ecosystem. Therefore, the present study evaluated the ecological footprint of Amerindians on macroinvertebrate communities in ADEs and the effects of modern human disturbance on soil invertebrates and soil quality in ADEs and REF soils. Soil sampling was undertaken in 18 paired sites (9 ADEs and 9 REF), with three levels of human disturbance: old forests (OF) consisting of secondary forests in advanced stage of regeneration (>20 years); young forests (YF) consisting of secondary forests in early stage of regeneration (<20 years); and agricultural systems (AS), located in three Central Amazonian states. Standard or well-known assessment methods were used for soil macroinvertebrate sampling, as well as soil chemical, physical and macro-morphological analyses. Over 9,000 soil invertebrates belonging to 667 morphospecies were found, most of which were ants, beetles and spiders, but also with high richness of termites, millipedes, true bugs, cockroaches and earthworms. Although total species richness was not different in ADEs than REF soils, their communities were very different, and a tenacious pre-Columbian footprint was observed, with 43% of species found exclusively in ADEs. Biological activity was also higher in ADEs compared to REF soils, indicating significant changes in ecosystem services in these anthropogenic soils. Furthermore, some invertebrates such as earthworms were very abundant in ADEs, and their communities were adapted to human disturbance, with higher populations even in agricultural fields compared to REF soils, mainly due to the high nutrient and organic matter contents of the ADEs. Overall soil quality was highest in ADEs than in REF soils and in OF than in YF and AS. The soil quality in ADEs was also more resilient to land-use change that REF soils. Modern agriculture decreased soil biodiversity in both ADE and REF soils, with lowest species richness in AS, and highest in OF. Hence, ADEs represent distinct and important habitats for soil biodiversity in Amazonia, particularly in OF, and may act as refuges for a high number of rare/exclusive soil invertebrate species which are absent or present only in low populations in REF soils. Furthermore, the high quality of these soils, and the negative effects of modern land uses implies the need for proper management and enhanced conservation efforts in ADEs in Amazonia.

Keywords: Soil biology. ADEs. Soil macrofauna. Ecosystem services. Tropical forest. Land-use change.

SUMMARY

1 GENERAL INTRODUCTION

Soil biota play a fundamental role in the terrestrial ecosystems, delivering ecosystem services that are essential for the maintenance of life on earth (LAVELLE et al., 2006). The soil biota includes hundreds of thousands of species ranging from microorganisms (e.g. bacteria and fungi) to large animals such as vertebrates (ORGIAZZI et al., 2016; BROWN et al., 2018). Among these, the soil macroinvertebrates deserve special attention due their ability to affect soil physical properties and processes, regulate microbial communities, and alter organic matter decomposition and nutrient cycling in soils (LAVELLE et al., 1997). Furthermore, soil animals represent more than 25% of all known species on earth (DECAËNS et al., 2006). Moreover, some macroinvertebrates such as earthworms, ants and termites physically modify soil characteristics, affecting the availability of resources to other animals and plants, and have therefore been called “ecosystem engineers” (LAVELLE et al., 1997). These engineers are usually the most representative group of soil macrofauna, due the high abundance of social insects (ants and termites) and the large biomass of earthworms compared to other soil invertebrates (BROWN et al., 2004). However, although crucially important for soil processes, these invertebrates are very sensitive to land-use change and environmental disturbances, meaning that they can be powerful tools to evaluate soil quality and/or health, especially in human-disturbed areas (PAOLETTI, 1999; ROUSSEAU et al., 2013).

Deforestation is one of the major reason for the loss of biodiversity on Earth, especially in Amazonia, one of the largest continuous and relatively well-preserved tracts of tropical forest on the planet, and host to around 10% of the world’s biodiversity (LEWINSOHN; PRADO, 2005). Around 0.5 % of Amazonia is deforested year^{-1}, and much of this area is used for annual cropping and pastures for cattle (INPE, 2018). However, humans have been modifying biodiversity patterns throughout Amazonia for over 10,000 years (ROOSEVELT, 2013). Besides the earthworks (e.g., geoglyphs) and archaeological sites of pre-Columbian settlements widespread over the Amazonia basin (WATLING et al., 2017), Amerindians also built high fertility soils commonly called Amazonian dark earths (ADEs) or Terra preta de Índio (CLEMENT et al., 2015; MCMICHAEL et al., 2014; WATLING et al., 2018). These soils have high contents of Ca, Mg, P and black carbon converting them into a highly contrasting environment compared to natural low fertility Amazonian soils

(LEHMANN et al., 2003). Additionally, the agricultural practices of pre-Columbian people also modified biodiversity in ADEs, promoting the occurrence of useful plants (e.g., manioc, brazil nut, papaya, guava), and generating a distinct signature of soil microbial communities (GROSSMAN et al., 2010; LEVIS et al., 2018). However, their soil invertebrate communities are virtually unknown (CUNHA et al., 2016).

Although ADEs are archaeological sites protected by national laws (e.g., BRAZIL, 1961), these areas have been extensively used for agricultural proposes (JUNQUEIRA; SHEPARD; CLEMENT, 2010), raising concerns about the effects of modern agricultural practices on soil quality and biodiversity in these anthropogenic soils. It is well known that land use change in Amazonia strongly affects the belowground biota (FRANCO et al., 2018), extinguishing native species and allowing the invasion and colonization of exotic/opportunist invertebrates (e.g., BARROS et al., 2004), and potentially modifying soil processes and ecosystem services in Amazonia rainforest (DECAËNS et al., 2018; LAVELLE et al., 2016). However, soil invertebrate communities have only been studied in non-anthropic Amazonian soils, and nothing is known of the impacts of land use on soil quality and on their macrofauna populations in ADEs.

Therefore, the present study was undertaken, to evaluate the ecological footprint of pre-Columbian people on soil macroinvertebrate communities in Central Amazonia and assess the impact of land-use on macrofauna communities, with a particular emphasis on earthworms, and other soil quality indicators in ADEs and non-anthropic Amazonian soils. The work was undertaken with the financial support of various bilateral cooperation projects (Brazil-UK, Brazil-USA), and had the contribution of a large number of researchers, students and institutions from Brazil and abroad, and was part of the activities of the Terra Preta de Índio Network (TPINetwork; tpinet.org).

2 CHAPTER I: A “DIRTY” FOOTPRINT: ANTHROPOGENIC SOILS PROMOTE BIODIVERSITY IN AMAZONIAN RAINFORESTS

2.1 RESUMO

As florestas tropicais da Amazônia que se pensavam serem intocadas e selvagens, são cada vez mais conhecidas por terem sido densamente habitadas por populações que mostram uma cultura diversificada e complexa antes da chegada dos europeus. Ainda não é claro até que ponto essas sociedades impactaram e modificaram a paisagem. As Terras Pretas de Índio (TPIs) são solos férteis encontrados em toda a Bacia Amazônica, criados pelas sociedades pré-colombianas como resultado de hábitos sedentários. Muito se sabe da química desses solos, mas sua zoologia foi negligenciada. Sendo assim, caracterizamos comunidades de macroinvertebrados do solo e atividade nesses solos em nove sítios arqueológicos em três regiões amazônicas. Encontramos 667 morfoespécies e uma tenaz pegada pré-colombiana, com 43% das espécies encontradas exclusivamente em TPIs. A atividade biológica do solo é maior nas TPIs quando comparados aos solos de adjacentes, e está associada a maior biomassa e riqueza de organismos conhecidos pela sua alta capacidade de bioturbação. Os resultados também demonstram que as TPIs têm um conjunto único de espécies, no entanto, as mudanças no uso da terradas TPIs reduz da fertilidade e ameaça a biodiversidade nestes locais. Essas descobertas apoiam a ideia de que os seres humanos construíram e sustentaram um sistema fértil de alto contraste que persistiu até os nossos dias e alterou irreversivelmente os padrões de biodiversidade na Amazônia.

Palavras-chave: Invertebrados do solo. Biodiversidade do solo. Terra preta de índio. Engenheiros do ecossistema.

2.2 ABSTRACT

Amazonian rainforests once thought to hold an innate pristine wilderness, are increasingly known to have been densely inhabited by populations showing a diverse and complex cultural background prior to European arrival. To what extent these societies impacted their landscape is unclear. Amazonian Dark Earths (ADEs) are fertile soils found throughout the Amazon Basin, created by pre-Columbian societies as a result of more sedentary habits. Much is known of the chemistry of these soils, yet their zoology, have been neglected. Hence, we characterised soil macroinvertebrate communities and activity in these soils at nine archaeological sites in three Amazonian regions. We found 667 morphospecies and a tenacious pre-Columbian footprint, with 43% of species found exclusively in ADEs. The soil biological activity is higher in the ADEs when compared to adjacent reference soils, and it is associated with higher biomass and richness of organisms known to engineer the ecosystem. We show that these habitats have a unique pool of species, however, the contemporary land-use in ADEs drives nutrient decay and threats biodiversity. These findings support the idea that Humans have built and sustained a contrasting high fertile system that persisted until our days and irreversibly altered the biodiversity patterns in Amazonia.

Keywords: Soil invertebrates. Belowground biodiversity. Amazonian dark earths. Ecosystem engineers.

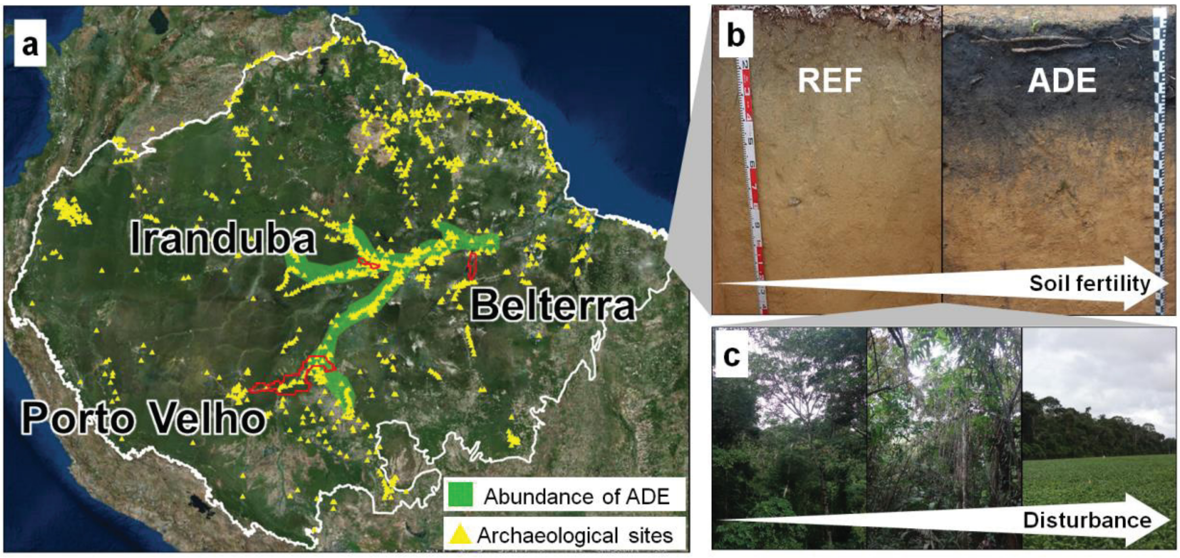
2.3 INTRODUCTION

The Amazon basin contains the largest continuous and relatively well-preserved tract of tropical forest on the planet. Although deforestation rates in Amazonia have been showing a generally decreasing trend over the last decade, human activities in the region were still responsible for losses of 7,900 km^2 of its natural vegetation in 2018 alone (INPE, 2018). Many forested areas have become highly fragmented, and may be reaching tipping points where biodiversity and ecosystem functions may be dramatically affected (BARKHORDARIAN et al., 2018; DECAËNS et al., 2018), potentially leading to cascading effects that impact ecosystem services over a much larger area (LATHUILLIÈRE et al., 2018; LAWRENCE; VANDECAR, 2015).

Humans have modified Amazonian biodiversity patterns over millennia, and Amerindians created areas with high concentrations of useful trees and hyperdominance of some species, often associated with archaeological sites (LEVIS et al., 2018) (Fig. 1a). Furthermore, occupations of some indigenous societies', beginning at least 6,500 years ago, created fertile anthropogenic soils, locally called "Terra Preta de Índio" (TPI) or Amazonian Dark Earths – ADEs (CLEMENT et al., 2015; MCMICHAEL et al., 2014; WATLING et al., 2018) (Fig. 1b). The ADEs may occupy up to 3% of the surface area of Amazonia (MCMICHAEL et al., 2014), and appear to be more common along major rivers (Fig. 1a), but are also abundant in interfluvial areas (CLEMENT et al., 2015). ADE sites tend to have high soil P, Ca and pyrogenic C contents (GLASER; BIRK, 2012; LIMA et al., 2002; SOMBROEK et al., 2004), and particular communities of plants and soil microorganisms (BROSSI et al., 2014; TAKETANI et al., 2013), but up to now, soil animal communities in these historic anthropogenic soils were not previously known.

Soil macroinvertebrates represent as much as 25% of all known described species (DECAËNS et al., 2006), and are a huge source of biodiversity that may easily surpass 1 million species (BROWN et al., 2018). However, soil animal communities have been little studied in megadiverse regions, such as the Amazonian rainforest (BARROS et al., 2006; FRANCO et al., 2018), and these habitats may be home to thousands of species (BROWN et al., 2006; MATHIEU, 2004), particularly smaller invertebrates such as nematodes and mites (FRANKLIN; MORAIS, 2006; HUANG; CARES, 2006), but also of macroinvertebrates.

FIGURE 1 - SAMPLING STRATEGY TO ASSESS SOIL FAUNA AND SOIL FERTILITY IN CENTRAL (IRANDUBA), SOUTHWESTERN (PORTO VELHO) AND LOWER (BELTERRA) AMAZON. (A) BOUNDARY OF AMAZON BASIN (WHITE LINE), BOUNDARIES OF MUNICIPALITIES WHERE SAMPLES WERE TAKEN (RED LINES), ARCHAEOLOGICAL SITES (YELLOW TRIANGLES), AND AREAS WITH HIGH CONCENTRATION OF AMAZONIAN DARK EARTHS (ADE, SHADED IN GREEN) AT ARCHAEOLOGICAL SITES. ARCHAEOLOGICAL AND ADE SITES MODIFIED FROM Clement et al. (2015) AMAZONIA MAP BACKGROUND: ESRI, DIGITALGLOBE, GEOEYE, EARTHSTAR GEOGRAPHICS, CNES/AIRBUS DS, USDA, USGS, AEX, GETMAPPING, AEROGRID, IGN, IGP, SWISSTOPO, AND THE GIS USER COMMUNITY. (B) SOIL PROFILES OF ANALYTICALLY PAIRED ADE AND NEARBY REFERENCE (REF) SOILS; PHOTOS G.C. MARTINS, R. MACEDO. (C) LAND USE SYSTEMS (LUS) SAMPLED IN EACH REGION, CONSISTING IN AN INTENSIFICATION/DISTURBANCE GRADIENT INCLUDING OLD SECONDARY RAINFOREST (>20 yrs. UNDISTURBED), YOUNG SECONDARY FOREST (<20 YRS. OLD), AND RECENT AGRICULTURAL SYSTEMS (PASTURE, SOYBEAN, MAIZE); PHOTOS G.C. MARTINS, M. BARTZ.



Hence, the aim of this study was to assess soil invertebrate macrofauna communities and their activity in ADEs at nine archaeological sites and adjacent reference soils (REF) under three land-use systems (LUS: old and young secondary forest and recent agricultural/pastoral systems), in order to evaluate anthropic effects on Amazonian soil biodiversity. We predicted that 1) soil biodiversity composition and soil enrichment in anthropogenic soils would reflect a pre-Colombian footprint but also, that 2) animal richness, biomass, activity, and nutrient contents in these soils would be determined by present-day land-use.

2.4 MATERIAL AND METHODS

2.4.1 STUDY SITES

The municipalities of Iranduba (IR) in Central Amazon, Belterra (BT) in Lower Amazon and Porto Velho (PV) in Southwestern Amazon, were chosen for this study (Fig. 1a). All sites have a tropical monsoon climate (Köppen's Am), with a mean annual temperature of 24 ºC and precipitation between 2,000 and 2,280 mm year^{-1} (ALVARES et al., 2014). In each region, paired sites with ADEs and nearby reference (REF) non-anthropogenic soils (Fig. 1b) were selected under different LUS (Fig. 1c): native secondary vegetation (dense ombrophilous forest) classified as old forest (OF) when >20 years old, or young forest (YF) when <20 years old, and agricultural systems (AS) of maize in IR, soybean in BT, and introduced pasture in PV. The REF sites were within a minimum distance of 150 m (soybean at BT) to a maximum distance of 1.3 km (pasture at PV) from the ADE sites, and maximum distance between paired sites within a region was 14 km (Embrapa sites to Tapajós National Forest sites in BT).

One of the OF in BT was at the Embrapa Amazônia Oriental Belterra Experiment Station, while the other one was at the Tapajós National Forest, a site of previous work on ADEs (MAEZUMI et al., 2018a). Both OFs at IR were at the Embrapa Amazônia Ocidental Caldeirão Experiment Station, and have been extensively studied in the past for soil fertility and pedogenesis (ALHO et al., 2019; MACEDO et al., 2017), as well as microbial diversity (GROSSMAN et al., 2010; O'NEILL et al., 2009). ADE formation in IR was estimated to have begun ~1,050 - 950 years BP (NEVES et al., 2004) and at BT ~530-450 years BP (MAEZUMI et al., 2018b). At PV, ADE formation began much earlier (~6500 years BP) (WATLING et al., 2018).

The AS fields with annual crops were under continuous (at least 6 years) annual row cropping of maize (IR) and soybean (BT) and had been planted < 60 d prior to sampling, using conventional tillage (IR), or reduced tillage (BT). The crops received the recommended doses of inorganic fertilizers and pest management practices for each crop, which was planted using certified commercial seeds. The pastures at PV were around 9 (REF) and 12 yr old (ADE) and planted with *Brachiaria* (REF) and *Paspalum* (ADE) grasses. Soils at most REF sites were classified according to FAO (IUSS WORKING GROUP WRB, 2015) as dystrophic Ferralsols and Acrisols (Supplementary Table 8), the two most common soil types in Amazonia

(FAO/UNESCO (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS), 1992). At one YF site in PV, both ADE and REF soils were overlying a plinthic horizon and the REF soil was classified as a Plinthosol. All ADEs were classified as Pretic Clayic Anthrosols. with dark organic matter-rich surface soil horizons, generally >20 cm deep. All soils had greater than 50% clay and had either clay or heavy clayey texture. General details on the sampling sites chosen are provided in Supplementary Table 1.

2.4.2 SOIL MACROINVERTEBRATE SAMPLING

We performed field sampling in April (IR) and May (BT) of 2015, and in late February/early March of 2016 (PV), at the end of the main rainy season, which is the best time to collect soil macroinvertebrates (SWIFT; BIGNELL, 2001). Soil and litter macrofauna were collected using the standard method recommended by the Tropical Soil Biology and Fertility (TSBF) Program of the United Nations Educational, Scientific and Cultural Organization (UNESCO) (ANDERSON; INGRAM, 1993), also considered by the International Organization for Standardization (ISO) as the appropriate method for evaluating soil macrofauna populations in the tropics (ISO, 2017). At each sampling site, five sampling points were located within a 1 ha plot, at the corners and the centre of a 60 x 60m square, resulting in an “X” shaped sampling design (Supplementary Fig. 1). At each of these points, a soil monolith (25 x 25 cm up to 30 cm depth) was initially delimited with a 10 cm deep steel template, and then divided into surface litter and three 10 cm-thick layers (0-10, 10-20, 20-30 cm). Macroinvertebrates (i.e., invertebrates with > 2mm body width) were collected in the field by hand-sorting both the soil and litter, and were immediately fixed in 92% ethanol. Collected invertebrates were identified to species or genus level (earthworms, ants, termites), or sorted into morphospecies considering external morphological characteristics (e.g., antenna, mouthparts, body format) with higher taxonomic level assignations (e.g., order and/or family) for other groups.

2.4.3 ADDITIONAL SAMPLES FOR ECOSYSTEM ENGINEERS

We performed additional sampling for ecosystem engineers (earthworms, termites and ants), in order to better estimate their species richness, especially in forest sites where higher diversity is normally expected. Earthworms were collected at four additional cardinal points of the grid (Supplementary Fig. 1), hand-sorted from holes of similar dimensions as the TSBF monoliths, and preserved in 96% ethanol. Termites were sampled in five 10 m2 (2 x 5 m) plots (Supplementary Fig. 1) by manually digging the soil and looking for termitaria in the soil, as well as in the litter and on trees using a modification of the transect method (JONES; EGGLETON, 2000). The termite samples were taken in all OF and YF (except one of the REF YF at PV), but not in the agricultural fields (maize, soybean and pasture), as these tend to have very few termite colonies. Ants were sampled in 10 pitfall traps (300 ml plastic cups) set up as two 5-trap transects on the sides of each 1 ha plot (Supplementary Fig. 1), as well as in two traps to the side of each TSBF monolith (distant ~5 m). Each cup was filled to a third of its volume with water, salt and detergent solution. The pitfall traps remained in the field for 48h. Pitfall traps were set up in only in the forest systems of IR and BT (not at PV). Termites and ants were preserved in 80% ethanol and the alcohol changed after cleaning the samples within 24 h. All the animals (earthworms, ants, termites) were identified to species level or morphospecies level (with genus assignations) by Samuel James/Marie Bartz (earthworms), Agno Acioli (termites) and Alexandre Ferreira/Rodrigo Feitosa (ants).

2.4.4 SOIL PHYSICAL AND CHEMICAL ATTRIBUTES

After hand-sorting the soil from each TSBF monolith, 2 to 3 kg samples were collected from each depth (0-10, 10-20, 20-30 cm) for chemical and soil particle size analysis, and while analysed separately, mean values were calculated over 0-30 cm depth. The following soil properties were assessed following standard methodologies: pH ($CaCl_2$); Ca^{2+}, Mg^{2+}, Al^{3+} (KCl 1 mol L^{-1}); K^+ and P (Mehlich-1); total nitrogen (TN) and carbon (TC) using an element analyser (CNHS) (TEIXEIRA et al., 2017). Soil texture was obtained using the FAO soil texture triangle (IUSS WORKING GROUP WRB, 2015), and base saturation and cation exchange capacity (CEC) were calculated using standard formulae (TEIXEIRA et al., 2017).

In order to assess functional differences induced by soil fauna activity in the ADE and REF soils, soil macromorphology samples were taken 2 m from each monolith (Supplementary Fig. 1) using a 10 × 10 × 10 cm metal frame. The collected material was separated into different fractions including: living invertebrates, litter, roots, pebbles, pottery shards, charcoal (biochar), non-aggregated/loose soil, physical aggregates, root-associated aggregates, and fauna-produced aggregates using the method of Velásquez et al. (VELASQUEZ et al., 2007). Each fraction was oven dried at 60°C for 24h and weighed. This method allows estimating the relative contribution of soil macrofauna, roots and soil physical processes to soil macroaggregation (VELASQUEZ et al., 2007) and structure, which determines the delivery of several important soil-based ecosystem services (ADHIKARI; HARTEMINK, 2016).

2.4.5 TREATMENT OF SOIL FAUNA DATA

Density (number of individuals) and biomass of the soil macrofauna surveyed using the TSBF method were extrapolated per square meter considering all depths evaluated. Density and biomass of immature forms of insects (nymphs and larvae) were grouped in the respective taxonomic group. The following taxonomic groups, representing 2% or less of total density were grouped as “Others”: Araneae, Hemiptera, Orthoptera, Diptera (larvae), Gastropoda, Dermaptera, Isopoda, Blattaria, Scorpionida, Opiliones, Lepidoptera (larvae), Uropygi, Solifuga, Thysanoptera, Geoplanidae, Neuroptera (larvae), Hirudinea and Embioptera. To calculate the beta (β) diversity index we removed singleton species (species represented by single individuals, i.e., one individual among all the 9,380 individuals collected).

2.4.6 STATISTICAL ANALYSES

To compare species diversity between ADE and REF, we plotted rarefaction and extrapolation curves using the iNEXT (HSIEH; MA; CHAO, 2018) package for total macroinvertebrate, ant, termite and earthworm species diversity, using the number of TSBF monolith samples as a measure of sampling effort intensity. The same procedure was used for all earthworm data (9 samples per site), termite data obtained from both the 10-m^2 plots and TSBF monoliths, and ant data obtained from both pitfall traps and TSBF monoliths.

We used the betapart package (BASELGA; ORME, 2012) in R to decompose β-diversity (calculated using the Sørensen dissimilarity index) into its Turnover (Simpson index of dissimilarity) and Nestedness components using all soil+litter macroinvertebrate, ant, termite and earthworm data from monolith samples. The average β-diversity was calculated to highlight LUS effect, by comparing all LUS (OF, YF and AS) within each soil type (REF and ADE) and region. The soil type effect was assessed comparing the diversity between REF and ADE soils within each LUS in each region. To identify the effect of geographical distance on species turnover we also calculated the average β-diversity among the three replicates of each LUS within each soil type.

Due to non-normal distribution of both the faunal variables (i.e., density and biomass of invertebrates collected using the TSBF method) and soil properties, we used General Linear Models (GLM) to adjust data to other probability distributions. The best adjustment was quasi-Poisson (overdispersion) and Gamma for invertebrate density and biomass, respectively. Soil chemical properties were adjusted in Gamma distribution but particle size fractions could not be adjusted. ANOVA tests were performed with the multcomp package (HOTHORN; BRETZ; WESTFALL, 2008) of R, adopting a factorial design with the following factors: soil type (ADE and REF) and LUS (old forests, young forests and agricultural systems). When factor interactions were significant ($P<0.05$), the data were analysed comparing the effects of soil type within the LUS and the effects of LUS within each soil type. Significant differences were tested using Tukey's test at 95% probability ($P<0.05$) for GLM, or with non-parametric Kruskal-Wallis tests when data could not be adjusted with GLM.

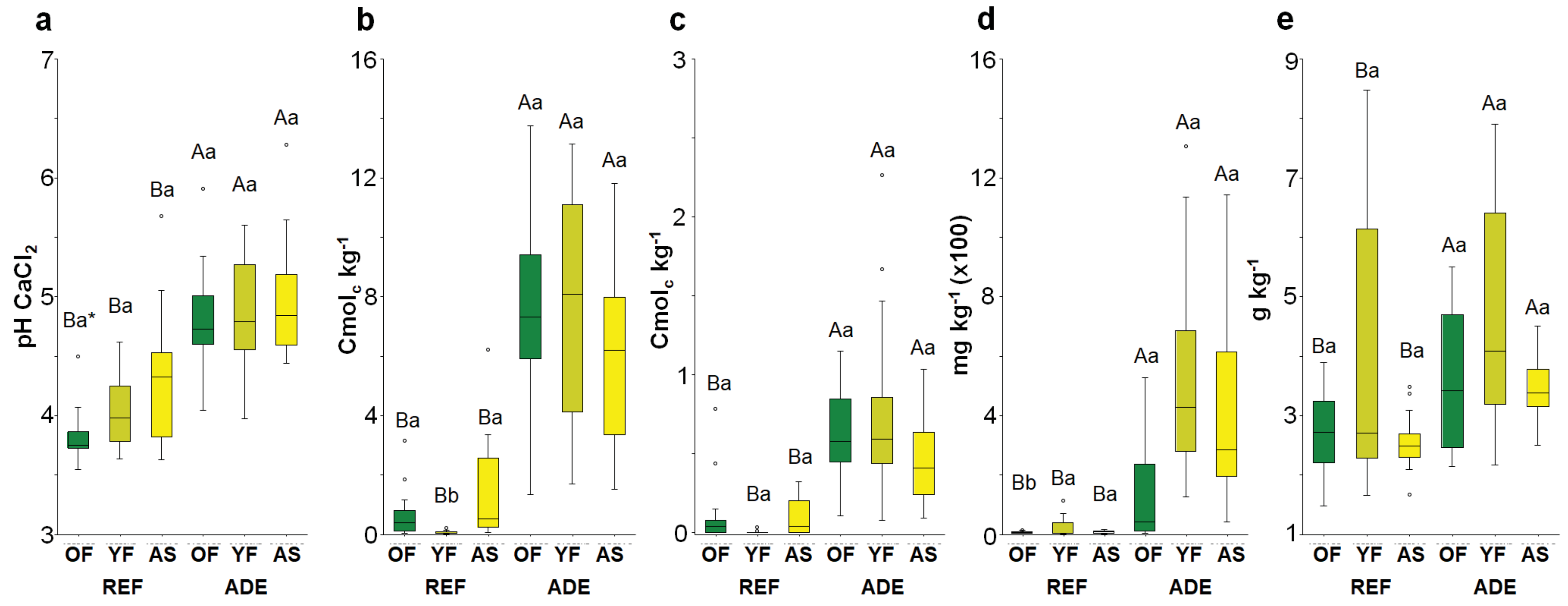
A Principal Component Analysis (PCA) was performed using the density of earthworms, termites, ants and overall (total) soil fauna density and biomass, together with the results of five variables from soil micromorphology (non-aggregated soil, pottery shards and fauna, root and physical aggregates) and ten variables from soil chemical and textural analyses (pH, Al^{3+}, P, SB, T, TC, TN, and sand, silt and clay fractions). The significance of the PCA model (soil type and LUS) was assessed using Monte Carlo test permutations ($P<0.05$), using the ADE-4 package (DRAY; DUFOUR, 2007) for R.

2.5 RESULTS

2.5.1 ADES ARE DISTINCT SOILS WITH DISTINCTIVE MACROINVERTEBRATE COMMUNITIES

The ADEs at all the sites had higher soil pH and were enriched in Ca, Mg, P and total C compared to REF soils within each LUS (Fig. 2), following trends typically observed in ADE sites throughout Amazonia (LEHMANN et al., 2003; SOMBROEK et al., 2004). Significantly lower amounts of exchangeable Al were also found in the ADEs (Supplementary Table 2). Soil texture at the sites was similar in both ADE and REF soils (Supplementary Table 2), so the enrichment was not due to differential clay contents, but the result of ancient anthropogenic activities (LEHMANN et al., 2003; SMITH, 1980). Some differences in soil fertility among land-use systems were also observed (Supplementary Table 2), where plots under agricultural or pastoral use (AS) had higher K contents (due to fertilization) than old forest (OF) and lower N contents, probably due to soil erosion processes, denitrification, and leaching (BUSTAMANTE; KELLER; SILVA, 2009; LUIZÃO et al., 2009).

FIGURE 2 - SOIL CHEMICAL PROPERTIES AT THE COLLECTION SITES IN AMAZONIA: (A) PH, (B) EXCHANGEABLE CA, (C) EXCHANGEABLE MG, (D) AVAILABLE P, AND (E) TOTAL CARBON IN THE TOPSOIL LAYER (0-30 CM DEPTH; MEAN VALUES FOR THE THREE REGIONS) SHOWING DIFFERENCES DUE TO SOIL TYPE (REF VS. ADE SOILS) IN EACH OF THE LAND-USE SYSTEMS (OF, YF, AS). *DIFFERENT UPPER-CASE LETTERS INDICATE SIGNIFICANT DIFFERENCES (*P*< 0.05) AMONG SOIL CATEGORIES WITHIN EACH LAND-USE SYSTEM, WHILE DIFFERENT LOWER-CASE LETTERS INDICATE SIGNIFICANT DIFFERENCES AMONG LAND USE SYSTEMS WITHIN THE SAME SOIL TYPE. ADE: AMAZONIAN DARK EARTH; REF: REFERENCE SOILS; OF: OLD FORESTS; YF: YOUNG FORESTS; AS: AGRICULTURAL SYSTEMS. VALUES SHOWN ARE MEDIAN (BLACK LINE), 1ST AND 3RD QUARTILES (BOX) AND MAX/MIN OBSERVATIONS (UPPER AND LOWER LINES) AND THE OUTLIERS (SMALL OPEN CIRCLES), WHEN PRESENT.

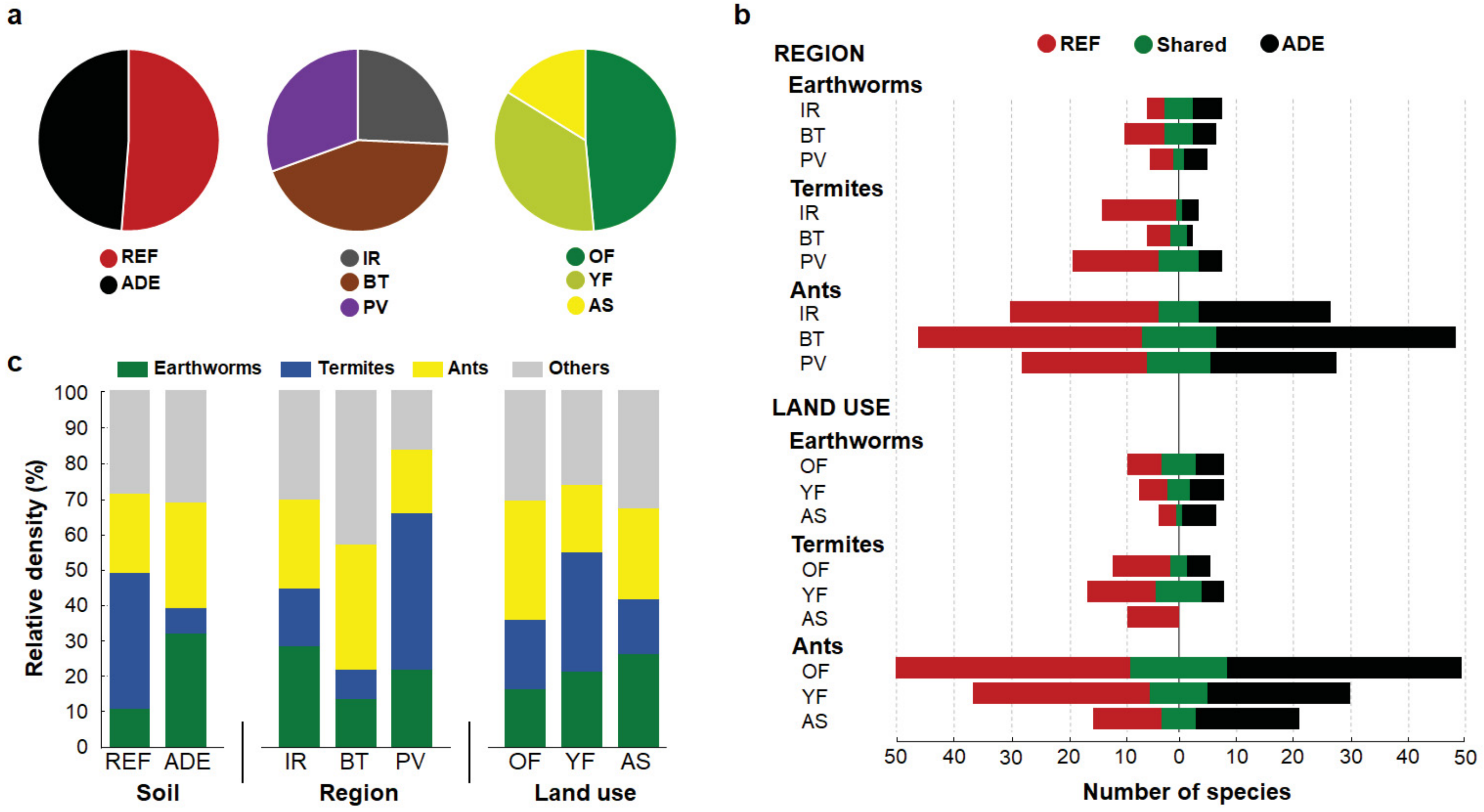


We collected 9,380 macroinvertebrates in soil monoliths, of 667 different morphospecies, belonging to 24 higher taxa (Fig. 3a; Supplementary Table 3). Ants (Formicidae) were the most diverse group collected (154 spp.), followed by spiders (86 spp.), beetles (78 spp.), millipedes (53 spp.), true bugs (42 spp.), termites (37 spp.), cockroaches (34 spp.), and earthworms (32 spp.) (Supplementary Table 2). The number of singleton species (one individual in the total sample of 9,380) was very high (328 spp.), representing around 49% of the total macroinvertebrate richness (Supplementary Table 4).

Similar numbers of species were found in ADEs (382 spp.) and REF (399 spp.) soils. The proportion of unique morphospecies was high in both soils: 48.5% in ADEs and 51.5% in REF soils (Fig. 3a; Supplementary Fig. 2), particularly for ants (75 spp. ADE, 70 spp. REF) and earthworms (22 spp. ADE, 20 spp. REF) (Fig. 3b; Supplementary Figs 3-5). Termites had a high number of unique species in REF soils (21 spp.; see Fig. 3b). These trends for ants, earthworms and termites remained similar even after singleton species were removed. Centipede and Opiliones richness was also high in REF soils (14 and 14 spp., respectively), while millipede and snail richness (37 spp. and 12 spp., respectively) was high in ADEs (Supplementary Table 2), possibly due to the higher soil Ca levels (COLEMAN; CROSSLEY; HENDRIX, 2004). The high number of species unique to each soil (Fig. 3a) was reflected in high β-diversity values and species turnover, ranging from 67-79% for all of the soil macroinvertebrates (Supplementary Table 6). Furthermore, among the ecosystem engineers collected, we found an important number of species new to science (>20 earthworm species, >20 termite species and >30 ant species) that still need to be described.

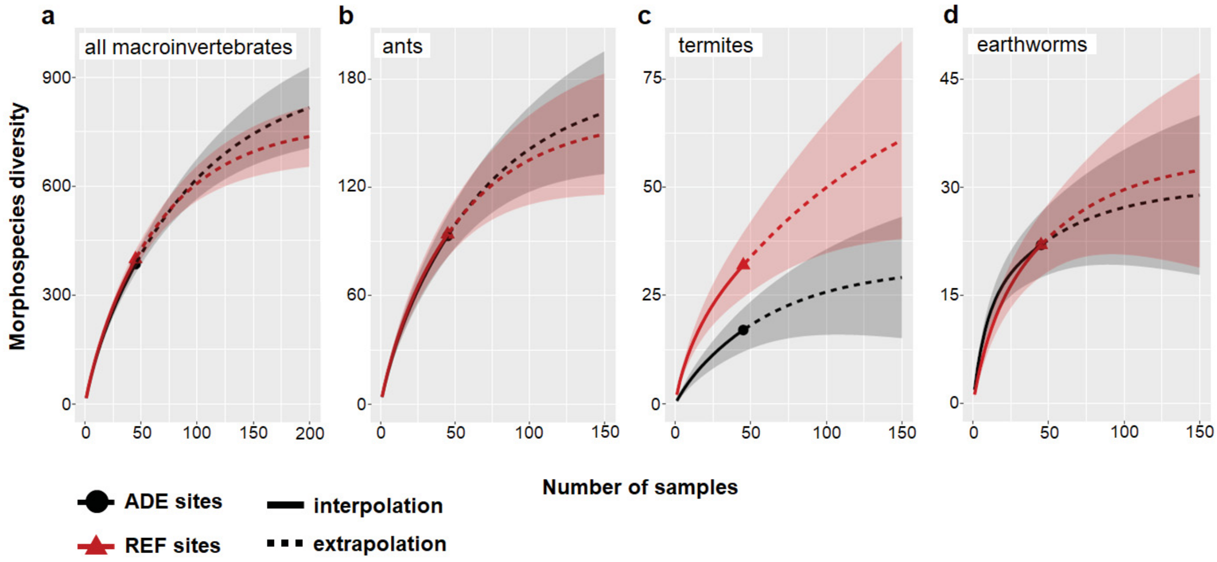
ADEs were home to 95 rare (doubleton and rare individuals) and to 18 non-rare or abundant macroinvertebrate morphospecies not found in REF soils (Supplementary Table 4). Interestingly, within the non-rare/abundant taxa, 19 species (mainly ant and earthworm species) had greater abundance of individuals in ADEs, while 13 species (mainly ant species) were more prevalent in REF soils (Supplementary Table 4).

FIGURE 3. - MORPHOSPECIES DIVERSITY AND ABUNDANCE PATTERNS IN SOIL COMMUNITIES AT COLLECTION SITES IN AMAZONIA: (A) DISTRIBUTION OF UNIQUE MORPHOSPECIES (INCLUDING SINGLETONS) OF ALL MACROINVERTEBRATES ACCORDING TO SOIL TYPE (ADE, REF), REGION (IR, BT, PV) AND LAND USE SYSTEMS (OF, YF, AS). (B) NUMBERS OF MORPHOSPECIES OF EARTHWORMS, TERMITES AND ANTS OBSERVED IN BOTH SOIL CATEGORIES (GREEN BARS) OR UNIQUELY IN ADE (BLACK BARS) OR IN REF (RED BARS) SOILS, IN THE DIFFERENT REGIONS AND LAND USE SYSTEMS ACROSS REGIONS. (C) RELATIVE DENSITY (%) OF EARTHWORMS, TERMITES, ANTS AND OTHER SOIL MACROINVERTEBRATES (SUM OF ALL OTHER TAXA) FOUND IN THE DIFFERENT SOIL CATEGORIES (ADE VS. REF), REGIONS, AND LAND USE SYSTEMS; ADE: AMAZONIAN DARK EARTH; REF: REFERENCE SOILS; IR: IRANDUBA; BT: BELTERRA; PV: PORTO VELHO; OF: OLD FORESTS; YF: YOUNG FORESTS; AS: AGRICULTURAL SYSTEMS.



Estimated richness for total macroinvertebrates, ants and earthworms (Fig. 4a, b, d) was not different between REF and ADE soils but for termites was two-times higher in REF soils (Fig. 4c). These results were confirmed with the more intensive sampling effort performed for ants, termites, and earthworms (Supplementary Fig. 6). The monolith samples' collected around 65-75% of the estimated richness of total soil macroinvertebrates and ants in both soil types and of termites in REF soils (Supplementary Fig. 7 a, b, c). Earthworm richness in both soil categories and termite species in ADEs were relatively well sampled by the monoliths, which collected 70-80% of the estimated total diversity (Supplementary Fig. 7c, d). The use of complementary sampling methods increased the number of collected species for ants in both soils and for termites in REF soils (Supplementary Fig. 6a, b), revealing an important un-sampled species pool of these soil engineers (particularly of ants) in the forests of each region, especially in REF soils.

FIGURE 4. MORPHOSPECIES RAREFACTION AND EXTRAPOLATION CURVES, SHOWING HOW MORPHOSPECIES QUANTITIES INCREASE IN BOTH ADE AND REF SOILS DEPENDING ON SAMPLING INTENSITY (NUMBER OF SAMPLES) FOR: (A) ALL SOIL MACROINVERTEBRATES, (B) ANTS, (C) TERMITES AND (D) EARTHWORMS. DATA CORRESPOND TO INVERTEBRATES COLLECTED IN SOIL MONOLITHS FROM ALL SITES AND LAND USE SYSTEMS. DARK GREY AND RED AREAS REPRESENT 95% CONFIDENCE INTERVALS. ADE: AMAZONIAN DARK EARTH; REF: REFERENCE SOIL.



Land-use effects on species turnover rates were slightly higher for all soil macroinvertebrates (0.79 and 0.74 within REF and ADEs, respectively) than for soil type comparisons (0.70, 0.67 and 0.71 for OF, YF and AS, respectively), indicating that species turnover was more closely related to LUS than to soils (Supplementary Table 6). Similar results were observed for earthworms, with much higher turnover rates (0.84 and 0.62 within REF and ADEs, respectively) due to LUS than due to soil, particularly in OF and YF. Conversely, soil type had a greater impact on ant and termite species turnovers than land-use (0.78 for ants and 0.72 for termites in OF). The species turnover among regions was also very high, mainly for overall macroinvertebrates and earthworms in AS (Supplementary Table 7).

2.5.2 ECOSYSTEM ENGINEERS DOMINATE THE SOIL FAUNA COMMUNITIES

Ecosystem engineers (termites, ants and earthworms) (LAVELLE et al., 1997) represented on average 72% and 69% of the soil macroinvertebrate individuals in ADE and REF soils, respectively (Fig. 3c). The proportion of ecosystem engineers was significantly higher in PV than IR and BT, mainly due to the higher proportion of termites in PV (Fig. 3c). Ecosystem engineers represented 62 to 75% of total invertebrate biomass in the different LUS and soil categories, and was not significantly different between ADE and REF soils (Supplementary Table 5). Termite populations were significantly higher in REF soils with populations over 1000 individuals m^{-2}, while earthworms, ants, and other invertebrates were proportionally more prevalent in ADE (Fig. 3c; Supplementary Table 5). Ants were proportionally more abundant at BT, and termites in IR and PV (Fig. 3c). In biomass, earthworms represented from 44% (AS, REF) to 92% (AS, ADE) of the total macroinvertebrate biomass, and their abundance and biomass were significantly higher in ADE (particularly in YF and AS) than in REF soils (Supplementary Table 5). No other soil animal represented more than 35% of the biomass in any given soil type or LUS.

2.5.3 MODERN LAND USE ERODES SOIL BIODIVERSITY

A total of 349, 278, and 152 morphospecies of macroinvertebrates were found in OF, YF and AS, respectively, of which 249, 181, and 83 species were unique to the respective LUS (Fig. 3a). Removing singleton species, morphospecies richness was 137 (OF), 98 (YF) and 47 (AS) in ADE, and 122 (OF), 102 (YF) and 54 (AS) in REF soils. Hence, richness was 56% and 46% lower in modern AS compared with OF and YF, respectively. This trend was also observed for most of the groups of soil animals, and was particularly marked (>60% decrease in spp. richness) for opilionids, centipedes, isopods and cockroaches in both REF and ADEs, and for earthworms in REF and termites in ADEs (Supplementary Table 3). Species richness decreases in AS compared to OF were slightly (but not significantly) higher for ADE (66%) and REF (56%) soils.

2.5.4 SOIL BIOTA INFLUENCE ADE SOIL STRUCTURE

Soil macromorphology revealed a significantly higher proportion of fauna-produced aggregates (Fig. 5) in ADE soils compared with REF soils, and likewise, in the same LUS, a lower proportion of non-aggregated soil (Supplementary Table 8) in ADEs than REF soils, implying important changes in soil structure in ADEs. Fauna-produced aggregates were also more abundant in OF compared to YF and AS systems (Fig. 5), which tended to have higher proportions of loose, non-aggregated soil and physical aggregates (Supplementary Table 8). The proportions of other aggregate fractions were not affected by soil type and LUS (Supplementary Table 5).

Multivariate analysis (PCA) confirmed the importance of soil fertility associated with ADE (nutrient contents aligned with x-axis) and REF soils as a regulator mainly of earthworm and termite abundance, and land use disturbance or intensification (LUS aligned with y-axis) as a regulator of ant and overall soil fauna abundance and biodiversity (Fig. 6).

FIGURE 5. PROPORTION OF FAUNA-PRODUCED AGGREGATES IN TOP-SOIL (0-10 CM LAYER) IN TWO DIFFERENT AMAZONIAN SOILS (REF: NON-ANTHROPOGENIC REFERENCE SOILS; ADE: FROM AMAZONIAN DARK EARTH) AND THREE DIFFERENT LAND USE SYSTEMS (OF: OLD FORESTS, YF: YOUNG FORESTS, AS: AGRICULTURAL SYSTEMS). VALUES SHOWN ARE MEDIAN (BLACK LINE), 1ST AND 3RD QUARTILES (BOX) AND MAX/MIN OBSERVATIONS (UPPER AND LOWER LINES) AND THE OUTLIERS (SMALL OPEN CIRCLES), WHEN PRESENT. *DIFFERENT LETTERS INDICATE SIGNIFICANT DIFFERENCES (*P*< 0.05) WITHIN SOIL OR LAND USE COMPARISONS.

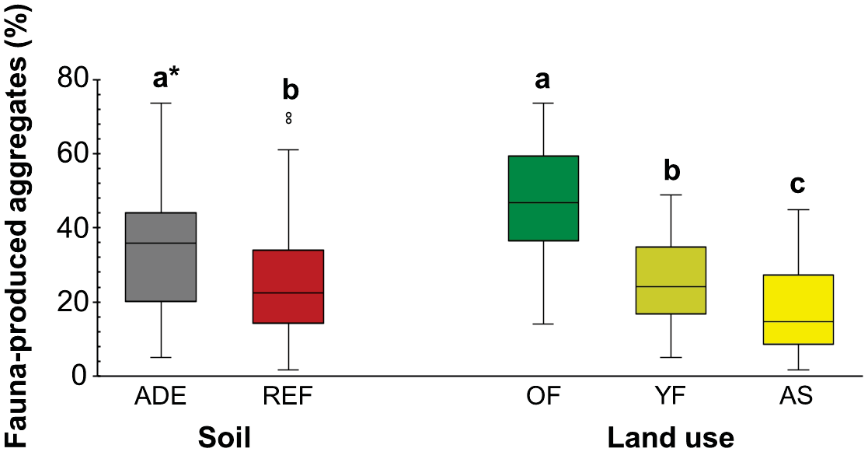
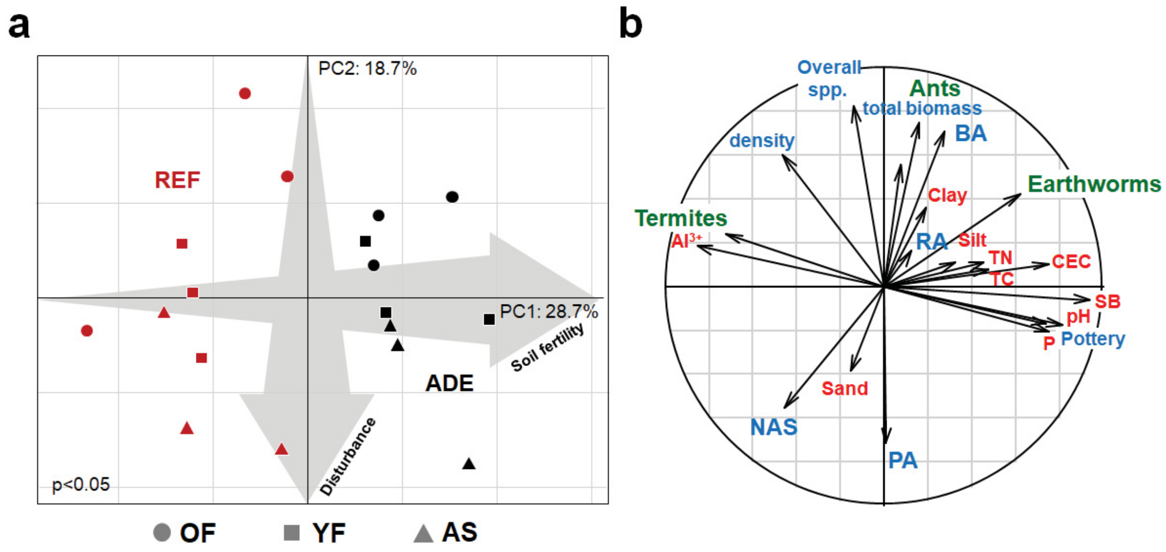


FIGURE 6. PRINCIPAL COMPONENT ANALYSIS (PCA) OF SOIL MACROINVERTEBRATE DATA, COMBINED WITH SOIL MACROMORPHOLOGY FEATURES AND SOIL CHEMICAL AND PHYSICAL PROPERTIES: (A) POSITION OF SAMPLING SITES ON THE PLANE DEFINED BY THE FIRST TWO PCA AXES; ADE: AMAZONIAN DARK EARTH; REF: REFERENCE SOILS; OF: OLD FORESTS; YF: YOUNG FORESTS; AS: AGRICULTURAL SYSTEMS. SIGNIFICANCE OF MONTE-CARLO TEST FOR SOIL TYPE (ADE AND REF) AND LAND USE SYSTEMS (OF, YF AND AS) *P*< 0.05. (B) CORRELATION CIRCLE REPRESENTING THE CORRELATION BETWEEN INDIVIDUAL VARIABLES AND THE FIRST TWO PCA AXES. BLUE ARROWS: MACROMORPHOLOGICAL FRACTIONS (NAS=NON-AGGREGATED SOIL; PA=PHYSICAL AGGREGATES; RA=ROOT AGGREGATES; FA=FAUNA-PRODUCED AGGREGATES, POTTERY), TOTAL SOIL FAUNA DENSITY (NUMBER OF IND. m^{-2}), BIOMASS (FRESH BIOMASS IN g m^{-2}) AND OVERALL MORPHOSPECIES RICHNESS. (SEE METHODS). GREEN ARROWS: DENSITY (NO. IND. m^{-2}) OF ANTS, TERMITES AND EARTHWORMS. RED ARROWS: SOIL CHEMICAL PROPERTIES (SB=SUM OF BASES, CEC=CATION EXCHANGE CAPACITY, TC=TOTAL CARBON, TN=TOTAL NITROGEN) AND PARTICLE SIZE FRACTIONS (SAND, SILT, CLAY).



2.6 DISCUSSION

Our study found over 660 macroinvertebrate morphospecies in the 18 sites sampled in three Amazonian regions, including at least 70 new species of ecosystem engineers. We also found that although species richness is similar in ADE and REF soils, these two habitats harbour very different species pools, with few found in common to both habitats (Fig. 3b). Furthermore, although species rarefaction curves were still far from saturation using our current sampling effort, estimated richness showed similar trends, and showcased the wealth of species still to be discovered in both soils (Fig. 4). Finally, because these animals have been relatively poorly represented in taxonomic surveys in Amazonia (CONSTANTINO; ACIOLI, 2006;

FRANKLIN; MORAIS, 2006; JAMES; BROWN, 2006; VASCONCELOS, 2006), and because ADEs had never been sampled before, we believe that these anthropogenic soils represent a major gap in the knowledge of Amazonian biodiversity. Although ADEs occupy only a small fraction of the Amazonian surface area, they are scattered throughout the region (CLEMENT et al., 2015; KERN et al., 2017), representing thousands of localized special habitats for species. The high β diversity values and species turnovers between different ADEs mean that each of these patches may be home to distinctive soil animal communities, including many new species, judging by the number of new ecosystem engineers found. Hence, ADEs represent an immense underground zoo, which could easily include thousands of species that have not yet been studied and/or classified.

Soil provides chemical and physical support for vegetation, and as millennia of human activities created ADEs in the Amazon, this generated patches of higher contents of nutrient and organic resources in a matrix of poorer soils (KERN et al., 2017). The formation processes and human management of these soils results in distinct plant and microbial communities (BROSSI et al., 2014; CLEMENT et al., 2015; LEVIS et al., 2018; TAKETANI; TSAI, 2010). Here we show that current soil animal abundance and diversity also reflect the impact of these ancient anthropogenic activities. The ADEs developed a different pool of species compared with REF soils. Similar biological selection processes probably occurred and are likely operating in other anthropogenic soils, either already created or being formed in various regions of the world (e.g., West Africa, Europe, Central America etc.) (MACPHAIL et al., 2017; SOLOMON et al., 2016; WIEDNER et al., 2014). Studying the pathways to species selection (and possibly diversification) in ADEs and other anthropogenic soils requires further work, particularly expanding microbial and invertebrate biodiversity inventories. Fire may be one of the important factors to consider (MAEZUMI et al., 2018a): the anthropogenic alterations of ADE generally included frequent burning that led to the formation of highly stable charcoal (GLASER; BIRK, 2012), and higher C and plant nutrient resources (Fig. 2) (LEHMANN et al., 2003; SOMBROEK et al., 2004). Fire, in other contexts, has been documented to generate unique habitats that promote local biodiversity (KELLY; BROTONS, 2017).

The functional differences observed in biotic communities of ADEs also mean that these soils could provide different ecosystem services in the landscape.

Higher earthworm populations and an improved soil structure mainly due to fauna-produced aggregates (as occurs in ADE) could positively affect primary productivity, litter decomposition and nutrient cycling (LAVELLE et al., 2006), pedogenetic processes (MACEDO et al., 2017), and could help stabilize soil organic carbon in these soils (CUNHA et al., 2016). These processes have been little studied, and merit further attention, both in forested and agriculturally managed ADE soils.

As archaeological sites, ADEs are protected by Brazilian law (BRAZIL, 1961), but throughout Amazonia they are intensively used for agricultural and horticultural purposes (FRASER et al., 2011; JUNQUEIRA et al., 2016; KERN et al., 2017). Soil macrofauna are threatened by modern land use change (particularly intensive annual cropping and livestock production), independently of the soil type. The biodiversity in both ADE and REF soils decreased with increasing environmental disturbance (Fig. 3a, Fig. 6), and negative impacts on populations of selected taxa were higher in ADE than in REF soils. Modern human activity has been associated with negative environmental impacts in the Amazon (DECAËNS et al., 2018; FRANCO et al., 2018), but on the other hand, historical human footprints associated with ADEs appear to have “positive” effects on the Amazonian ecosystem (BALÉE, 2010). For instance, we found that old forests on ADEs were the most biodiverse LUS.

Soil invertebrates are known to display high endemism (BALÉE, 2010), and hence high β-diversity values, mainly due to their low dispersal ability (DECAËNS et al., 2016). Still, the high turnover rates between communities of ADE and REF soils suggest that ADEs may represent refuges for large numbers of specialist species that have been overlooked in previous work in the region (BARROS et al., 2006; CONSTANTINO; ACIOLI, 2006; FRANCO et al., 2018; FRANKLIN; MORAIS, 2006), where ADEs were not targeted. This persistent anthropogenic footprint promotes biodiversity (HECKENBERGER et al., 2007) and modifies its distribution patterns in the Amazonian basin, making humans an endogenous part of the environment. This footprint is a prevailing driver in our study and as such, should be integrated into future ecological research in Amazonia. Finally, considering their distinctive below-ground communities, and the negative effect of modern land-use intensification, ADEs deserve special attention and management, in order to protect their biological resources and promote more sustainable uses of Amazonian soils (GLASER, 2007).

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3 CHAPTER II: EARTHWORM COMMUNITIES IN AMAZONIAN DARK EARTHS AND NON-ANTHROPIC SOILS

3.1 RESUMO

Durante milênios a floresta amazônica vem sendo modificada por seres humanos. Um dos vestígios mais interessantes dos povos pré-Colombianos são as férteis Terras Pretas de Índio (TPIs). As TPIs vem sendo estudadas ao longo dos anos, e atualmente vários de seus atributos físicos e químicos já são conhecidos, entretanto, há uma falta de conhecimento sobre a biodiversidade do solo nessas áreas. As minhocas são um dos invertebrados mais importantes do solo, com várias espécies associadas à perturbação humana, altamente sensíveis a alterações da paisagem, no entanto, suas comunidades são praticamente desconhecidas nas TPIS. Neste estudo, nós avaliamos as comunidades de minhocas em TPIs e solos não-antrópicos (REF) e os efeitos do uso moderno do solo (agricultura) nas populações desses invertebrados em TPIs e solos REF em três regiões da Amazônia Central. Foram encontradas 38 espécies/morfoespécies de minhocas, a maioria delas espécies novas, sendo 12 spp. associadas apenas as TPIs, indicando que as terras pretas representam um hábitat único, abrigando muitas espécies desconhecidas. As comunidades de minhocas foram mais afetadas pelo uso moderno da terra nos solos referência do que nas TPIs, com menor densidade, biomassa, riqueza e diversidade de espécies nos sistemas agrícolas/ pastagens. Nas TPIs, a riqueza e diversidade das minhocas foi menor, mas a densidade e biomassa não foram afetadas pela agricultura moderna, indicando que as espécies predominantes nas TPIs são oportunistas. Espécies invasoras como a *Pontoscolex corethrurus* também foram encontradas em florestas antigas (florestas secundárias em estágio avançado de regeneração com >20 anos de idade) tanto nas TPIs quanto nos solos REF, indicando forte interferência humana na floresta amazônica.

Palavras-chave: Biologia dos solos. Terra preta de Índio. Mudança do uso da terra. Oligochaeta.

3.2 ABSTRACT

During millennia the Amazon rainforest has been modified by humans. One of the most interesting footprints of Pre-Columbian people are the very fertile Amazonian Dark Earths (ADEs). ADEs have been studied over decades, with several physical and chemical attributes already known, but, there is little knowledge of the belowground diversity in these soils. Earthworms are one the most important soil dwelling invertebrates with several species associated with human disturbance, and highly sensitive to landscape alteration, however, their communities are practically unknown in ADEs. In this study, we evaluated the earthworm communities in ADEs and non-anthropic soils (REF) and the effects of the modern land-use (agriculture) on their populations in both ADE and REF soils across three regions of Central Amazonia. We found 38 earthworm species/morphospecies, most of them new to science, and 12 spp. associated only with ADEs, indicating that ADEs are a unique environment, hosting many unknown species. Earthworm communities were more affected by land-use change in REF than ADEs, with lower density, biomass, richness and diversity in agricultural/pastoral systems. In ADEs earthworm richness and diversity decreased, but density and biomass were not affected by modern land use, implying that the dominant species in ADEs are opportunistic. Invasive earthworms like *Pontoscolex corethrurus* were found in old forests (secondary forests in advanced stage of regeneration, >20 years old) in ADE and REF soils, indicating strong human interference on the Amazonia rainforest.

Keywords: Soil biology. Amazonian dark earths. Terra preta de Índio. Land-use chance. Oligochaeta.

3.3 INTRODUCTION

The Amazonian rainforest holds around 10 % of the world's diversity (DA SILVA; RYLANDS; DA FONSECA, 2005; LEWINSOHN; PRADO, 2005), and many of these species are invertebrates associated with soil for at least part of their life-cycle (BROWN et al., 2006). As many as 2,200 species of soil macroinvertebrates may live in a lowland Amazonian rainforest site (MATHIEU, 2004), but few sites have been studied throughout the 5 million km^2 of Amazonia (BARROS et al., 2006), that contains as many as 23 diverse ecoregions (OLSON et al., 2001; BORSATO et al., 2015). Furthermore, deforestation has once again increased in Amazonia, particularly with the advancement of agricultural frontiers, generating an estimated loss about 0.5% year^{-1} of Brazilian Amazonian territory (INPE, 2018), with potentially catastrophic effects on biodiversity.

Deforestation has drastic affects not only on aboveground biodiversity (e.g. plants, large animals, insects), but also soil organisms (DECAËNS et al., 2018). This can also affect ecosystem services (MARICHAL et al., 2014; LAVELLE et al., 2016), as belowground invertebrates help maintain ecosystem functioning (BROWN et al., 2018; LAVELLE et al., 2006). Ecosystem engineers such as termites, earthworms and ants are particularly important, as they can modify their soil habitat through feeding and bioturbation, mixing organic and mineral particles in the soil profile, changing organic matter decomposition and nutrient cycling, ultimately also affecting plant growth (LAVELLE et al., 1997).

Around 200 earthworm species have been reported from Amazonia (FEIJOO; BROWN; JAMES, 2017), but as many as 2000 are estimated to occur in the region (LAVELLE; LAPIED, 2003). Conversion of rainforest to pastures and polyculture agroforestry systems often increases earthworm populations, mainly because of exotic earthworm invasion (RÖMBKE & VERHAAGH, 1992, RÖMBKE; MELLER; GARCIA, 1999, CHAUVEL et al., 1999; BARROS et al., 2004, 2006; MARICHAL et al., 2010, 2014). On the other hand, conversion to annual crops often has a drastic negative effect on both earthworm abundance and species richness (LAVELLE; PASHANASI, 1989; FRAGOSO et al., 1995).

The invasive species *Pontoscolex corethrurus* is widespread in Amazonia (JAMES; BROWN, 2006), and is particularly associated with modern human disturbance (BARROS et al., 2002; MARICHAL et al., 2010). However, humans have been altering Amazonian forests for over 10,000 years (ROOSEVELT, 2013). Pre-Colombian people intensively modified the landscape, generating persistent footprints in this environment, such as the Amazonian Dark Earths (ADEs) also known locally as Terra Preta de Índio (SMITH, 1980). ADEs were formed by centuries of Amerindian occupation, and are characterized by their dark colour and high levels of carbon, calcium and pH (LEHMANN et al., 2003; MACEDO et al., 2017). Due to their high chemical fertility compared to non-anthropic soils, ADEs are commonly utilized for agricultural purposes, being largely cultivated for high value crops like papaya and melons but also for other widely grown crops like maize, soybean, manioc, as well as perennial pastures for cattle production (LEVIS et al., 2018; TEIXEIRA et al., 2009).

Although much is known of the chemical, physical and mineralogical characteristics of ADEs, few studies focused on soil organisms in these soils (BROSSI et al., 2014; GROSSMAN et al., 2010; SOARES et al., 2011; TAKETANI et al., 2013), and all of them targeted only microbes. Information on soil macroinvertebrates such as earthworms are scarce, with only one published study (CUNHA et al., 2016). Hence, the present study evaluated earthworm communities in ADEs and REF soils under different vegetation types (forest, agriculture), to shed light on the role of ancient and modern human impacts on earthworm abundance and diversity in Central Amazonia.

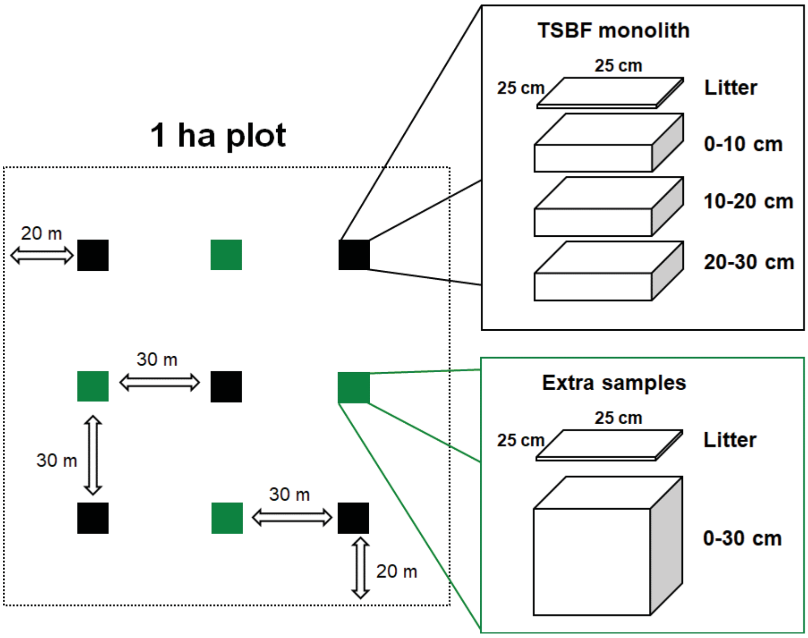
3.4 MATERIAL AND METHODS

Earthworm communities were surveyed in three regions of Central Brazilian Amazonia: Iranduba (IR), Belterra (PA) and Porto Velho (PV). In each municipality, paired ADE and REF soils were selected under three different land use systems (LUS): old secondary forest (OF) (>20 yr without human disturbance); young secondary forest (<20 yrs disturbance); and agricultural fields (currently cultivated with maize, soybean and perennial pastures). More description and information about the sites can be found on Supplementary Table 1.

3.4.1 EARTHWORM SAMPLING

At each site (1 ha plot), nine samples (30 m distance from each other) were collected on a square grid, of which 4 main samples were collected at the corners, and one of them at the centre of the square (Fig. 1). For the five main samples, an adaptation of the Tropical Soil Biology and Fertility (TSBF) method (ANDERSON; INGRAM, 1993) proposed as standard method by ISO norm 23611-1 (ISO, 2017) was used. The surface litter and the top 10 cm soil layer were isolated with a 25 × 25 cm x 10 cm deep steel frame. The surface litter was removed and handsorted, and the top 10 cm layer placed into a plastic bag and taken for handsorting nearby. The remaining two soil layers were subsequently removed (10-20 and 20-30 cm) and also handsorted on-site. The remaining four samples, also handsorted on-site were of the same size, but not separated into the three depth layers (Fig. 1).

FIGURE 1 - SCHEMATIC DIAGRAM OF THE SAMPLING DESIGN USED AT EACH SITE, BASED ON THE TSBF-ISO METHOD (ANDERSON; INGRAM, 1993).



Earthworms separated from the soil monoliths were preserved in 92% ethanol. In the laboratory, they were identified to species, genus or morphospecies level, using the available taxonomic keys (BLAKEMORE, 2002; MICHAELSEN, 1900; RIGHI, 1990, 1995). Earthworm fresh (preserved) biomass was measured using a digital balance (0.0001g).

3.4.2 SOIL ANALYSES

After hand-sorting, 500 g of soil from the five main monoliths was collected and submitted to standard chemical and particle size analyses. The samples (dried at 45 ºC) were sieved at 2 mm and analysed according to Teixeira et al. (2017), for: pH ($CaCl_2$), exchangeable Al, Ca, Mg (KCl 1M), P and K (Mehlich-1). Total carbon (TC) and nitrogen (TN) were determined by dry combustion (Vario EL III), and particle size analysis (clay, silt and sand contents) was obtained following Teixeira et al. (2017).

3.4.3 STATISTICAL ANALYSES

Mean earthworm species richness (mean no. species found), species distribution within samples (no. species sample^{-1}) and Shannon diversity index were calculated using standard formulae (MAGURRAN, 2004). Earthworm data (density, total and mean individual earthworm biomass and ecological indices) were submitted to Shapiro-Wilk’s normality test. Due to non-normal distribution, General Linear Models (GLM) were used to adjust the data distribution. Using GLM, a factorial ANOVA was performed considering soil type (ADE and REF) and LUS (OF, YF and AS) as factors. When the ANOVA was significant ($P< 0.05$) Tukey’s test was used to determine differences between treatments using multcomp package in R software (HOTHORN; BRETZ; WESTFALL, 2008). When GLM was unable to adjust the data to known distribution models (e.g., earthworm density data), non-parametric Kruskal-Wallis’ test was used, following the factors cited above. Soil data was similarly analysed, and results are presented in Supplementary Table 2.

Using species occurrence and disregarding singletons (species represented by single individuals) Beta-diversity (β) indices were calculated to assess the turnover components. Using Betapart package (BASELGA; ORME, 2012) we calculated β Sørensen ($\beta_{\text{Sør}}$) dissimilarity index (max. diversity) and β Simpson (β_{Sim}) dissimilarity index (turnover) and Nestedness ($\beta_{\text{Sør}} – \beta_{\text{Sim}}$). β diversity values were partitioned according to the following effects: LUS (mean of beta-diversity indices obtained within a region in the same soil category); regional/spatial (obtained comparing the same LUS within each soil category); and soil category effect (result from comparisons between ADEs and REF soils in the same LUS within each region). We also calculated the rarefaction curves of species/morphospecies (data including singletons) for LUS in each soil category using the iNEXT package (HSIEH; MA; CHAO, 2018).

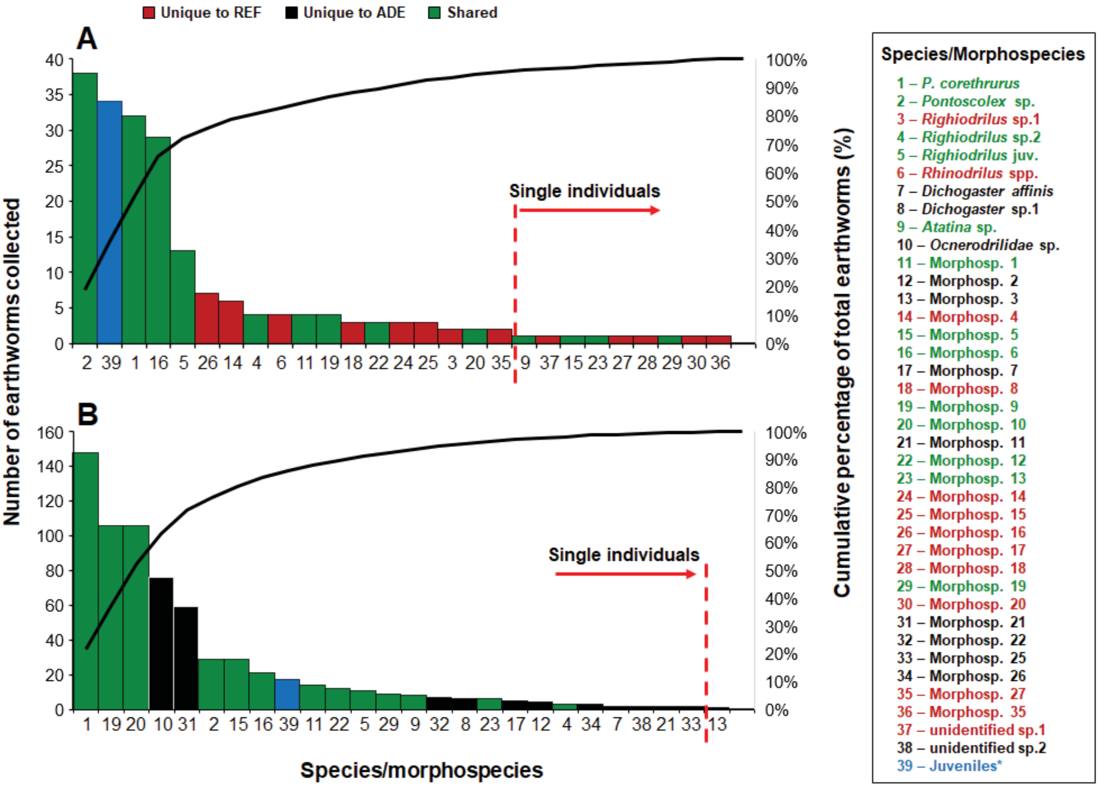
Additionally, a Principal Component Analysis (PCA) was performed using the earthworm data (density, biomass and diversity indices) and chemical and particle size fractions obtained with the five main TSBF monoliths using ADE-4 package (DRAY; DUFOUR, 2007) in R software.

3.5 RESULTS

A total of 1,079 earthworms were collected, belonging to 38 morphospecies, with at least 20 species new to the science which will be described in future publications. From this total, 13 morphospecies were unique to REF soils (red bars), 12 to ADEs (black bars) and 13 shared between both soils (green bars) (Fig. 2A, B). Highest earthworm richness was found in OF sites, with 23 and 17 spp. (unique +

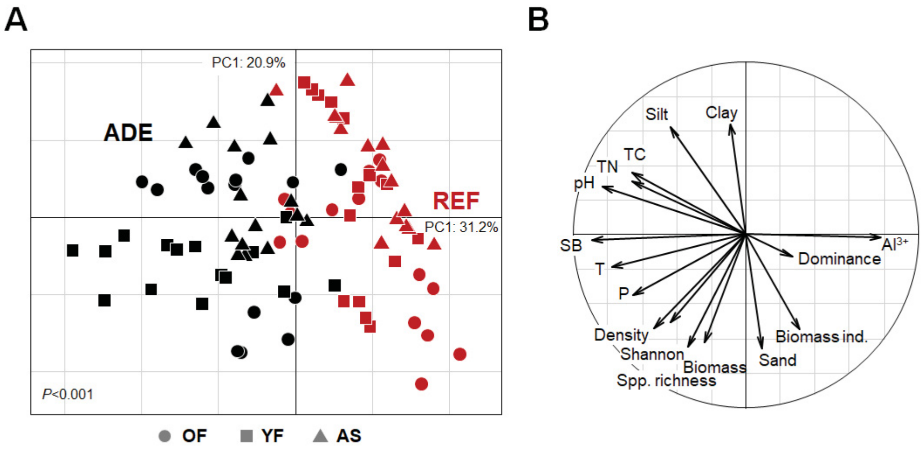
shared species) in REF and ADE soils, respectively (Fig. 2A, B). Additional samples (n = 4) increased the number of morphospecies sampled, especially in OF (35%) and AS fields (20%). The most common genera found was *Pontoscolex* (Rhinodrilidae family), which was collected in 10 (five in REF and five in ADEs) of the 18 areas sampled. Interestingly, in all OF sites *P. corethrurus* specimens were found, a peregrine earthworm of worldwide distribution (TAHERI; PELOSI; DUPONT, 2018).

FIGURE 2 - SPECIES/MORPHOSPECIES DISTRIBUTION ACCORDING THE NUMBER OF EARTHWORMS COLLECTED IN (A) REF SOILS AND (B) ADES (TOTAL N=9 SAMPLES PER SITE; 81 SAMPLES EACH FOR ADE AND REF), INCLUDING SINGLE INDIVIDUALS. *UNIDENTIFIED JUVENILE EARTHWORMS THAT LIKELY BELONG TO THE SPECIES FOUND IN EACH LOCATION.



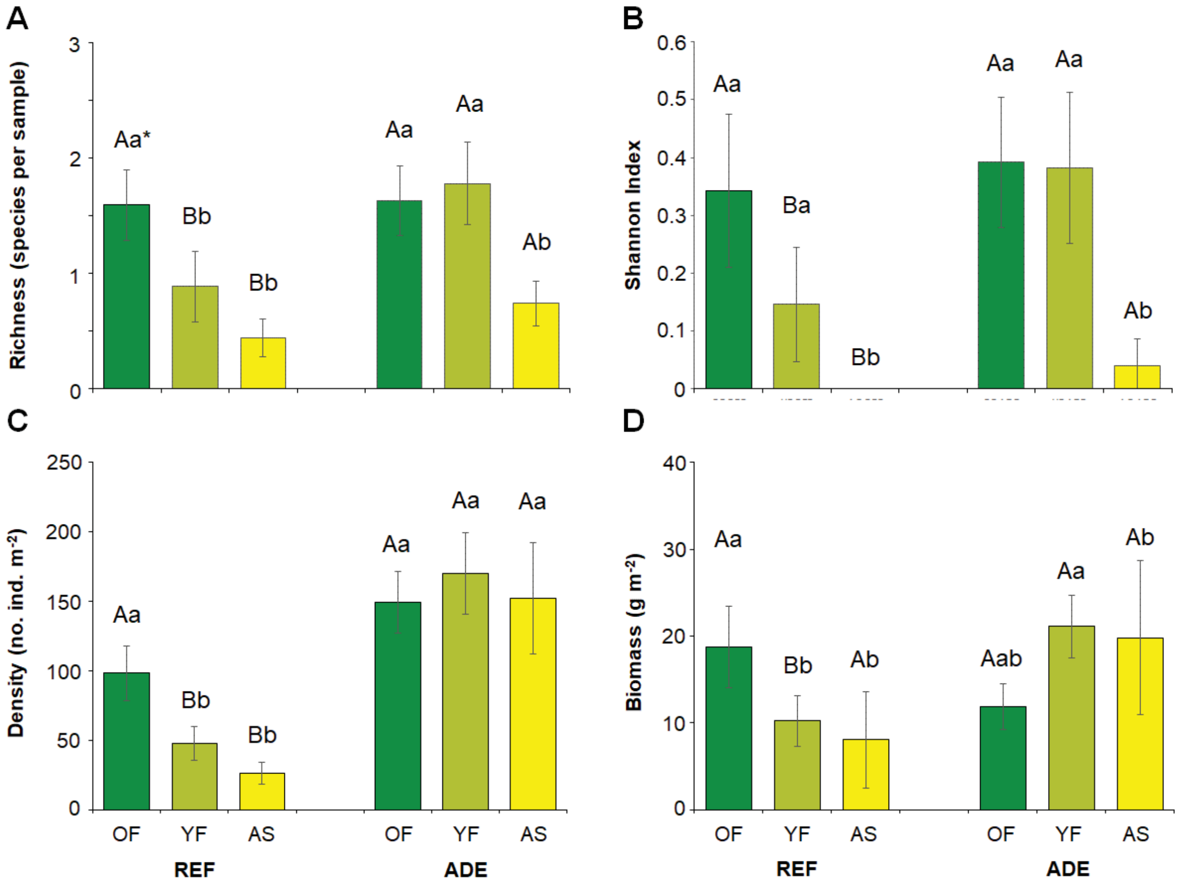
The PCA analysis showed a clear separation between ADEs and REF soils (Fig. 3A). Axis 1 (PC1) explained 30.5% of the variance and separated the samples based on soil fertility, with the X-axis (Fig. 3B) related mainly to levels of P, to SB (Ca^{2+} + Mg^{2+} + K^{+}), CEC, total carbon and nitrogen and pH. Axis 2 (PC2) separated the samples regarding earthworm biomass (total, biomass mean per individual and species richness) and soil texture (clay, sand contents). Earthworm density, diversity (Shannon) and species richness were related to OF and YF on ADEs, while individual biomass (bigger earthworms) was related to REF soils (OF, YF). AS sites, mainly on REF soils, were inversely associated to all earthworm data.

FIGURE 3 - PRINCIPAL COMPONENT ANALYSIS OF EARTHWORM DATA (DENSITY, TOTAL AND MEAN INDIVIDUAL EARTHWORM BIOMASS, SHANNON INDEX AND NUMBER OF SPECIES) COMBINED WITH SOIL CHEMICAL AND PARTICLE SIZE ANALYSIS OF NON-ANTHROPIC SOILS (REF: RED COLOR) AND AMAZONIAN DARK EARTHS (ADES: BLACK COLOR) UNDER THREE LAND USE SYSTEMS (LUS). A) FACTORIAL MAP SHOWING SAMPLE DISPERSION ACCORDING THE SOIL TYPE (ADE, REF) AND LUS (OF=OLD FOREST; YF=YOUNG FOREST; AS=AGRICULTURAL/PASTORAL SYSTEM). SIGNIFICANCE OF THE MODEL (SOIL CATEGORY OR LAND-USE SYSTEMS) OBTAINED USING MONTE-CARLO TEST (999 PERMUTATIONS). B) RELATIONSHIP BETWEEN THE RESPONSE VARIABLES AND THE TWO MAIN AXES.



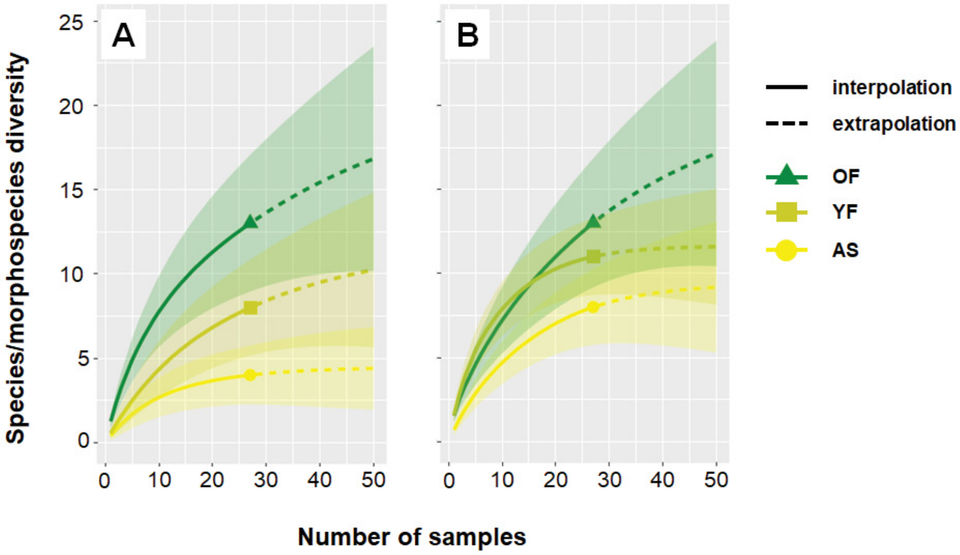
The number of earthworm species collected per sample (mean richness sample^{-1}) also showed differences among the LUS within each soil category (Fig. 4A). In REF soils, the richness was greater in OF (1.6 spp. sample^{-1}) than YF (0.9) and AS (0.4), while in ADEs both OF (1.6) and YF (1.7) had higher richness than AS (0.7 spp. sample^{-1}). Earthworm communities were also affected by soil type, with mean richness greater in ADEs under YF and AS than these LUS in REF soils. Shannon index showed the same trend, but diversity in AS in ADEs was higher than in REF soils (Fig. 4B). Species rarefaction curves were similar in both ADE and REF soils (Fig. 5a, b), showing a higher number of earthworm species expected in OF than YF and AS. Species saturation in both soils were almost achieved with the sampling effort in YF and AS, but for OF a three or four times larger sampling effort would be needed in order to fully assess expected species richness (Fig. 5A, B).

FIGURE 4 - EARTHWORM COMMUNITIES IN AMAZONIAN DARK EARTHS (ADE) AND NON-ANTHROPIC SOILS (REF): A) MEAN EARTHWORM RICHNESS PER SAMPLE, B) SHANNON DIVERSITY INDEX, C) EARTHWORM DENSITY (n=9, IND. m^{-2}), D) EARTHWORM BIOMASS (n=9, g m^{-2}). *DIFFERENT LETTERS INDICATE SIGNIFICANT DIFFERENCES (P< 0.05) BETWEEN SOILS WITHIN THE SAME LUS (CAPITAL LETTERS) AND AMONG LUS WITHIN EACH SOIL (SMALL LETTERS). BARS INDICATE STANDARD ERRORS.



In REF soils, earthworm density was higher in OF (98 ind. m^{-2}) than YF (47 ind. m^{-2}) and AS (26 ind. m^{-2}), while in ADEs no significant differences among LUS were found, with 149, 170 and 152 ind. m^{-2} in OF, YF and AS, respectively (Fig. 4C). However, earthworm density in ADEs was significantly higher than in REF soils for both YF and AS (Fig. 4C). Earthworm biomass showed similar trends as density values, with means of 11.8, 21.1 and 19.7 g m^{-2} for OF, YF and AS in ADEs, respectively (Fig. 4D), with significant difference only between YF and AS within ADES. In REF soils, biomass was higher in OF (18.7 g m^{-2}) than in YF (10.2 g m^{-2}) and AS (8.1 g m^{-2}). Comparing soil types, the YF and AS in ADEs had higher biomasses than these LUS in REF soils (Fig. 4D).

FIGURE 5 - EARTHWORM SPECIES/MORPHOSPECIES RAREFACTION AND EXTRAPOLATION CURVES IN (A) NON-ANTHROPIC SOILS (REF) AND AMAZONIAN DARK EARTHS (ADE) UNDER OLD (OF) AND YOUNG FORESTS (YF), AND AGRICULTURAL/PASTORAL SYSTEMS (AS). LIGHT COLORED AREAS REPRESENT 95% CONFIDENCE INTERVALS.



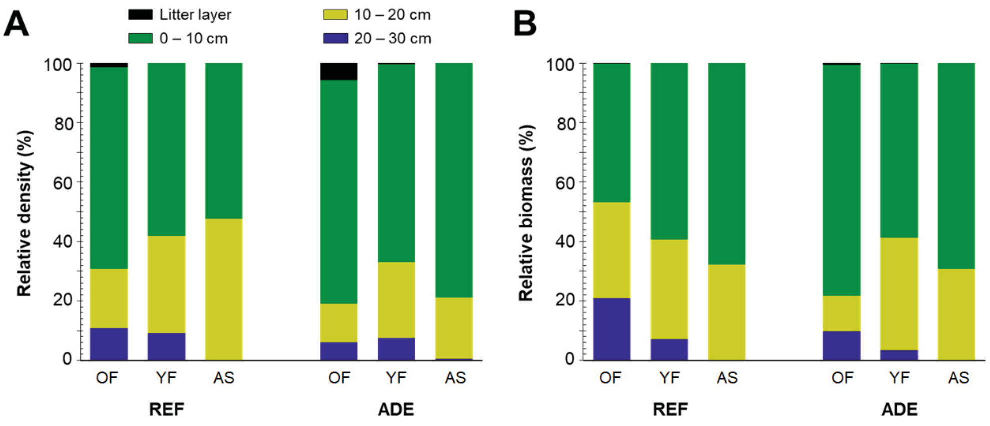
The partition of beta-diversity values showed important effects of LUS on earthworm species turnover in REF soils (0.85), though these were slightly lower in ADEs (0.60) (Table 1). Regional effect, which show the diversification of species as result of the spatial/geographical distance, were particularly significant for YF in REF soils and for AS in both REF soils and ADEs, with turnover values of around 1 (Table 1).

TABLE 1 - PARTITION OF BETA-DIVERSITY OF EARTHWORM SPECIES INTO β SØRENSEN (OVERALL DIVERSITY), SPECIES TURNOVER (β SIMPSON DISSIMILARITY INDEX) AND NESTEDNESS ACCORDING THE EFFECTS OF LAND-USE SYSTEMS (OF=OLD FOREST; YF=YOUNG FOREST; AS=AGRICULTURAL/PASTORAL SYSTEM), REGION (WITHIN LUS AND SOIL CATEGORY) AND SOIL TYPE (WITHIN EACH LUS); ADE: AMAZONIAN DARK EARTHS; REF: NON-ANTHROPIC SOILS.

Partitioned effect	Max div. ($\beta_{Sorensen}$)	Turnover ($\beta_{Simpson\ dis.}$)	Nestedness
LUS effect			
REF	0.9	0.85	0.05
ADE	0.7	0.60	0.1
Region effect			
OF			
REF	0.64	0.54	0.10
ADE	0.75	0.71	0.04
YF			
REF	1	1	0
ADE	0.73	0.69	0.04
AS			
REF	1	1	0
ADE	1	1	0
Soil effect			
in OF	0.50	0.46	0.04
in YF	0.72	0.66	0.06
in AS	0.83	0.66	0.17

Earthworms was concentrated within the top 10 cm of the soil profile in both soil types (ADE, REF), although they tended to be more superficial in ADEs than in REF soils (Fig. 6A). In AS in REF soils, distribution was more even within the top two soil layers (0-10, 10-20 cm). Still, more than 90% of all individuals were collected in the 0-20 cm of the soil. Very few earthworms were found in the surface litter, and mainly in OF sites (ADE and REF). Earthworm biomass was distributed in the soil profile similar to density. However, larger earthworms were found deeper in OF in REF soils, so that biomass at 20-30 cm depth represented up to 20% of the total found in this LUS (Fig. 6B).

FIGURE 6 - RELATIVE DISTRIBUTION OF EARTHWORMS IN SOIL PROFILE (0-30 CM). (A) DISTRIBUTION OF DENSITY AND (B) BIOMASS OF EARTHWORMS IN SOIL PROFILE UNDER OLD AND YOUNG FORESTS (OF AND YF, RESPECTIVELY) AND AGRICULTURAL FIELDS (AS) IN NON-ANTHROPIC SOILS (REF) AND AMAZONIAN DARK EARTHS (ADE).



3.6 DISCUSSION

Our results suggest that historical Amerindian landscape modification not only changed soil fertility and plant community composition (GROSSMAN et al., 2010; LEVIS et al., 2018), it also profoundly transformed the earthworm populations and their distribution in archaeological sites with ADEs (Fig. 2, 3C). Few species were found in both ADE and REF soils (34% of total), and 32% of all species were found exclusively in ADEs, indicating this was a unique habitat for several unique earthworm species. Furthermore, species turnover due to soil type (ADE vs. REF) in OF was close to 50% (Table 1), indicating that even in these old secondary forests, major species changes occurred due to previous Amerindian occupation and more traditional land uses such as slash and burn agriculture, practiced over centuries in ADE sites (MAEZUMI et al., 2018).

The selection processes of earthworm species in ADEs likely began with habitat interference/disturbance by the Amerindians, followed by the reduction in populations of susceptible native species, the introduction of opportunistic/exotic earthworm species and finally, the colonization of vacant niche spaces by the exotic species (KALISZ; WOOD, 1985). Interestingly, a large number of native and undescribed species were found in ADEs, despite intensive modification of the habitat (slash and burn agriculture, human settlement) and soil environment (, higher pH, P and Ca contents due to input of bones and organic materials

LEHMANN et al., 2003; NEVES et al., 2003; SMITH, 1980), over centuries of Amerindian use. Soil characteristics of ADEs are very different from the natural REF soil conditions which led to the evolution of the original native Amazonian earthworms. Therefore, the high species turnover observed between ADEs and REF soils was not surprising, as well as the high turnover associated with LUS effect for both soil categories, mainly in REF soils (Table 1).

The species most commonly encountered in ADEs was *P. corethrurus* (Fig. 2B), although the species was also quite frequent in REF soils (Fig. 2A), together with other native *Pontoscolex* spp. The widespread presence of this species in both ADEs and REF forest soils indicates a rather high level of anthropic disturbance in both OF and YF, and the role of humans in dispersing *P. corethrurus* (a good indicator of human disturbance; MARICHAL et al., 2010; TAHERI et al., 2018a). However, *P. corethrurus* has several cryptic lineages, so a molecular approach is needed in order to properly identify the individuals collected. This should be compared with the molecular data of the *P. corethrurus* neotype (JAMES et al., 2019), and of several other lineages found in Latin America (TAHERI et al., 2018b). The collection sites are within the native range of the *Pontoscolex* genus, and other species were found (Fig. 2), some of which were morphologically similar to *P. corethrurus*.

Unlike most native species, exotic earthworms show high ecological plasticity, being able to survive under a wide range of soil and habitat conditions, with variable contents of sand or clay and high or low soil organic matter content (GONZÁLEZ et al., 2006; LAVELLE et al., 1987). Their abundance in ADEs prompted Cunha et al. (2016) to propose an important role of earthworms in soil processes and the genesis of ADEs. Ponge et al. (2006) showed that *P. corethrurus* actively ingested charcoal and mixed it with soil mineral particles, burying these material in the top soil of slash and burn Amazonian agricultural fields. This behaviour may increase soil carbon stabilization, promoting contact between organic material and soil minerals, improving the protection of organic C in macro and microaggregates (LEHMANN; KLEBER, 2015). In fact, the burrowing activities of earthworms over centuries in ADEs could have contributed to increased organic C content in these soils.

Contrasting with pre-Columbian disturbances, modern agricultural practices had severe negative effects on earthworm species richness and diversity, both in

REF soils and ADEs (Fig. 4A, B). This confirms previous observations on the negative effects of land use change and intensification on earthworm communities in the region (BARROS et al., 2004; FRAGOSO; LAVELLE, 1992; MARICHAL et al., 2014; DECAËNS et al., 2018). Deforestation and soil disturbance tend to negatively affect forest earthworms, mainly native species, due to decreases in available food and to changes in the soil environment (e.g. lower soil moisture and higher temperature due to absence of litter layer and tree cover). Additionally, the conversion of forests to agriculture fields cultivated with maize and soybean affects earthworms more than permanent pastures due to constant soil disturbance and use of pesticides (BROWN et al., 2018).

However, although earthworm densities were lower in AS than forests in REF soils, they were not in ADEs (Fig. 3c). This result reinforces the hypothesis that earthworm communities in anthropic soils are dominated by opportunistic species, both native and exotic, that are probably *r*-strategists, able to quickly colonize disturbed environments (BOUCHÉ, 1977). The higher nutrient resources (particularly organic matter) in ADEs, as well as the additional microhabitats created by abundant charcoal and pottery may also be important, though the direct relationship between the latter two components and earthworms have not yet been tested experimentally (CUNHA et al., 2016). The high earthworm density and biomass (close to 20 g m^{-2}) in AS in ADEs (Fig. 4D), also means that they may be contributing to several important ecosystem services in these soils, including plant root and shoot growth (VAN GROENIGEN et al., 2015). Further research on this is warranted, particularly considering the extensive use of ADEs for agriculture throughout Amazonia (KAWA; RODRIGUES; CLEMENT, 2011).

Although earthworms are major soil bioturbators, and probably have been influencing the soil properties and processes of ADEs since their formation began over 6,500 years ago (WATLING et al., 2018), no information is available on their functional role in these anthropic soils. Our results show that ADEs are a unique environment within the Amazonian rainforest, with a unique pool of earthworm species, but further research should assess how widespread this phenomenon is, and the roles of these unique earthworm communities in ADEs.

3.7 CONCLUSION

ADEs represent an important niche for earthworms that differs from adjacent REF soils. Furthermore, they are sensitive to modern agricultural practices, which can reduce species richness, although density and biomass values are maintained, compared to forest systems. Hence, earthworm populations seem to be more resistant to LUS modification in ADEs than REF soils, although nothing is known of the functional consequences of these changes, which deserve further attention. A better description of the earthworm communities across a broad range of ADEs and reference soils in Amazonia, accompanied with more detailed studies (field, laboratory and greenhouse), on the functional roles of earthworms in these soils is necessary in order to improve the conservation and sustainable management of ADEs throughout Amazonia.

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4 CHAPTER III: SOIL QUALITY AND ORGANIC MATTER HUMIFICATION IN AMAZONIAN DARK EARTHS AND NON-ANTHROPIC SOILS

4.1 RESUMO

As Terras Pretas de Índio (TPIs) são solos férteis formados por séculos de ocupação de povos pré-Colombianos espalhadas na bacia Amazônica. Atualmente, muitas TPIs estão sendo usadas para produção agrícola moderna, no entanto, não há informações sobre como essas práticas estão afetando a qualidade do solo nas TPIs. Portanto, este trabalho avaliou a qualidade do solo em nove TPIs e nove solos não-antrópicos (REF) na Amazônia Central, usando os atributos químicos, macromorfológicos e biológicas do solo para gerar o Índice Geral da Qualidade do Solo (GISQ). Além disso, avaliou-se o efeito de sistemas de uso do solo (florestas em estágio inicial e avançado de regeneração e agricultura) sobre o GISQ, a matéria orgânica do solo (MOS) e o índice de humificação da MOS nas diferentes frações de macroagregados. A qualidade geral do solo foi maior nas TPIs do que nos solos referência, e as propriedades físicas e biológicas das TPIs foram mais resistentes às mudanças no uso da terra em comparação com os solos naturais da Amazônia. Além disso, a fauna do solo não modificou os teores totais de carbono e nitrogênio nem o índice de humificação da MOS nos agregados biogênicos. O índice de humificação da MOS foi menor nas TPIs que nos solos REF e nas florestas secundárias que a área agrícola, respectivamente. Isso sugere que as TPIs são mais resistentes à perturbação humana em comparação aos solos REF, mas as propriedades biológicas e físicas desses solos ainda são afetas negativamente pela mudança no uso da terra. Finalmente, este trabalho confirma diferenças na qualidade da MOS nas TPIs, indicando diferenças na dinâmica da MOS em solos antrópicos.

Palavras-chave: Espectroscopia de fluorescência. GISQ. Agregados do solo. Mudança do uso da terra. Macrofauna do solo.

4.2 ABSTRACT

Amazonian dark earths (ADEs) are fertile soils formed by centuries of Amerindian occupation throughout the Amazon basin. Currently, many of these soils are being used for modern crop production, but little is known of how these practices affect the quality of these soils. Therefore, the present study evaluated overall soil quality in nine ADEs and nine nearby non-anthropic soils (REF) in Central Amazonia, using soil chemical, macromorphological and biological properties to generate the General Index of Soil Quality (GISQ). Furthermore, the effects of land-use systems (old and young secondary forests and agricultural fields) on GISQ and on soil organic matter (SOM) and humification index of SOM in different fractions of soil aggregates were also assessed. Overall soil quality was higher in ADEs than REF soils, and the physical and biological properties of these anthropogenic soils were more resilient to land-use change compared to natural Amazonian soils. Furthermore, soil fauna did not modify the total carbon and nitrogen contents neither the humification index of SOM in biogenic aggregates. The humification index of SOM was lower in ADEs than REF soils and in secondary forests compared with pastures, respectively. This suggests that ADEs are more resilient to human disturbance than REF soils, but biological and physical properties of these soils are still negatively affected by land-use change. Additionally, this study confirms differences in SOM quality of in ADEs than REF soils, indicating differences in SOM dynamics in anthropic soils.

Keywords: Fluorescence spectroscopy. GISQ. Soil aggregates. Land-use change. Soil macrofauna.

4.3 INTRODUCTION

The conversion of forests to agricultural fields is a constant concern in the Amazonian rainforest region, which lost about 5% of its natural vegetation in the last decade (INPE, 2018). This loss can have important negative impacts on above and belowground species diversity (FRANCO et al., 2018), and lead to important changes in chemical, physical and biological soil properties, further exacerbated by the simplification of the vegetation in row-crop or pastoral agroecosystems. However, human activity in Amazonia is not recent and Amerindians have been modifying neotropical rainforests for thousands of years (MAEZUMI et al., 2018; NEVES et al., 2004), leaving a significant ecological footprint, easily identified by the high occurrence of useful plant species and archaeological remains found throughout Amazonia (KAWA; RODRIGUES; CLEMENT, 2011). Pre-Columbian settlements also strongly modified natural soils, generating very fertile anthrosol called Amazonian dark earths (ADEs). These soils have higher pH and contents of carbon, calcium, magnesium and phosphorous in relation to non-anthropic reference (REF) soils (ALHO et al., 2019). Although ADEs are archaeological sites, these soils have been used extensively for agricultural purposes, mainly by small-farmers, being cultivated with maize, manioc and soybean (ARROYO-KALIN, 2010; JUNQUEIRA; SHEPARD; CLEMENT, 2010). However, the effects of forest to agriculture conversion on overall soil quality in ADEs has not yet been investigated, particularly using a suite of biological, chemical and physical soil quality indicators.

Soil invertebrate communities, particularly the macrofauna are highly sensitive to changes in vegetation and land-use systems, often showing reduced abundance and diversity in agricultural fields (MARICHAL et al., 2014; MATHIEU et al., 2009). Soil macrofauna, especially ecosystem engineers (earthworms, ants, termites and burrowing beetles) are important ecosystem services providers

(LAVELLE et al., 1997, 2006). Invertebrate fauna affects the four main types of ecosystem services (e.g. provisioning, cultural, regulating, and supporting services) due to their effects on multiple soil processes and properties such as soil structure (e.g. burrowing and casting activities), soil organic matter decomposition and nutrient cycling, biological control and plant growth (BROWN et al., 2018). The reduction in soil macroinvertebrate communities with land-use change is also frequently accompanied by changes in soil aggregation, leading to decreased soil porosity, affecting water storage and infiltration in agroecosystems (DE SOUZA BRAZ; FERNANDES; ALLEONI, 2013), and hence reducing soil quality compared to natural systems.

The assessment of the impact of land management on soil quality is made difficult by the wide range of chemical, physical and biological variables related to this concept (DORAN; PARKIN, 1994). Hence, in recent years broader indicators, that integrate these soil properties into their measurement have been proposed, such as the general indicator of soil quality (GISQ; VELÁSQUEZ; LAVELLE; ANDRADE, 2007). The GISQ uses multivariate analysis and variables well-known to be good for soils, to generate a value (ranging from 0 to 1) that indicates the overall soil quality (VELASQUEZ; LAVELLE; ANDRADE, 2007). This indicator is obtained using sub-indicators of soil quality, separated into different groups, such as chemical attributes (usually soil pH and plant nutrient contents) associated with soil fertility, biological variables usually represented by the soil macrofauna communities, and soil characteristics related to good physical structure such as aggregate types (measured by micromorphology) which indicates the contribution of soil fauna to soil structure and ecosystem services (VELASQUEZ et al., 2007).

Besides these indicators, soil organic matter (SOM) is also frequently used to indicate soil quality (SIKORA; STOTT, 1996). Some properties of SOM such as total C and N contents, C stocks, and stabilisation are related to vital soil process, mainly greenhouse gas emissions and nutrient turnover (LAL, 2015; LEHMANN; KLEBER, 2015; PAUSTIAN et al., 2016). The distribution of C in different soil aggregates is also essential for its sequestration, as C found in microaggregates within macro-aggregates tends to be better conserved and protected from microbial degradation (BOSSYUT et al., 2005). Several spectroscopy techniques can also give qualitative information on SOM and its quality in natural and agricultural environments. These include Laser-induced fluorescence spectroscopy (LIFS), that has been successfully applied to characterize SOM humification in non-treated soil samples (DIECKOW et al., 2009; RAPHAEL et al., 2016). LIFS can identify the chemical recalcitrance of SOM due to the fluorescence characteristics of aromatic organic matter compounds such as aromatic rings. Hence, their utilization in soils has been increasing in the last few years due to the ease and speed of measurement and low analysis cost compared to other spectroscopic methods (MILORI et al., 2006).

In the present study we evaluated overall soil quality in ADEs and non-anthropic (REF) soils in three regions of central Amazonia under secondary forests with different ages of regeneration and agricultural fields, using chemical, physical and biological indicators, and identified the effects of soil macrofauna activity and land use on soil organic matter in various aggregate fractions, and its humification levels in ADEs and REF Amazonian soils.

4.4 MATERIAL AND METHODS

Were evaluated 18 paired sites, with nine ADEs and nine nearby REF soils in three Central Amazonian regions: Iranduba–AM, Belterra–PA and Porto Velho–RO, with three land-use systems (LUS): old forest (OF), i.e., secondary dense ombrophilous forest in an advanced stage of regeneration (> 20 years old), young forest (YF), i.e., dense ombrophilous forest in an early stage of regeneration (< 20 years old) and agricultural systems (AS), i.e., areas currently used for agricultural/pastoral proposes. In each site a 60m x 60m area within a 1 ha plot was chosen to assess chemical and physical soil properties and soil macrofauna communities (Supplementary Figure 1). More details on each sampling site can be found in Supplementary Table 1.

4.4.1 SOIL INVERTEBRATE SAMPLING

Macroinvertebrate communities were collected using a modified version of the standard Tropical Soil Biology and Fertility (TSBF) method (ANDERSON; INGRAM, 1993). In each site five soil monoliths with dimensions 25 x 25 cm and 30 cm depth (divided in 0 – 10, 10 – 20 and 20 – 30 cm layers) were dug, being four monoliths in the corners and one in the centre of the area (Supplementary Figure 1).

Soil invertebrates were hand-sorted from the monoliths in the field and fixed in 92% ethanol. In the laboratory, earthworms, ants and termites were identified to species or genus level, and other invertebrates were grouped into higher taxonomic levels (e.g., order and/or family). Less abundant taxonomic groups (accounting for <2% of total invertebrates collected) were grouped into a category of “Others”, that included the following taxa: Araneae, Hemiptera, Orthoptera, Diptera (larvae), Gastropoda, Dermaptera, Isopoda, Blattaria, Scorpionida, Opiliones, Lepidoptera (larvae), Uropygi, Solifuga, Thysanoptera, Geoplanidae, Neuroptera (larvae), Hirudinea and Embioptera. Total taxa richness and dominance (Simpson) were calculated per site, using abundance data.

4.4.2 SOIL ANALYSES

Soil samples for chemical and particle size analyses were collected after hand-sorting the TSBF monoliths from the three soil layers (0 – 10, 10 – 20 and 20 – 30 cm). The following soil properties: pH ($CaCl_2$); Ca^{2+}, Mg^{2+}, Al^{3+} (KCl 1 mol L^{-1}); K^+ and P (Mehlich-1) were obtained following standard Brazilian methods described in Teixeira et al. (2017); total nitrogen (TN) and total carbon (TC) were determined by dry combustion using an element analyser (Vario EL). To obtain the sum of bases (SB) and cation exchange capacity (CEC) standard formulae were used (TEIXEIRA et al., 2017). Values used were the means of all three layers (0 – 30 cm) from each site.

4.4.3 SOIL MACROMORPHOLOGY FRACTIONS

Five soil macromorphology samples were collected nearby each soil monolith (approximately 2 m distance) using a 10 × 10 × 10 cm metal frame. Soil samples were separated into four main aggregate fractions: non-aggregated/loose soil (NAS); physical aggregates (PA); root-associated aggregates (RA); and fauna-produced aggregates (FA), following the methodology proposed by Velasquez et al. (2007b). Each aggregate fraction was oven dried at 60°C for 24h and weighed, for further determination of the relative mass contribution (%). In order to assess the role of biological activity (roots and fauna) in affecting C and N distribution in the aggregate fractions, the samples from both YF and the pasture in Porto Velho were used to obtain TC and TN by dry combustion.

4.4.4 GENERAL INDICATOR OF SOIL QUALITY (GISQ)

All variables obtained were separated into three data sets: soil fertility (pH, SB, CEC, P, Al, P, TC, TN); soil physical properties (aggregate fractions obtained by macromorphology analysis; PA, FA, RA, NAS); and macrofauna data (density of earthworms, ants, termites, beetles, centipedes, millipedes, others and total fauna; total biomass, richness of taxa and dominance index). To generate the GISQ index four steps were followed (VELASQUEZ; LAVELLE; ANDRADE, 2007): (i) Principal Component Analysis (PCA) were performed using each data set separately; (ii) the variables related to soil quality were identified and given relative weights and directions (positive/negative relationships based on well-known good soil characteristics); (iii) sub-indicators were created and calculated (using inertia values/contribution of each variable to first two axes of the PCAs) for soil physical quality, chemical fertility and biological diversity, obtaining values between 0 and 1; (iv) all three sub-indicators were combined (using the respective inertia values/contribution of each subindicator to Factors 1 and 2 of the PCA), to obtain the general index of soil quality for each site, with values ranging from 0 (lowest quality) to 1 (highest quality).

4.4.5 LASER-INDUCED FLUORESCENCE SPECTROSCOPY (LIFS) OF SOIL AGGREGATES

LIFS analysis was performed on soil macroaggregate fraction (FA, PA, RA, NAS) from both YF and the pasture from Porto Velho. Two replicates of each soil macromorphology fraction (0.5 g) from each of the five samples were ground to pass through a 250-mm mesh, and them pressed (3-ton cm^{-2}) into pellets of 1 cm diameter and 2-mm thickness. These pellets were inserted into a locally-assembled apparatus to run LIF measurements. Both sides of the samples were excited with 351-nm ultraviolet radiation emitted by an Ar laser equipment (Coherent Innova 90–6, Coherent Inc., Santa Clara, CA) at Embrapa Instrumentation, in São Carlos, following the methodology proposed by Milori et al. (2006), totalling four measurements for each sample. The spectra generated by the fluorescence of aromatic structures of soil organic matter were obtained using locally-developed software. The area of organic matter fluorescence was calculated by the integration of the spectra between 475 nm – 800 nm. To calculated the humification index (H_{LIF}), we used the ratio between the area of organic matter fluorescence and the TC values.

4.4.6 Statistical analysis

Chemical, physical and biological sub-indicators, total carbon and nitrogen contents of soil macroaggregates fractions and the H_{LIF} values were submitted to Shapiro-Wilk normality test. Due to non-normal distribution of the data set, General linear models (GLM) were used to perform ANOVA. For the sub-indicators of soil quality and GISQ the ANOVA was performed considering a factorial design (2 × 3), with two soil categories (ADE and REF) and three LUS (OF, YF and AS). The H_{LIFs} and soil macroaggregate fractions data were analysed in a 2 × 2 factorial design, with two soil categories and two LUS (YF and AS). Factors with significant *P* values (<0.05) were tested using Tukey's HSD test (*P*<0.05). GLM and PCA analyses were performed in R software using the Multcomp (HOTHORN; BRETZ; WESTFALL, 2008) and ADE-4 packages (DRAY; DUFOUR, 2007), respectively.

4.5 RESULTS

The biological sub-indicator responded both to LUS and soil category, being higher in ADEs (0.68) than REF soils (0.52; mean of all land-use systems), and lower in AS (0.48-0.62) and YF (0.48-0.65) sites than in OF (0.61-0.77) in both soil categories (Table 1). The physical sub-indicator showed differences only between LUS, being higher in OF (0.82-0.84) and YF (0.76-0.78) than AS (0.46-0.55) sites (Table 1). The chemical sub-indicator showed higher mean values in ADEs (0.80) than REF soils (0.61), but little difference between LUS. The GISQ values were affected by both soil category and LUS (*P*< 0.05). Higher GISQ values were found on OF than YF and AS, and significant differences were found between YF and AS sites (Table 1). The overall soil quality was higher in ADEs than REF soils with 0.68 and 0.48, respectively.

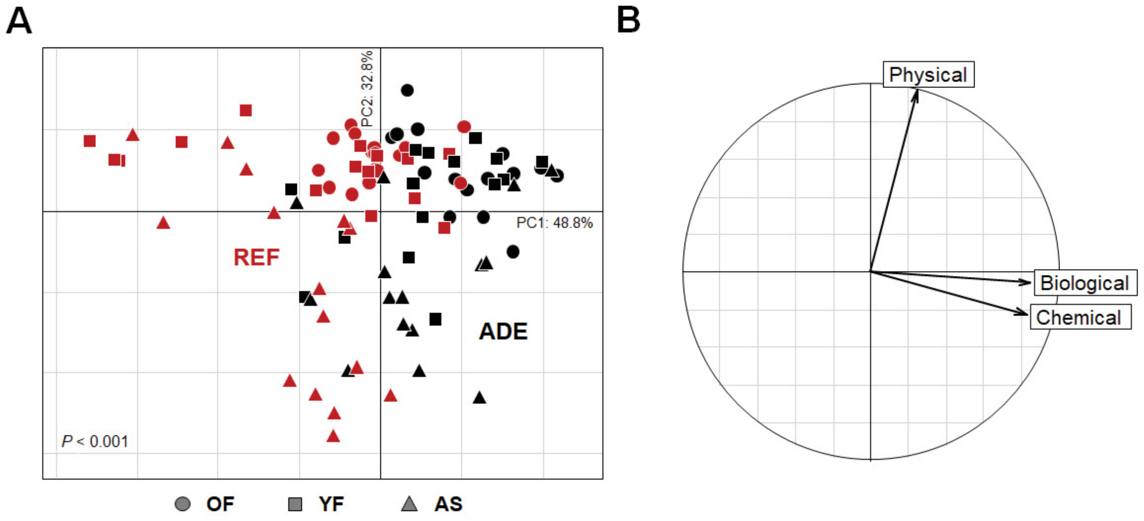
TABLE 1 - GENERAL INDICATOR OF SOIL QUALITY (GISQ) AND THE BIOLOGICAL, PHYSICAL AND CHEMICAL SUB-INDICATORS OF SOIL QUALITY IN THREE LAND-USE SYSTEMS IN AMAZONIAN DARK EARTHS (ADES) AND NON-ANTHROPIC/REFERENCE SOILS (REF) UNDER THREE LAND-USE SYSTEMS (OF: OLD FORESTS; YF: YOUNG FORESTS; AS: AGRICULTURAL SYSTEMS) IN CENTRAL AMAZONIA.

Land-use systems	**Subindicadors**						**GISQ**	
	Biological		**Physical**		**Chemical**			
	REF	ADE	REF	ADE	REF	ADE	REF	ADE
Old forest	0.61Ba*	0.77Aa	0.82Aa	0.84Aa	0.64Ba	0.77Aa	0.65Ba	0.82Aa
Young forest	0.48Bb	0.65Ab	0.78Aa	0.76Aa	0.58Ba	0.79Aa	0.51Bb	0.70Ab
Agricultural systems	0.48Bb	0.62Ab	0.46Ab	0.55Ab	0.61Ba	0.84Aa	0.28Bc	0.54Ac
Mean	0.52B	0.68A	0.68A	0.71A	0.61B	0.80A	0.48B	0.68A

*Different capital letters indicate statistical differences between soil category within each land-use system; small letters indicate statistical differences among the land-use systems within each soil category according to Tukey' test (*P*< 0.05).

The first two axes of the PCA (PC1 and PC2) performed using the physical, chemical and biological sub-indicators accounted for 81.6% of the explained variance (Fig. 1A). The PC1 was related with the biological and chemical sub-indicators, separating the two soil categories (ADE and REF) according to their chemical fertility (higher in ADEs) and macroinvertebrate populations (better in ADEs), while the PC2 was correlated with the physical sub-indicator, separating most AS from the secondary forest sites on the top of the factorial map (Fig. 1A).

FIGURE 1 - PRINCIPAL COMPONENT ANALYSIS (PCA) OF CHEMICAL, PHYSICAL AND BIOLOGICAL SUB-INDICATORS USED TO CALCULATED THE GENERAL INDICATOR OF SOIL QUALITY (GISQ) IN AMAZONIAN DARK EARTHS (ADES) AND NON-ANTHROPIC/REFERENCE SOILS (REF) UNDER THREE LAND-USE SYSTEMS (OF: OLD FORESTS; YF: YOUNG FORESTS; AS: AGRICULTURAL SYSTEMS) IN CENTRAL AMAZONIA, BRAZIL. SIGNIFICANCE OF THE MODEL (SOIL CATEGORY OR LAND-USE SYSTEMS) WERE OBTAINED USING MONTE-CARLO TEST (999 PERMUTATIONS).



The soil macroaggregates fractions showed similar contents of TC and TN independent of soil category and LUS, with no statistical differences them (Table 2). However, TC and TN contents were affected by soil category and LUS (P<0.05): mean values of TC were 23 and 34% higher in ADEs than in REF soils in the YFs and AS, respectively. Land use also affected TC, with around 70-85% more TC in YF (61-75 g kg^{-1} C) than AS (33 to 44 g kg^{-1} C), respectively (Table 2). As with soil carbon, TN contents were 28-53% higher in ADEs than REF soils. The LUS also affected TN, with 60-91% more N in YF (4-6.1 g kg^{-1}) than AS sites (2.5-3.2 g kg^{-1}). The C:N ratio showed a significant interaction between soil categories and LUS (P<0.05). In REF soils, YF sites had higher C:N ratio (15.0) than AS (12.9), while in ADEs the C:N ratio was higher in AS (13.5) compared to YF (12.2) (Table 2). Comparing the soil category within each LUS, in YF sites, REF soils had higher C:N ratio than ADEs, but in AS the C:N ratio was higher in ADEs compared to REF soils (Table 2).

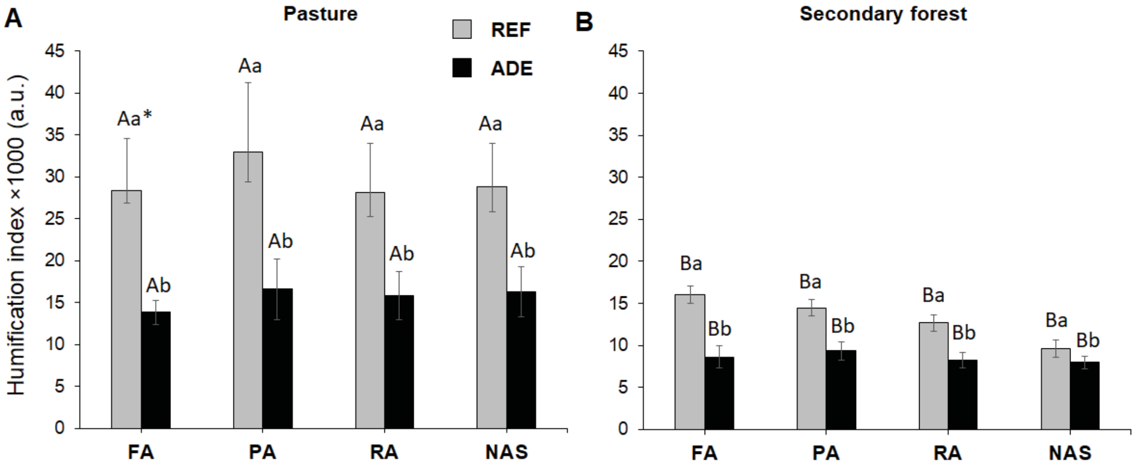
TABLE 2 - TOTAL CARBON AND NITROGEN CONTENTS AND C:N RATIO IN DIFFERENT SOIL MACROAGGREGATE FRACTIONS (FA=FAUNA PRODUCED-AGGREGATES; PA=PHYSICAL AGGREGATES; RA=ROOT-ASSOCIATED AGGREGATES; NAS=NON-AGGREGATED SOIL) IN TWO LAND-USE SYSTEMS IN AMAZONIAN DARK EARTHS (ADE) AND NON-ANTHROPIC/REFERENCE SOILS (REF) IN PORTO VELHO-RO.

Land-use	Soil	Aggregates[1]	Total carbon	SE	Total nitrogen	SE	C:N ratio	SE
Young forest	REF	FA	53.7^{ns}	±2.0	3.7^{ns}	±0.4	14.1^{ns}	±0.5
		PA	57.8	±1.7	3.8	±0.5	14.8	±0.6
		RA	62.7	±7.2	3.8	±0.3	16.2	±0.6
		NAS	69.1	±2.2	4.6	±0.4	14.8	±0.4
		Mean	**60.8 Ab**		**4.0 Ab**		**15.0 Aa**	
	ADE	FA	81.2	±4.1	6.5	±0.7	12.2	±0.3
		PA	69.3	±4.9	5.6	±0.4	12.3	±0.3
		RA	73.8	±6.2	6.0	±0.4	12.3	±0.3
		NAS	74.0	±4.4	6.2	±0.4	12.0	±0.2
		Mean	**74.5 Aa**		**6.1 Aa**		**12.2 Bb**	
Agricultural system	REF	FA	32.3	±2.8	2.5	±0.2	13.0	±0.4
		PA	31.3	±2.9	2.4	±0.2	12.8	±0.3
		RA	33.7	±3.1	2.5	±0.2	13.3	±0.4
		NAS	31.8	±2.7	2.6	±0.3	12.4	±0.4
		Mean	**32.8 Bb**		**2.5 Bb**		**12.9 Bb**	
	ADE	FA	47.6	±2.5	3.4	±0.1	13.8	±0.3
		PA	41.9	±5.4	3.1	±0.4	13.6	±0.2
		RA	43.9	±4.6	3.3	±0.3	13.4	±0.1
		NAS	41.7	±4.5	3.1	±0.3	13.3	±0.2
		Mean	**43.8 Ba**		**3.2 Ba**		**13.5 Aa**	

*Different capital letters indicate statistical differences between soil category within each LUS; small letters indicate statistical differences between the LUS within each soil category (*P*< 0.05); ns=No statistical differences found among aggregate fractions.

The Humification index (H_{LIF}) resulting from the LIFS analyses ranged from 7,571 to 32,992 arbitrary units (a.u.), and showed similar trends observed for TC results, being affected by both soil category and LUS (Fig. 2A, B). Higher H_{LIF} values were observed for REF areas (mean of 21,381 a.u.) compared to ADE soils (mean of 12,096 a.u.). The values of H_{LIF} obtained considering the land-use systems were significantly different with 22,607 and 12,460 a.u. for AS and YF sites, respectively. (Fig. 2A, B) Soil aggregates fractions showed no difference in H_{LIF} of SOM in both soil categories and the LUS evaluated (Fig. 2A, B).

FIGURE 2 - HUMIFICATION INDEX OF SOIL ORGANIC MATTER IN DIFFERENT SOIL MACROAGGREGATE FRACTIONS (FA=FAUNA PRODUCED-AGGREGATES; PA=PHYSICAL AGGREGATES; RA=ROOT-ASSOCIATED AGGREGATES; NAS=NON-AGGREGATED SOIL) IN: A) PASTURE SYSTEM AND B) SECONDARY FOREST IN EARLY STAGE OF REGENERATION (<20 YEARS OLD) IN AMAZONIAN DARK EARTHS (ADE) AND NON-ANTHROPIC/REFERENCE SOILS (REF) IN PORTO VELHO-RO.



*Different small letters indicate significant differences (*P*<0.05) between ADE and REF soils within soil aggregates fractions and LUS; capital letters indicate significant differences (*P*<0.05) between LUS within aggregate fractions and soil category. No statistical differences were found between aggregate fractions.

4.6 DISCUSSION

It is well known that pre-Columbian activities that generated ADEs have important positive impacts on soil fertility (LEHMANN et. al., 2003), which is why these soils are frequently used for farming (KAWA; RODRIGUES; CLEMENT, 2011). Here, we show that both pre-Columbian settlement (ADE vs REF) and modern land use have significant impacts on soil biological quality (sub-indicators) as well as TC, TC and C:N ratios in macroaggregates (mean of all fractions), while modern land use negatively affected only physical soil quality and pre-Columbian activities positively affected only chemical soil quality in ADEs and REF Amazonian soils. Hence, soil macroinvertebrate communities and soil C and N in aggregate fractions appeared to be more sensitive indicators of land use (modern and ancient), than physical and chemical variables.

Modern agricultural practices negatively affected soil biological quality, i.e. the soil macrofauna community (especially density and biomass), independent of the soil category (Table 1). This confirms results of Rousseau et al. (2013), who also observed high sensitivity of biological sub-indicators to land-use modification using GISQ in tropical dry forests. Negative effects of human disturbance on soil invertebrate communities are well known, and have been reported in several previous studies across the Amazonian rainforest (DECAËNS et al., 2004, 2018; FRANCO et al., 2018; MATHIEU et al., 2005; ROUSSEAU; SILVA; CARVALHO, 2010). Lower macroinvertebrate abundance and diversity in agricultural fields, are usually associated with intense soil disturbance (e.g. management practices in conventional tillage agriculture), pesticide use, as well as habitat simplification particularly with monoculture cropping. The simplification and reduction of the litter layer, commonly used as home for a large number of epigeic invertebrates (e.g. millipedes, centipedes, some species of earthworms and ants, etc.) is also detrimental (SANTOS; FRANKLIN; LUIZAO, 2008; VOHLAND; SCHROTH, 1999).

The biological sub-indicator also showed higher values in ADEs than REF soils (Table 1), indicating their positive response to the higher fertility (pH, C, N, plant available nutrients) of ADEs. Several soil fauna groups particularly millipedes and earthworms had higher populations in ADEs than REF soils (Supplementary Table 5), and these organisms are known to positively respond to food (organic C and N resources) availability (BROWN, et al., 2003; SNYDER; BOOTS; HENDRIX, 2009) that is higher in ADEs (Table 2). The formation processes of ADEs involved intense human disturbance over long time-periods, and may have unintentionally selected more resistant/plastic or opportunist invertebrates. The colonization and invasion of opportunistic and exotic species after forest clearance has been frequently observed in Amazonian soils, especially by earthworms (CHAUVEL et al., 1999; MARICHAL et al., 2010), that have received more attention compared to other soil macroinvertebrates. In fact, the invasive earthworm species *Pontoscolex corethrurus*, found throughout the tropics and sub-tropics (TAHERI; PELOSI; DUPONT, 2018) was frequent in several of the ADE sites sampled (see Chapter II; CUNHA et al., 2016), and this species is known to have important impacts on soil properties and processes, including macroaggregate formation (BAROIS et al., 1993; BROWN et al., 1999; SÁNCHEZ-DE LÉON et al., 2014; TAHERI; PELOSI; DUPONT, 2018).

As observed by many other authors before (KERN et al., 2017; WOODS et al., 2009) chemical soil quality was higher in ADE than REF soils (Table 1) surpassing even the effects of fertilization practices utilized in AS sites. This is due to the higher values of pH, exchangeable cations (Ca and Mg) and P found in ADEs (Figure 2 on Chapter II and Supplementary Table 2), used to calculate the chemical sub-indicator. The slash and burn practices that added large amounts of Ca and Mg oxides, as well as the discarding of high amounts of bone residues and pottery in ADEs slowly increased soil pH and also the availability of various nutrients in ADEs, in contrast to the natural REF Amazonian soils (ARROYO-KALIN, 2010; SMITH, 1980; LIMA et al., 2002). The lack of differences in the chemical sub-indicator for land-use system was not expected, especially in REF soils, due to the low chemical fertility commonly observed in Amazonian soils and the high fertilizer applications normally used in annual crops, especially maize and soybean fields (LUIZÃO et al., 2009; RISKIN et al., 2013).

The physical soil quality was affected only by LUS, with lower quality in AS compared to YF and OF (Table 1). Forest soils tend to many large, water stable macro-aggregates, due to intense biological activities (fauna pedoturbation) and high soil organic matter contents (LEE; FOSTER, 1991). The AS studied here were managed with conventional tillage (maize), reduced tillage (soybean) and no-tillage (pasture), but their macroaggregate contents belied poorer soil structure (PAUSTIAN et al., 2000) compared with the forest soils. Interestingly, although ADEs had less non-aggregated soil and more fauna macroaggregates than REF soils (Supplementary Table 8), this did not reflect in higher physical quality in ADEs vs. REF soils (Table 1). This is also despite the higher C contents in both bulk soil (Figure 2, chapter 1) and in total soil macroaggregates in ADEs (Table 2).

The overall soil quality (GISQ) in ADE sites was significantly higher than REF soils, and in OF than YF and AS (Table 1), implying that both pre-Columbian activities and modern agriculture have important impacts on soil quality. This indicator has been successfully used to compare overall soil quality between natural ecosystems and agricultural fields in other Amazonian sites (VELÁSQUEZ et al., 2012; LAVELLE et al., 2017). Therefore, although there are few published studies using the GISQ, we were able to confirm its' usefulness to differentiate soil quality in different LUS and soil types. Furthermore, it may also be useful for monitoring soil quality changes over time (VELASQUEZ; LAVELLE; ANDRADE, 2007).

Macroaggregates associated with plant roots (RA) are expected to have higher C contents, due to the plant root sloughing off and mucilage production (TRAORÉ et al., 2008). Furthermore, biogenic aggregates associated with bioturbating soil fauna (FA) also often have higher C and N contents (LOSS et al., 2017), due to selective ingestion of soil particles by earthworms and selective feeding on C-rich food sources (fresh or decomposing organic matter), and relatively low assimilation efficiencies (LAVELLE et al., 2001). On the other hand, physical macroaggregates, produced to due physical soil phenomena (PA), and loose soil (NAS) are not expected to have higher C or N contents than the bulk soil. However, TC and TN contents as well the C:N ratio in the biogenic macroaggregate fractions (FA, RA) were not different than those of NAS and PA. Furthermore, the TC and TN contents of the individual macroaggregate fractions was not higher in ADEs than in REF soils, despite the higher overall content of C (but not N; Supplementary Table 2) in ADEs. Land use (YF vs. AS) also had no effect on the TC and TN contents of the different macroaggregate fractions. However, the mean TC and TN values of all macroaggregate fractions combined was significantly higher in ADEs than REF soils, and in YF than AS (Table 2). The reduction in SOM contents in AS compared to forests is well-known (BONINI et al., 2018; FIGUEIRA et al., 2016), and is with higher SOM mineralization rates in AS, as well as lower inputs of organic matter in these systems.

The role of soil fauna in C and N cycling, is highly dependent on their bioturbation activities, but also on their ecological category (LAVELLE et al., 1997). Litter feeding epigeic and anecic species tend to have a much more important role in litter decomposition and incorporation into the soil, leading to higher C and N contents in their faeces (BROWN et al., 2001). On the other hand, endogeic species produce much larger amounts of egesta (LAVELLE et al., 1997), but tend to contribute more towards C sequestration in their castings (BROWN et al., 2001), particularly in seasonally dry habitats (MARTIN et al., 1992). The large amount of FA found in the present study (Supplementary Table 8), indicates a prevalence of endogeic species, particularly in OF and YF, where the proportion of FA and the physical sub-indicator values were higher (Table 1).

H_{LIF} values were higher in REF soils compared to ADEs (Figure 2A, B), indicating lower chemical recalcitrance of SOM in anthropic soils. This result was unexpected, considering the high concentration of aromatic structures in charcoal, widely found in ADEs (GLASER et al., 2003). Several hypotheses have been proposed to explain the high resilience of soil organic C in ADEs and the presence of charcoal in these soils was pointed out as a critical factor for the maintenance of soil

C over centuries in these soils (GLASER et al., 2003, 2014). On the other hand, the lower H_{LIF} values in ADEs in this study indicates that other mechanisms are acting to stabilize OM in these soils, such as occlusion and organic-mineral interactions, which are considered the most important mechanisms for long-term stabilization of SOM (LEHMANN; KLEBER, 2015).

Land use was also important for SOM recalcitrance (Figure 2b), with relatively higher concentration of aromatic structures in SOM from AS compared with YF. This suggests that agricultural land-use changed the dynamics of SOM turnover. Alterations in the H_{LIF} of SOM due to land-use changes were also verified by Dieckow et al. (2009) and Bordonal et al. (2017). However, the differences related to H_{LIF} observed in this study do not indicate that the total of recalcitrant structures (e.g. lignin, suberin, etc.) in REF are higher than in ADEs, but rather suggest that the proportion of these structures are higher in SOM from natural soils and pasture systems compared to anthropic soils and secondary forests, respectively. The lower soil fauna density and diversity in AS (Supplementary table 5) may also affect OM decomposition rates, especially due to the reduction in detritivores such as millipedes and earthworms that are important for organic matter breakdown and incorporation into the soil (BROWN et al., 2018; SILVA et al., 2017). In fact, this study highlights how land use can change SOM dynamics and turnover, increasing chemical recalcitrance in disturbed soils and decrease soil carbon values (and likely their stocks as well) in disturbed environments compared to sites with native vegetation in ADEs, probably due to the low contribution of SOM recalcitrance compared to organic-mineral interactions and occlusion to SOM stabilisation processes in these soils (LEHMANN; KLEBER, 2015). However, further studies are needed to clarify this question, and reveal the relative importance of different SOM stabilization processes acting in anthropic Amazonian soils. Considering the importance of biological agents such as endogeic fauna (e.g., earthworms), to soil aggregate formation in these soils, studies involving the potential role of fauna in SOM stabilization processes will be of particular interest.

4.7 CONCLUSIONS

Overall soil quality is higher in ADES than REF soils, due to improvement in chemical and biological soil properties in anthropogenic soils, indicating that long-term human occupations in Amazonia left long-lasting footprints on soil biology and fertility. However, modern human disturbance with annual cropping and cattle pasture production reduces the overall soil quality, and particularly soil physical and biological properties; this phenomenon occurs in both ADE and REF soils. Soil macroaggregates (particularly biogenic aggregates) are important components of ADEs, and their TC and TN contents are also higher in ADEs than REF soils, but again modern land use (AS) enhances degradation of both soil types. Land use also changes SOM humification, increasing the proportion of chemical recalcitrance in pastures. Additionally, H_{LIF} of SOM also is g lower in ADEs than REF soils, although further work is warranted on these latter topics, particularly to assess the role and interaction of fauna with land use, and the relative importance of various SOM stabilization mechanisms, especially in ADEs.

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5 GENERAL CONCLUSION

Amazonian dark earths are unique habitats, created over time by pre-Columbian settlements in the Amazonian rainforest. The contrasting botanical characteristics and management history of these archaeological sites led to a high quality chemically fertile and well aggregated soil that hosts diverse macroinvertebrate communities, with a large number of unique species. The different invertebrate communities in ADEs can alter ecosystem services in these soils, increasing the contribution of ecosystem engineering animals (particularly earthworms) to soil aggregation, and altering SOM humification processes compared to REF. While ancient Amerindian activities that created ADEs generally have positive impacts on soil quality and biodiversity, modern agriculture has devastating effects on these attributes, highlighting the need for proper management practices in order to promote their sustainable use. However, as these are only the first results of soil macrofauna communities and soil quality in ADEs, further research is needed in order to ascertain if the results found here represent a generalized phenomenon over much of Amazonia and across other ADEs.

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SUPPLEMENTARY TABLE 1 – GENERAL GEOGRAPHIC, SOIL AND LAND USE INFORMATION ON THE SAMPLING SITES

Supplementary Table 1. General geographic, soil and land use information on the sampling sites. Land use system, age of modern human intervention, soil type, soil category according to WRV/FAO (2015) and location of the sites studied in the three regions of Brazilian Amazonia. Dark green colour represents old forests, pale green colour represents young forests and yellow colour represents agricultural systems.

Region	State	Land use	Human intervention[1]	Soil type[2]	Soil category (WRB)	Coordinates
Iranduba	AM	Old Forest	> 20 years old	REF	Xanthic Dystric Acrisol	3°14'49.00"S, 60°13'30.71"W
		Old Forest	> 20 years old	ADE	Pretic Clayic Anthrosol	3°15'11.05"S, 60°13'45.03"W
		Young Forest	< 20 years old	REF	Xanthic Dystric Acrisol	3°13'34.47"S, 60°16'23.60"W
		Young Forest	< 20 years old	ADE	Pretic Clayic Anthrosol	3°13'49.23"S, 60°16'7.43"W
		Agricultural	Current	REF	Xanthic Dystric Acrisol	3°13'31.31"S, 60°16'29.18"W
		Agricultural	Current	ADE	Pretic Clayic Anthrosol	3°13'46.13"S, 60°16'7.32"W
Belterra	PA	Old Forest	> 20 years old	REF	Xanthic Dystric Ferralsol	2°47'4.59"S, 54°59'53.28"W
		Old Forest	> 20 years old	ADE	Pretic Clayic Anthrosol	2°47'3.25"S, 54°59'59.77"W
		Old Forest	> 20 years old	REF	Xanthic Dystric Acrisol	2°41'13.90"S, 54°55'3.30"W
		Old Forest	> 20 years old	ADE	Pretic Clayic Anthrosol	2°41'7.18"S, 54°55'7.11"W
		Agricultural	Current	REF	Xanthic Dystric Acrisol	2°41'3.56"S, 54°55'12.75"W
		Agricultural	Current	ADE	Pretic Clayic Anthrosol	2°41'3.79"S, 54°55'7.90"W
Porto Velho	RO	Young Forest	< 20 years old	REF	Xanthic Dystric Plinthosol	8°52'11.50"S, 64° 3'18.16"W
		Young Forest	< 20 years old	ADE	Pretic Clayic Anthrosol	8°51'51.92"S, 64°03'48.03"W
		Young Forest	< 20 years old	REF	Xanthic Dystric Ferralsol	8°50'49.52"S, 64° 3'59.20"W
		Young Forest	< 20 years old	ADE	Pretic Clayic Anthrosol	8°51'1.18"S, 64° 4'3.07"W
		Agricultural	Current	REF	Xanthic Dystric Ferralsol	8°52'35.30"S, 64°03'58.58"W
		Agricultural	Current	ADE	Pretic Clayic Anthrosol	8°51'56.53"S, 64°03'40.67"W

[1] Age of modern human disturbance (land management);

[2] REF –reference soil, ADE – Amazonian Dark Earth

SUPPLEMENTARY TABLE 2 – SOIL ANALYSES

Supplementary Table 2. Soil analyses from the topsoil layers (0-30 cm depth) in reference (REF) and Amazonian Dark Earth (ADE) soils under each of the land-use systems (OF: old forests, YF: young forests, AS: agricultural systems). Values represent means from the three study regions.

Soil type	Land use	Al[1]	K[1]	CEC[1]	Base saturation[1]	Total N[1]	Sand[2]	Silt[2]	Clay[2]	Texture class (FAO)
		cmol$_c$ kg^{-1}				%				
REF	OF	2.3±0.14Aa	0.06±0.01Ab	3.16±0.22Ba	7.6±2.1Ba	0.22±0.01Aab	23±7^{ns}	15±2Ab	62±5Aa	Heavy clay
	YF	2.8±0.09Aa	0.05±0.0Aab	2.89±0.09Ba	1.2±0.1Bb	0.27±0.03Aa	26±4	22±2Aa	52±2Ab	Clay
	AS	1.6±0.22Aa	0.09±0.01Aa	3.16±0.34Ba	19.5±5.7Ba	0.21±0.01Ab	21±4	19±9Aa	60±3Aa	Heavy clay
ADE	OF	0.5±0.20Ba	0.07±0.01Ab	8.76±0.78Aa	55.1±4.9Aa	0.28±0.02Aab	23±5	17±2Ab	60±4Aa	Heavy clay
	YF	0.6±0.13Ba	0.10±0.01Aab	8.85±1.0Aa	49.2±4.6Aa	0.36±0.03Aa	27±2	23±2Aa	50±2Ab	Clay
	AS	0.4±0.11Ba	0.12±0.02Aa	6.95±0.69Aa	50.3±4.8Aa	0.25±0.01Ab	19±3	22±1Aa	59±2Aa	Clay

*Upper case letters compare soil categories (ADE vs. REF), within each land-use system, while lower-case letters compare land use systems within the same soil type (ADE or REF). Different letters mean significant differences resulting from GLM or Kruskal-Wallis non-parametric tests. [1]GLM; [2]KW.

SUPPLEMENTARY TABLE 3 – MORPHOSPECIES RICHNESS

SUPPLEMENTARY TABLE 3. MORPHOSPECIES RICHNESS OF THE MAIN SOIL MACROINVERTEBRATE TAXA AND GROUPS. NUMBER OF SPECIES/MORPHOSPECIES OF EACH OF THE MAJOR SOIL MACROINVERTEBRATE TAXA COLLECTED IN THE SOIL MONOLITHS (LITTER AND 0-30 CM), IN EACH OF THE THREE MAIN LAND-USE SYSTEMS (OF: OLD FORESTS, YF: YOUNG FORESTS, AS: AGRICULTURAL SYSTEMS) IN BOTH REFERENCE (REF) AND AMAZONIAN DARK EARTH (ADE) SOILS (SUM OF ALL THREE REGIONS), AND TOTAL OBSERVED RICHNESS OF EACH TAXON COLLECTED OVER ALL SAMPLES. TOTAL NUMBERS DO NOT ALWAYS REPRESENT SUM OF ALL SPECIES IN EACH LAND-USE SYSTEM DUE TO SHARED SPECIES/MORPHOSPECIES.

Group	Number of morphospecies collected (soil monoliths)								
	REF				ADE				Overall observed richness
	OF	YF	AS	Total	OF	YF	AS	Total	
Earthworms	12	9	4	22	11	10	7	22	32
Termites	13	20	9	32	9	12	0	16	37
Ants	58	41	18	94	58	35	24	93	154
Beetles	16	21	15	47	25	11	12	43	78
Spiders	22	15	10	47	21	15	10	44	86
Millipedes	12	9	7	25	14	23	5	37	53
Centipedes	8	8	2	14	10	5	3	11	17
True bugs	9	5	11	24	8	11	4	21	42
Cockroaches	11	7	2	20	8	12	1	20	34
Opiliones	6	8	0	14	5	5	0	9	21
Isopods	6	6	1	11	7	5	2	13	21
Snails	3	2	2	7	7	3	2	12	17
Others*	20	14	10	42	25	14	7	44	75

*This group includes Orthoptera, Diptera (larvae), Dermaptera, Scorpionida, Lepidoptera (larvae), Uropygi, Solifuga, Thysanoptera, Geoplanidae, Neuroptera (larvae), Hirudinea and Embioptera

SUPPLEMENTARY TABLE 4 –SINGLETON, DOUBLETON, RARE, AND ABUNDANT SPECIES/MORPHOSPECIES

Supplementary Table 4. Number of singleton, doubleton, rare, and abundant species/morphospecies of all soil macroinvertebrate taxa and of selected macrofauna taxa collected in reference (REF) and Amazonian Dark Earth (ADE) soils (sum of all three regions and land use systems). Rare species represent taxa with fewer than 10 ind. over all samples. Non-rare and abundant species represent taxa with ≥10 ind. over all samples. Unique species were found in either ADE or REF; shared species were found in both soil categories. Species more abundant were considered to be those with quantities at least three times greater in ADE or REF soils, respectively. Locally distributed species were those found only in one region but in more than one land-use system. Widely distributed species were those found in more than one region.

Soil type	Taxon	Singletons	Doubletons		Rare		Non-rare or Abundant						
			Unique	Shared	Unique	Shared	Unique	Shared	More abundant in REF	Locally distributed (Total)	More abundant in REF	Widely distributed (Total)	More abundant in REF
REF													
	Ants	29	8	2	15	11	8	20	6	12	2	16	4
	Termites	6	3	-	7	3	8	5	2	9	1	4	3
	Earthworms	5	-	-	5	2	-	10	-	7	-	3	-
	Beetles	28	4	3	4	4	-	4	1	1	1	3	-
	Millipedes	5	3	-	8	4	-	5	2	4	2	1	-
	Total invertebrates	171	34	14	61	42	19	56	13	48	4	27	9
ADE									More abundant in ADE		More abundant in ADE		More abundant in ADE
	Ants	26	10	2	17	11	8	20	7	9	2	19	5
	Termites	-	2	-	2	3	1	5	1	2	-	4	1
	Earthworms	2	2	-	3	2	2	10	7	1	5	3	2
	Beetles	20	4	3	4	4	1	4	1	1	-	3	1
	Millipedes	14	6	-	6	4	2	5	1	6	1	1	-
	Total invertebrates	157	43	14	52	42	18	56	19	44	9	30	10

SUPPLEMENTARY TABLE 5 – SOIL MACROINVERTEBRATE DENSITY AND BIOMASS

Supplementary Table 5. Soil macroinvertebrate density and biomass. Mean density (Den.; number of individuals m^{-2}) and biomass (Bio.; fresh mass in g m^{-2}) of the main soil macroinvertebrate taxa collected in each of the land-use systems (OF: old forests, YF: young forests, AS: agricultural systems) studied in reference (REF) and Amazonian Dark Earth (ADE) soils. Different lower-case letters indicate significant differences between land-use systems within the same soil type, while different upper-case letters indicate significant differences between soil categories within each land-use system. Significance determined using GLMs or Kruskal-Wallis (KW) non-parametric tests.

GROUPS		REF			ADE		
		OF	YF	AS	OF	YF	AS
Earthworms[1]	Den.	152.5±28.2Aa	60.8±17.5Ba	44.8±12.4Bb	193.1±29.7Aa	268.8±35.2Aa	234.7±58.5Aa
	Bio.	19.34±4.48Aa	10.30±3.46Bab	3.58±1.09Bb	13.39±3.15Aa	27.34±5.11Aa	18.07±7.99Aa
Termites[2]	Den.	1758.9±989.2Aa	1057.1±365.2Aa	1241.6±858Aa	141.9±123.9Bab	168.5±80.3Ba	1.1±1.0Bb
	Bio.	3.87±2.58Aa	2.58±0.99Aa	2.11±0.96Aa	0.17±0.14Bab	0.34±0.18Ba	0.00±0.0Bb
Ants[1]	Den.	476.8±158.1Aa	210.1±69.4Aa	137.6±60.3Aa	416.0±111.9Aa	231.5±80.3Aa	533.3±316.3Aa
	Bio.	0.68±0.21Aa	0.41±0.11Aa	0.14±0.04Ab	0.76±0.26Aa	0.54±0.22Aa	0.47±0.19Aa
Ecosystem engineers[1]	Den.	2388.3±956.4Aa	1328±362.2Aa	1424±367.7Aa	750.9±193.9Ba	668.9±172.7Ba	769.1±198.6Ba
	Bio.	23.89±5.66Aa	13.28±3.41Bab	5.83±1.41Ab	14.32±3.08Aa	28.22±5.03Aa	18.54±7.96Aa
Beetles[1]	Den.	52.3±9.6Ba	58.7±11.2Aa	57.6±16.4Aa	137.6±33.0Aa	52.3±14.0Ab	21.3±6.0Bb
	Bio.	1.73±1.07Ba	2.60±1.18Aa	0.58±0.18Aa	3.11±0.96Aa	0.58±0.20Ab	0.21±0.11Ab
Millipedes[2]	Den.	25.6±8.8Aa	24.5±6.6Ba	52.3±20.7Aa	37.3±6.0Aab	97.1±32.1Aa	13.9±5.2Ab
	Bio.	1.02±0.50Aa	0.24±0.07Ba	0.75±0.29Aa	0.63±0.21Aab	3.50±1.54Aa	0.24±0.10Ab
Centipedes[2]	Den.	50.1±11.6Aa	38.4±9.0Aa	4.3±1.8Ab	67.2±10.3Aa	58.7±20.4Aab	19.2±8.2Ab
	Bio.	0.49±0.23Aa	0.29±0.09Aa	0.03±0.01Ab	0.27±0.05Aa	0.31±0.11Aab	0.11±0.06Ab
Others[2]*	Den.	103.5±13.5Ba	58.7±10.3Aa	106.7±30.4Aa	168.5±28.7Aa	113.1±28.7Aab	68.3±22.1Ab
	Bio.	6.47±2.29Aa	0.34±0.10Bb	0.94±0.37Ab	3.82±0.78Aa	1.23±0.34Ab	0.61±0.20Ab
Total[2]	Den.	2619.7±951.4Aa	1508.3±358.5Aa	1644.8±851Aa	1161.6±187.5Aa	989.9±156.4Aa	891.7±318Aa
	Bio.	33.59±6.97Aa	16.75±3.69Bab	8.13±1.35Ab	22.15±3.65Aa	33.84±5.44Aa	19.72±7.82Aa

[1] GLM; [2] KW; *This group includes Orthoptera, Diptera (larvae), Dermaptera, Hemiptera, Isopoda, Blattaria, Gastropoda, Lepidoptera (larvae), Uropygi, Solifuga, Opiliones, Scorpionida, Thysanoptera, Geoplanidae, Neuroptera (larvae), Hirudinea, and Embioptera

SUPPLEMENTARY TABLE 6 – EFFECTS OF LAND-USE SYSTEMS ON BETA-DIVERSITY

Supplementary Table 6. Effects of land-use systems (LUS) and soil type (REF and ADE) on β-diversity (without singletons), species turnover rates and nestedness of total soil macrofauna (339 morphospecies), ants, termites and earthworm communities. Richness values used for the calculations are from the soil monoliths (TSBF). REF: Reference soil, ADE: Amazonian Dark Earth, OF: old forests, YF: young forests, AS: agricultural systems.

	Max div. ($\beta_{Sorensen}$)	Turnover ($\beta_{Simpson\ dis.}$)	Nestedness
Macroinvertebrates			
LUS effect			
on REFs	0.86	0.79	0.07
on ADEs	0.83	0.74	0.09
Soil effects			
in OF	0.72	0.70	0.02
in YF	0.70	0.67	0.03
in AS	0.77	0.71	0.06
Ants			
LUS effect			
on REFs	0.86	0.72	0.14
on ADEs	0.83	0.75	0.08
Soil effects			
in OF	0.80	0.78	0.28
in YF	0.80	0.74	0.06
in AS	0.78	0.67	0.11
Termites			
LUS effect			
on REFs	0.81	0.65	0.16
on ADEs	0.81	0.39	0.42
Soil effects			
in OF	0.79	0.72	0.07
in YF	0.64	0.53	0.12
in AS	-	-	-
Earthworms			
LUS effect			
on REFs	0.90	0.84	0.06
on ADEs	0.72	0.62	0.10
Soil effects			
in OF	0.34	0.11	0.23
in YF	0.50	0.38	0.13
in AS	0.78	0.67	0.11

SUPPLEMENTARY TABLE 7 – EFFECTS OF REGION ON BETA-DIVERSITY

Supplementary Table 7. Effects of region on Beta diversity (without singletons), species turnover rates and nestedness of total soil macrofauna (339 morphospecies), ants, termites and earthworm communities, among each land-use system (OF: old forest; YF: young forest; AS: agricultural systems) within each soil type (REF and ADE). Richness values used for the calculations are from the soil monoliths (TSBF). REF: Reference soil, ADE: Amazonian Dark Earth.

	Max div. ($\beta_{Sorensen}$)	Turnover ($\beta_{Simpson\ dis.}$)	Nestedness
Macroinvertebrates			
Region effect			
OF - REF	0.85	0.81	0.04
ADE	0.82	0.81	0.01
YF - REF	0.86	0.81	0.05
ADE	0.85	0.79	0.06
AS - REF	0.91	0.90	0.01
ADE	0.90	0.86	0.04
Ants			
Region effect			
OF - REF	0.81	0.71	0.10
ADE	0.83	0.80	0.03
YF - REF	0.89	0.82	0.07
ADE	0.80	0.73	0.07
AS - REF	0.81	0.66	0.15
ADE	0.75	0.68	0.07
Termites			
Region effect			
OF - REF	0.82	0.72	0.10
ADE	0.83	0.75	0.08
YF - REF	0.71	0.63	0.08
ADE	0.81	0.77	0.04
AS - REF	0.88	0	0.88
ADE	-	-	-
Earthworms			
Region effect			
OF - REF	0.69	0.60	0.09
ADE	0.75	0.70	0.05
YF - REF	1	1	0
ADE	0.77	0.75	0.01
AS - REF	1	1	0
ADE	1	1	0

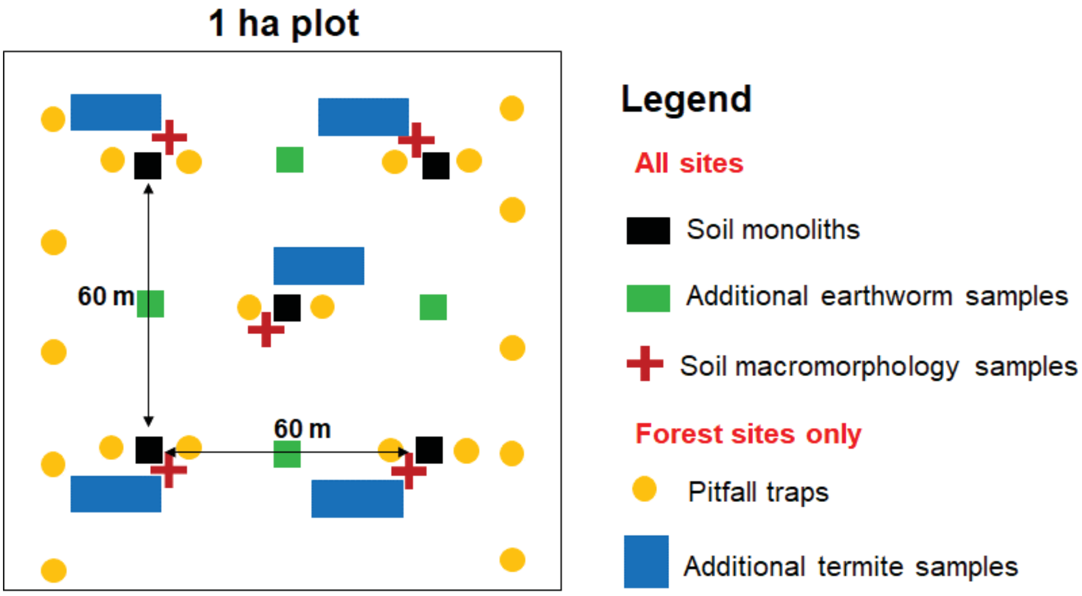
SUPPLEMENTARY TABLE 8 – AGGREGATE FRACTIONS FROM THE MICROMORPHOLOGY SAMPLES

Supplementary Table 5. Aggregate fractions from the micromorphology samples. Mean relative biomass (%) of the different aggregate fractions found in the soil macromorphology samples (0-10 cm) in each of the land-use systems (OF: old forests, YF: young forests, AS: agricultural systems) in reference (REF) and Amazonian Dark Earth (ADE) soils. Fractions measured were biogenic aggregates produced by ecosystem engineers (Fauna), rhizosphere aggregates (Root), physical aggregates (Physical), non-macroaggregated loose soil particles and unidentified aggregates less than 5 mm in size (NAS), coarse organic material such as leaves, roots, seeds, and woody pieces (Organic materials), soil invertebrates (Invertebrates), pottery shards (Pottery), stones and charcoal. Different lower-case letters indicate significant differences between land-use systems within the same soil type, while upper-case letters compare soil categories within each land-use system. Mean comparisons performed using ANOVA, GLMs or Kruskal-Wallis (KW) non-parametric tests.

Aggregate fraction	**REF**			**ADE**		
	OF	**YF**	**AS**	**OF**	**YF**	**AS**
Fauna[1]	43.6±5.3Ba	22.6±1.8Bb	14.2±1.1Bb	48.7±5.4Aa	28.8±0.4Ab	23.6±0.2Ab
Root[2]	4.4±1.1Aab	8.3±1.4Aa	5.9±2.1Ab	5.1±1.5Aab	10.5±2.4Aa	8.9±3.4Ab
Physical[2]	0.7±0.5Ac	7.5±1.6Ab	27.5±3.6Aa	0.0±0.0Ac	9.6±2.6Ab	20.3±2.7Aa
NAS*	41.7±5.4Ab	52.5±2.1Aa	50.4±3.0Aab	36.5±2.8Bb	45.9±2.9Ba	42.7±4.2Bab
Organic materials[1]	8.3±1.0Aa	4.0±1.0Ab	1.9±0.4Ab	5.8±1.0Aa	2.0±0.4Ab	1.9±0.3Ab
Invertebrates[2]	1.0±0.4^{ns}	0.0±0.0	0.1±0.4	1.4±0.8	0.0±0.0	0.2±0.1
Pottery[2]	0.2±0.2^{ns}	0.0±0.0	0.0±0.0	1.8±0.6	1.0±0.3	2.3±0.9
Stones[2]	0.0±0.0^{ns}	4.4±2.1	0.0±0.0	0.5±0.3	2.1±2.0	0.1±0.1
Charcoal[2]	0.20±0.2^{ns}	0.59±0.3	0.00±0.0	0.003±0.0	0.06±0.1	0.01±0.0

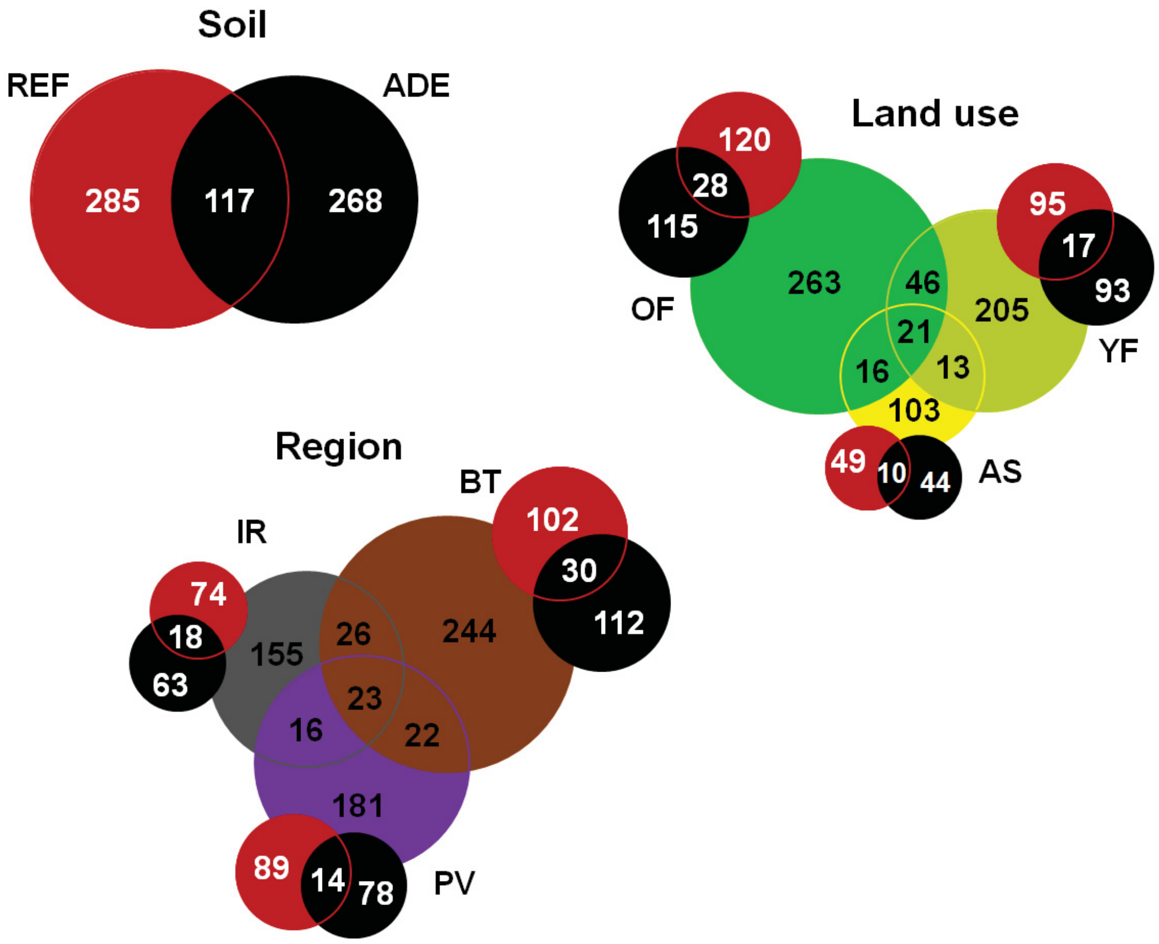
[1] GLM; [2] KW; *ANOVA

SUPPLEMENTARY FIGURE 1 – SAMPLING DESIGN



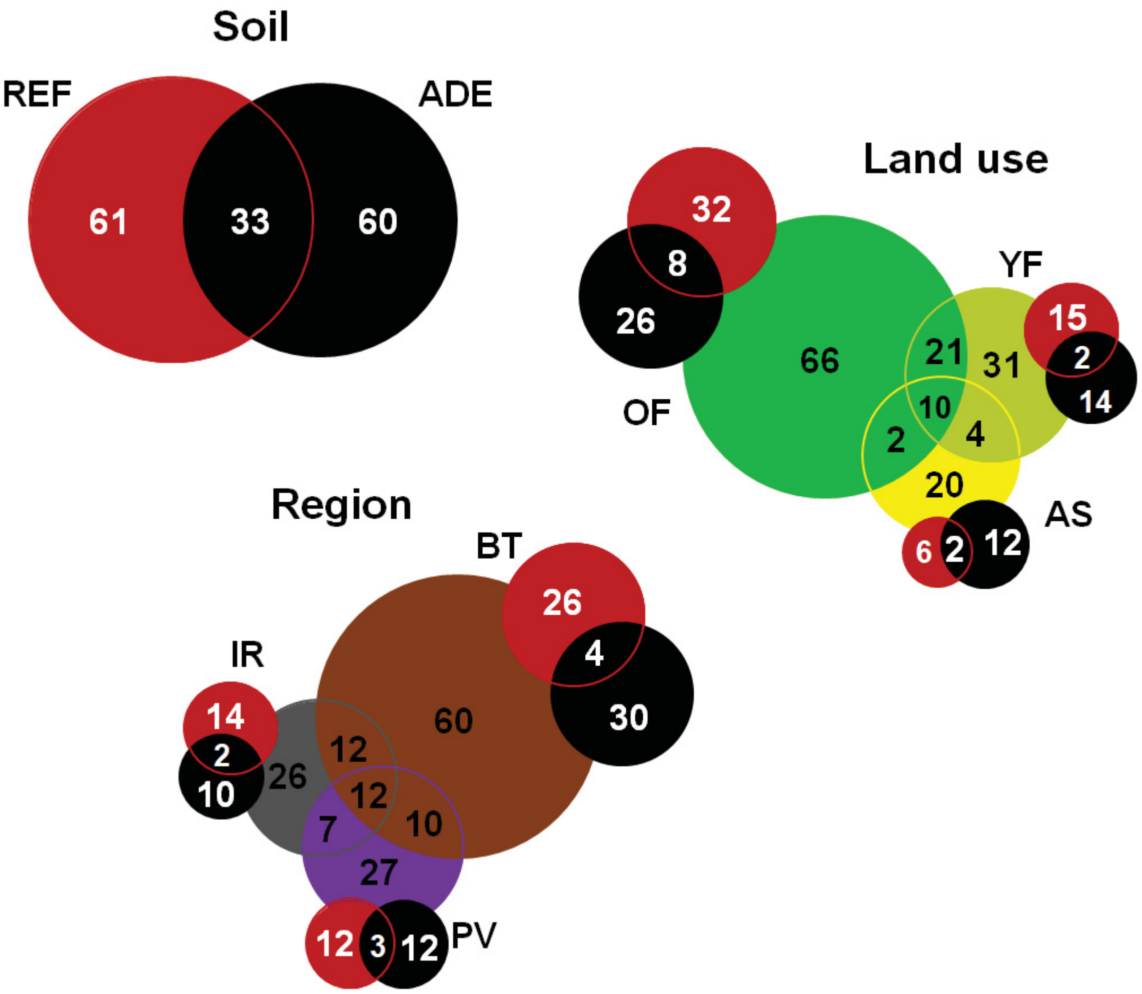
Supplementary Figure 1. Scheme used for soil and fauna sampling for each plot in each land use system. Distribution of the different samples in the 1 ha plot at all sites, showing the types of samples taken: monoliths for soil fertility and total soil macrofauna, soil macromorphology samples and additional samples for earthworms, and forest-only samples for termites (2 x 5 m plots) and ants (pitfall traps).

SUPPLEMENTARY FIGURE 2 – VENN CHARTS OF TOTAL MACROINVERTEBRATES SPECIES RICHNESS



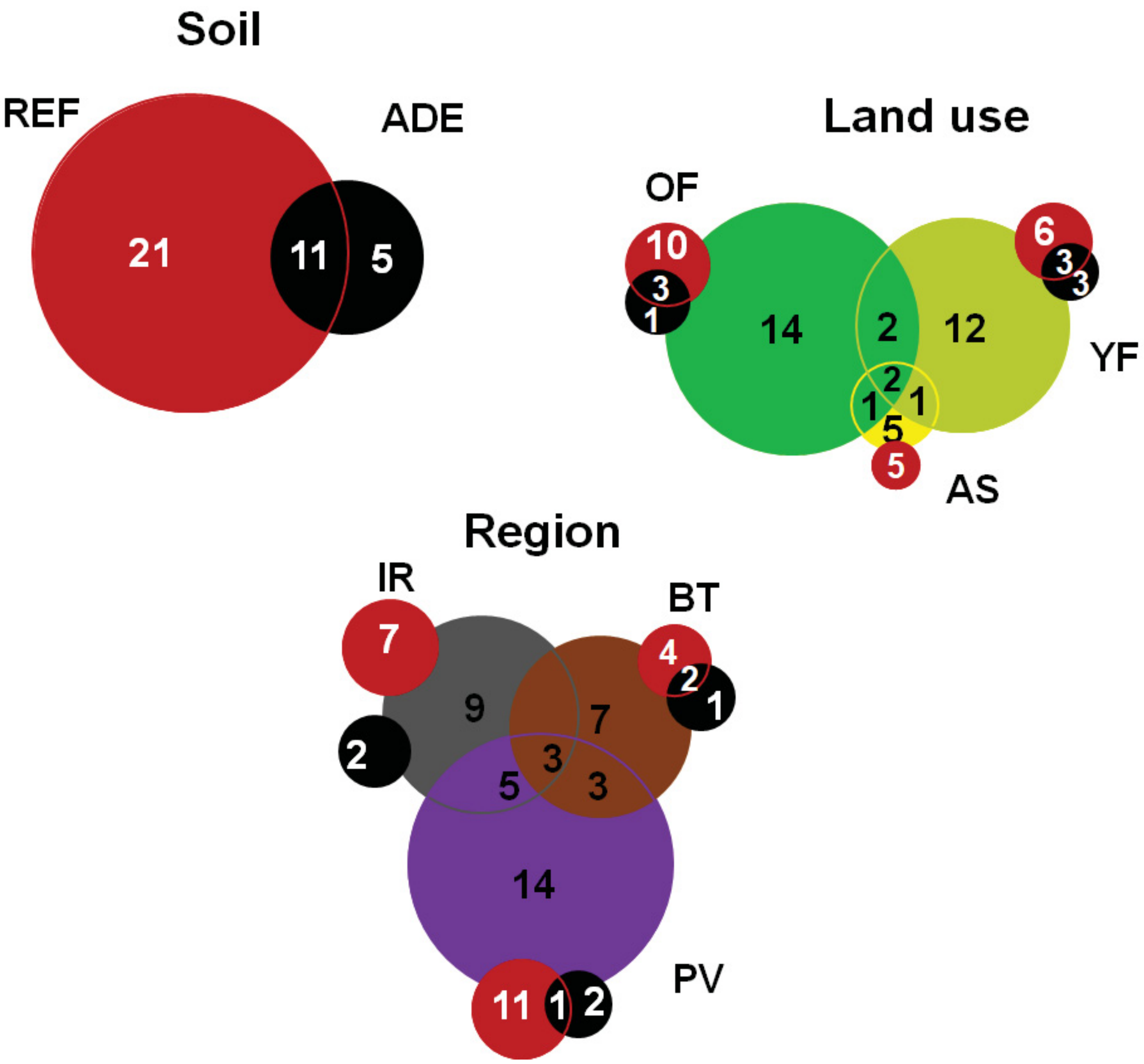
Supplementary Figure 2. Venn charts of total macroinvertebrate species richness (from soil monolith samples), and overlaps according to soil (REF: Reference soil, ADE: Amazonian Dark Earth), to geographic region (IR: Iranduba; BT: Belterra; PV: Porto Velho), and land-use systems (OF: old forest; YF: young forest; AS: agricultural systems) (ADE in black, REF in red). Numbers for species richness in REF (in red) and ADE (in black) soils are only of the unique species in each region and land-use system.

SUPPLEMENTARY FIGURE 3 – VENN CHARTS OF ANT SPECIES RICHNESS



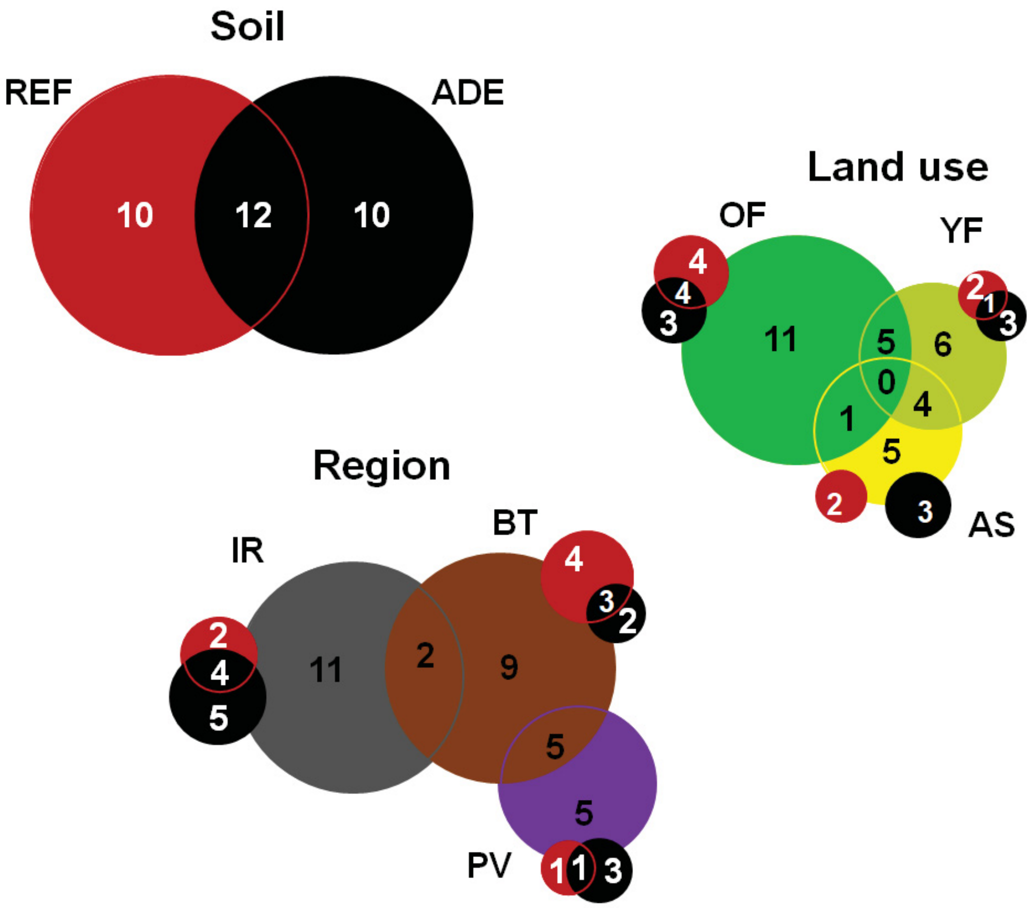
Supplementary Figure 3. Venn charts of ant species richness (from soil monolith samples), and overlaps according to soil (REF: Reference soil, ADE: Amazonian Dark Earth), to geographic region (IR: Iranduba; BT: Belterra; PV: Porto Velho), and land-use systems (OF: old forest; YF: young forest; AS: agricultural systems) (ADE in black, REF in red). Numbers for species richness in REF (in red) and ADE (in black) soils are only of the unique species in each region and land-use system.

SUPPLEMENTARY FIGURE 4 – VENN CHARTS OF TERMITE SPECIES RICHNESS



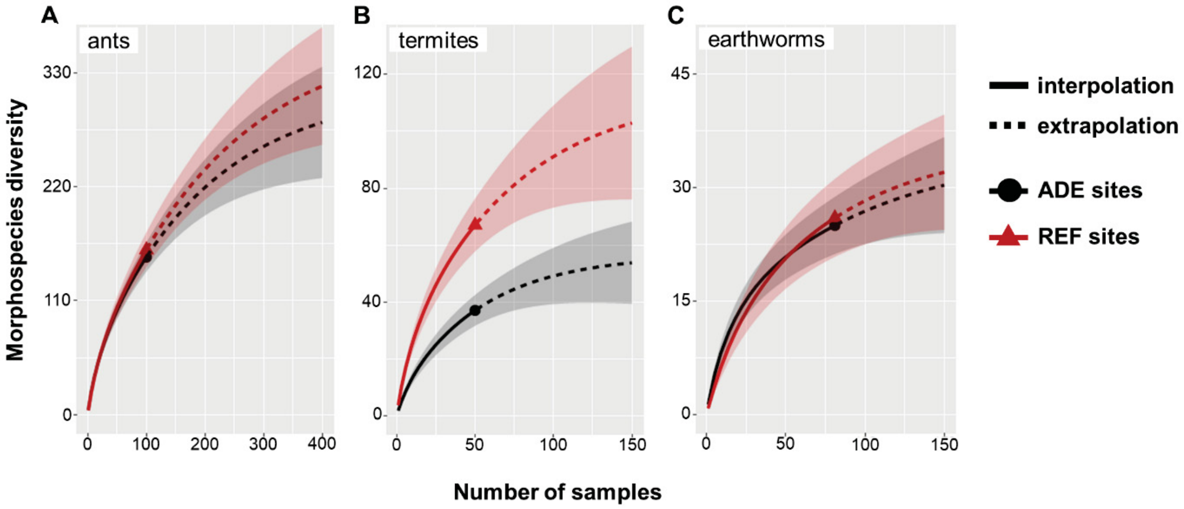
Supplementary Figure 4. Venn charts of termite species richness (from soil monolith samples), and overlaps according to soil (REF: Reference soil, ADE: Amazonian Dark Earth), to geographic region (IR: Iranduba; BT: Belterra; PV: Porto Velho), and land-use systems (OF: old forest; YF: young forest; AS: agricultural systems) (ADE in black, REF in red). Numbers for species richness in REF (in red) and ADE (in black) soils are only of the unique species in each region and land-use system.

SUPPLEMENTARY FIGURE 5 – VENN CHARTS OF EARTHWORM SPECIES RICHNESS



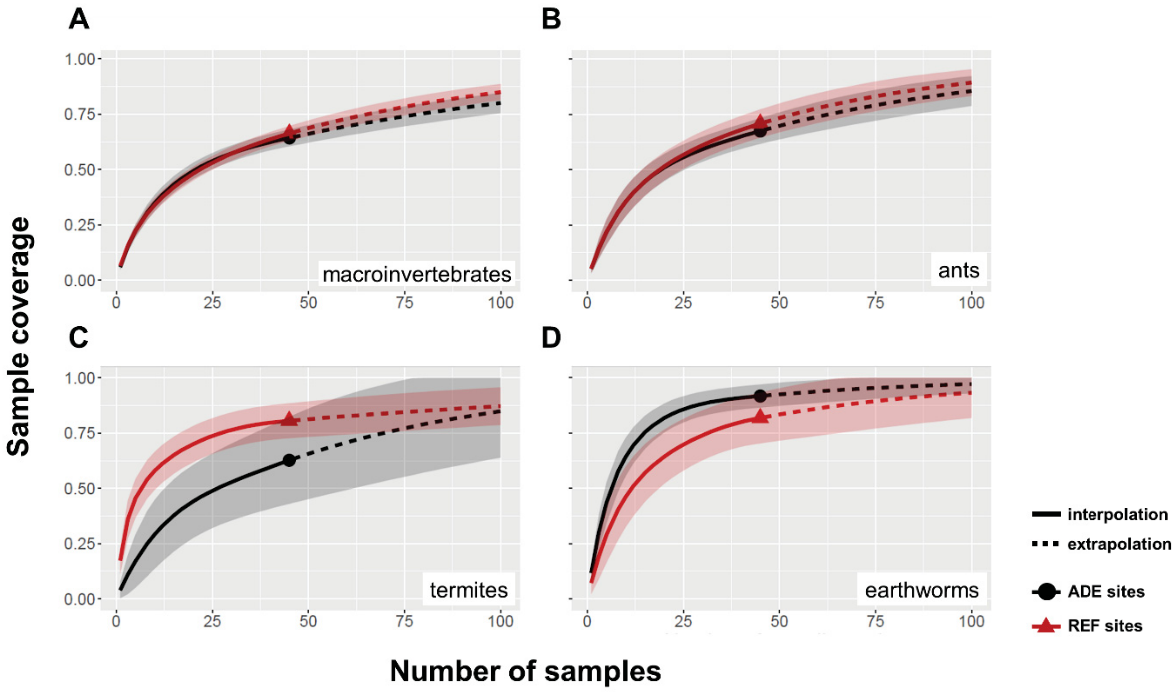
Supplementary Figure 5. Venn charts of earthworm species richness (from soil monolith samples), and overlaps according to soil (REF: Reference soil, ADE: Amazonian Dark Earth), to geographic region (IR: Iranduba; BT: Belterra; PV: Porto Velho), and land-use systems (OF: old forest; YF: young forest; AS: agricultural systems) (ADE in black, REF in red). Numbers for species richness in REF (in red) and ADE (in black) soils are only of the unique species in each region and land-use system.

SUPPLEMENTARY FIGURE 6 – MORPHOSPECIES RAREFACTION AND EXTRAPOLATION CURVES



Supplementary Figure 6. Morphospecies rarefaction and extrapolation curves, showing how morphospecies numbers increase in both ADE and REF soils depending on sampling intensity (number of samples) for: (**a**) ants collected in pitfall traps + TSBF in Iranduba and Belterra under old and young forests, (**b**) termites in TSBF samples + 10 m^2 plots in old and young forests (except YF1 in PV), and (**c**) earthworms from all (n=9 per plot) samples over all sites. Dark grey and red areas represent 95% confidence intervals. REF: Reference soil, ADE: Amazonian Dark Earth

SUPPLEMENTARY FIGURE 7 – MORPHOSPECIES RAREFACTION AND EXTRAPOLATION CURVES



Supplementary Figure 7. Sampling effort coverage, showing diversity collected depending on sampling intensity (number of samples) in ADEs and REF soils for: (**a**) All soil macroinvertebrates, (**b**) ants, (**c**) termites and (**d**) earthworms. Data correspond to invertebrates collected using soil monoliths, over all sites and land use systems. Dark grey and red areas represent 95% confidence intervals. REF: Reference